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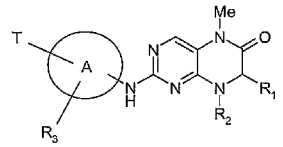
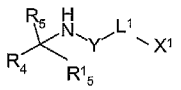
(54) **Title**: INHIBITORS OF PLK



(I)



(II)

(57) **Abstract**: Compounds of formula (I) are PLK inhibitors, useful for the treatment of cell proliferative diseases wherein R_1 is hydrogen, or an optionally substituted (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl or (C_3-C_6)cycloalkyl group; R_2 is hydrogen, or an optionally substituted (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl or (C_3-C_6)cycloalkyl group; R_3 is hydrogen, -CN, hydroxyl, halogen, optionally substituted (C_1C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl or (C_3-C_6)cycloalkyl, -NR_6R_7 or C_1-C_4 alkoxy, wherein R_6 and R_7 are independently hydrogen or optionally substituted (C_1C_6)alkyl; ring A is an optionally substituted mono- or bi-cyclic carbocyclic or heterocyclic ring or a ring system having up to 12 ring atoms; T is a radical of formula (II) wherein R_4 is a carboxylic acid group (-COOH), or an ester group which is hydrolysable by one or more intracellular esterase enzymes to a carboxylic acid group; R_5 and R^1_5 independently represent the side chain of a natural or non-natural alpha amino acid but neither of R_5 and R^1_5 is hydrogen, or R_5 and R^1_5 taken together with the carbon atom to which they are attached form a C_3-C_7 cycloalkyl ring; and Y, L^1 and X^1 are as defined in the claims.

Inhibitors of PLK

This invention relates to a series of amino acid esters, to compositions containing them, to processes for their preparation and to their use in medicine as Polo-like kinase 'PLK' inhibitors. Polo-like kinases (PLKs) are key enzymes that control mitotic entry of proliferating cells and regulate many aspects of mitosis necessary for successful cytokinesis. Of the four known human PLKs, PLK1 is the best characterized and is overexpressed in many tumour types with aberrant elevation frequently constituting a prognostic indicator of poor disease outcome. The compounds may be of use in the treatment of cell proliferative diseases such as cancer. The present invention encompasses compounds that are dihydropteridinine derivatives.

Background to invention

The PLKs, a family of Ser/Thr protein kinases named after their functional and sequence similarity with the archetypal *polo* kinase from *Drosophila melanogaster*, play a variety of roles in mitosis (*Nat. Rev. Mol. Cell Biol.*, **2001**, *2*, 21-32.). In yeasts *(Saccharomyces cerevisiae* and *S. pombe*) single PLKs exist, whereas four distinct PLKs have been identified to date in mammals. Human PLK1 (*Cell Growth Differ.*, **1994**, *5*, 249-257), PLK2 (serum-inducible kinase, SNK, *Mol. Cell. Biol.*, **1992**, *12*, 4164-4169), PLK3 (proliferation-related kinase, PRK *J. Biol. Chem.*, **1997**, *272*, 28646-28651) and PLK4 (*Oncol. Rep.*, **1997**, *4*, 505-510) are structurally homologous and contain two conserved domains, the N-terminal catalytic kinase domain, as well as a C-terminal region composed of the so-called polo boxes. Whereas PLK1, PLK2, and PLK3 are expressed in all tissues, PLK4 appears to possess unique physiological roles and the distribution of PLK4 mRNA in adults is restricted to certain tissues such as testes and thymus.

PLK1 is the best characterized member of the PLK family and it appears to fulfil most of the known functions of the single PLKs present in invertebrates (*Nat. Rev. Mol. Cell Biol.*, **2004**, *5*, 429-441). PLK1 protein levels fluctuate in a cell-cycle-dependent manner and its kinase activity peaks at the transition between the second gap phase and the mitosis phases (G2/M) of the eukaryotic cell division cycle. Upon exit from mitosis PLK1 levels drop as a result of ubiquitin-dependent proteolysis. PLK1 has been reported to be involved in the initiation of mitosis through activation of the cyclin-dependent kinase CDK1/cyclin B complex, *i.e.* the master switch for mitotic entry (mitosis-promoting factor, MPF, *Nature,* **1990**, *344*, 503-508).

This occurs when PLK1 phosphorylates, and thus activates, the dual specificity phosphatase CDC25C, which in turn relieves premitotic MYT1- and WEE1- mediated suppression of CDK1/cyclin B activity through dephosphorylation at the CDK1 pThr14 and pTyr15 sites (*Cell,* **1991**, *67*, 197 -211). Upon entry into mitosis, phosphorylation of CDC25C by PLK1 and PLK3

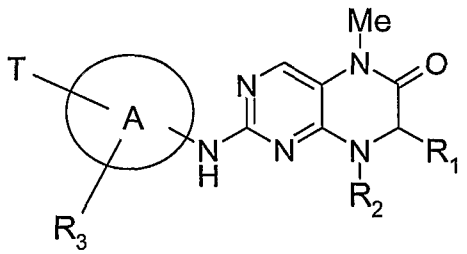
leads to its translocation into the nucleus. Apart from controlling entry into mitosis through CDK1 activation, PLK1 has additional roles in regulating progression through mitosis. It is involved in bipolar spindle formation, including centrosome maturation and regulation of the microtubule organizing centre, in the subsequent steps of mitosis involving sister chromatid separation, and finally in cytokinesis (*Dev. Cell,* **2003**, *5*, 127-138).

Brief Summary of the Invention

Compounds of the invention are related to compounds disclosed in WO 2004076454. They are inhibitors of PLK1 and the isoforms thereof. The compounds are thus of use in medicine, for example in the treatment of a variety of proliferative disease states, including cancers. The compounds are characterised by the presence in the molecule of an α,α-disubstituted glycine acid motif or an α,α-disubstituted glycine ester motif which is hydrolysable by an intracellular carboxylesterase. Compounds of the invention having the lipophilic α,α-disubstituted glycine ester motif cross the cell membrane, and are hydrolysed to the acid by the intracellular carboxylesterases. The polar hydrolysis product accumulates in the cell since it does not readily cross the cell membrane. Hence the PLK1 activity of the compound is prolonged and enhanced within the cell.

Detailed Description of the Invention

According to the invention there is provided a compound of formula (I), or a salt thereof:



(I)

wherein

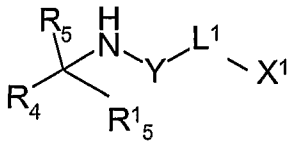
R_1 is hydrogen, or an optionally substituted (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_1-C_6)alkynyl or (C_3-C_6)cycloalkyl group;

R_2 is hydrogen, or an optionally substituted (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_1-C_6)alkynyl or (C_3-C_6)cycloalkyl group;

R_3 is hydrogen, -CN, hydroxyl, halogen, optionally substituted (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl or (C_3-C_6)cycloalkyl, -NR_6R_7 or (C_1-C_4)alkoxy, wherein R_6 and R_7 are independently hydrogen or optionally substituted (C_1-C_6)alkyl;

ring **A** is an optionally substituted mono- or bi-cyclic carbocyclic or heterocyclic ring or a ring system having up to 12 ring atoms;

T is a radical of formula (II)



(II)

wherein

R_4 is a carboxylic acid group (-COOH), or an ester group which is hydrolysable by one or more intracellular esterase enzymes to a carboxylic acid group;

R_5 and R^1_5 independently represent the side chain of a natural or non-natural alpha amino acid but neither of R_5 and R^1_5 is hydrogen, or R_5 and R^1_5 taken together with the carbon atom to which they are attached form a C3-C7 cycloalkyl ring;

Y is a bond, -C(=O)-, -$S(=O)_2$-, -C(=O)O-, -C(=O)NR_6-, -C(=S)-NR_6, -C(=NH)-NR_6 or -$S(=O)_2NR_6$- wherein R_6 is independently hydrogen or optionally substituted (C_1-C_6)alkyl;

L^1 is a divalent radical of formula -$(Alk^1)_m(Q^1)_n(Alk^2)_p$- wherein

m, **n** and **p** are independently 0 or 1,

Q^1 is (i) an optionally substituted divalent mono- or bicyclic carbocyclic or heterocyclic radical having 5 - 13 ring members, or (ii), in the case where p is 0, a divalent radical of formula -Q^2-X^2- wherein X^2 is -O-, -S- or NR^A- wherein R^A is hydrogen or optionally substituted C_1-C_3 alkyl, and Q^2 is an optionally substituted divalent mono- or bicyclic carbocyclic or heterocyclic radical having 5 - 13 ring members,

Alk^1 and Alk^2 independently represent optionally substituted divalent (C_3-C_6)cycloalkyl radicals, or optionally substituted straight or branched, (C_1-C_6)alkylene, (C_2-C_6)alkenylene, or (C_2-C_6)alkynylene radicals which may optionally contain or terminate in

an ether (-O-), thioether (-S-) or amino (-NR^A-) link wherein R^A is hydrogen or optionally substituted (C_1-C_3)alkyl;

X^1 represents a bond, -C(=O)-; or -$S(=O)_2$-; -$NR_6C(=O)$-, -$C(=O)NR_6$-, -$NR_6C(=O)$-NR_7- , -$NR_6S(=O)_2$-, or -$S(=O)_2NR_6$- wherein R_6 and R_7 are independently hydrogen or optionally substituted (C_1-C_6)alkyl.

In the compounds of the invention, when R_1 is other than hydrogen, the carbon atom to which the R_1 substituent is attached is asymmetric. Preferably the stereochemistry at that asymmetric center is (R).

In another broad aspect the invention provides the use of a compound of formula (I) as defined above, or an N-oxide, salt, hydrate or solvate thereof in the preparation of a composition for inhibiting the activity of PLK1.

The compounds with which the invention is concerned may be used for the inhibition of PLK1 activity *ex vivo* or *in vivo*.

In one aspect of the invention, the compounds of the invention may be used in the preparation of a composition for treatment of cell proliferative diseases such as cancer.

In another aspect, the invention provides a method for the treatment of the foregoing disease types, which comprises administering to a subject suffering such disease an effective amount of a compound of formula (I) as defined above.

Terminology

As used herein, the term "(C_a-C_b)alkyl" wherein a and b are integers, refers to a straight or branched chain alkyl radical having from a to b carbon atoms. Thus when a is 1 and b is 6, for example, the term includes methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, n-pentyl and n-hexyl.

As used herein, the term "divalent (C_a-C_b)alkylene radical", wherein a and b are integers, refers to a saturated hydrocarbon chain having from a to b carbon atoms and two unsatisfied valences.

As used herein, the term "(C_a-C_b)alkenyl" wherein a and b are integers, refers to a straight or branched chain alkenyl moiety with a to b carbon atoms; having at least one double bond of either E or Z stereochemistry where applicable. The term includes, for example, vinyl, allyl, 1- and 2-butenyl and 2-methyl-2-propenyl.

As used herein, the term "divalent (C_a-C_b)alkenylene radical" means a hydrocarbon chain having from a to b carbon atoms, at least one double bond, and two unsatisfied valences.

As used herein the term "C_a-C_b alkynyl", wherein a and b are integers refers to straight chain or branched chain hydrocarbon groups having from two to six carbon atoms and having in addition one triple bond. This term would include, for example, ethynyl, 1-propynyl, 1- and 2-butynyl, 2-methyl-2-propynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl and 5-hexynyl.

As used herein, the term "divalent (C_a-C_b)alkynylene radical", wherein a and b are integers refers to a divalent hydrocarbon chain having from two to six carbon atoms, and at least one triple bond.

As used herein, the term "carbocyclic" refers to a mono-, bi- or tricyclic radical having up to 16 ring atoms, all of which are carbon, and includes aryl and cycloalkyl.

As used herein, the term "cycloalkyl" refers to a monocyclic saturated carbocyclic radical having from 3-8 carbon atoms and includes, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl.

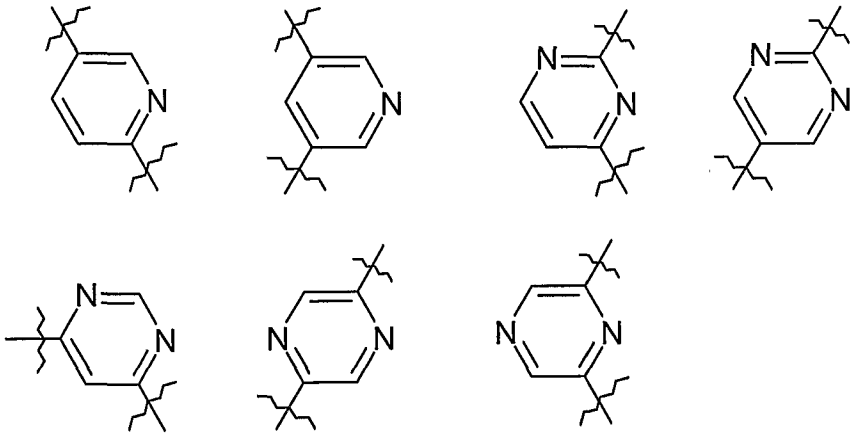
As used herein, the unqualified term "aryl" refers to a mono-, bi- or tri-cyclic carbocyclic aromatic radical, and includes radicals having two monocyclic carbocyclic aromatic rings which are directly linked by a covalent bond. Illustrative of such radicals are phenyl, biphenyl and napthyl.

As used herein, the unqualified term "heteroaryl" refers to a mono-, bi- or tri-cyclic aromatic radical containing one or more heteroatoms selected from S, N and O, and includes radicals having two such monocyclic rings, or one such monocyclic ring and one monocyclic aryl ring, which are directly linked by a covalent bond. Illustrative of such radicals are thienyl, benzothienyl, furyl, benzofuryl, pyrrolyl, imidazolyl, benzimidazolyl, thiazolyl, benzthiazolyl, isothiazolyl, benzisothiazolyl, pyrazolyl, oxazolyl, benzxazolyl, isoxazolyl, benzisoxazolyl,

isothiazolyl, triazolyl, benztriazolyl, thiadiazolyl, oxadiazolyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, indolyl and indazolyl.

As used herein, the unqualified term "heterocyclyl" or "heterocyclic" includes "heteroaryl" as defined above, and in its non-aromatic meaning relates to a mono-, bi- or tri-cyclic non-aromatic radical containing one or more heteroatoms selected from S, N and O, and to groups consisting of a monocyclic non-aromatic radical containing one or more such heteroatoms which is covalently linked to another such radical or to a monocyclic carbocyclic radical. Illustrative of such radicals are pyrrolyl, furanyl, thienyl, piperidinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, thiadiazolyl, pyrazolyl, pyridinyl, pyrrolidinyl, pyrimidinyl, morpholinyl, piperazinyl, indolyl, morpholinyl, benzofuranyl, pyranyl, isoxazolyl, benzimidazolyl, methylenedioxyphenyl, ethylenedioxyphenyl, maleimido and succinimido groups.

A "divalent phenylene, pyridinylene, pyrimidinylene, or pyrazinylene radical" is a benzene, pyridine, pyrimidine or pyrazine ring, with two unsatisfied valencies, and includes 1,3-phenylene, 1,4-phenylene, and the following:



Unless otherwise specified in the context in which it occurs, the term "substituted", as applied to any moiety herein, means substituted with up to four compatible substituents, each of which independently may be, for example, (C_1-C_6)alkyl, (C_1-C_6)alkoxy, hydroxy, hydroxy(C_1-C_6)alkyl, mercapto, mercapto(C_1-C_6)alkyl, (C_1-C_6)alkylthio, phenyl, halo (including fluoro, bromo and chloro), trifluoromethyl, trifluoromethoxy, nitro, nitrile (-CN), oxo, -COOH, -$COOR^A$, -COR^A, -SO_2R^A, -$CONH_2$, -SO_2NH_2, -$CONHR^A$, -SO_2NHR^A, -$CONR^AR^B$, -$SO_2NR^AR^B$, -NH_2, -NHR^A, -NR^AR^B, -$OCONH_2$, -$OCONHR^A$, -$OCONR^AR^B$, -$NHCOR^A$, -$NHCOOR^A$, -NR^BCOOR^A, -$NHSO_2OR^A$, -NR^BSO_2OH, -$NR^BSO_2OR^A$, -$NHCONH_2$, -NR^ACONH_2, -$NHCONHR^B$, -NR^ACONHR^B, -$NHCONR^AR^B$, or -$NR^ACONR^AR^B$ wherein R^A and R^B are independently a (C_1-C_6)alkyl, (C_3-C_6) cycloalkyl, phenyl or monocyclic heteroaryl having 5 or 6 ring atoms, or R^A and R^B when attached to the same nitrogen atom form a cyclic amino group (for example

morpholino, piperidinyl, piperazinyl, or tetrahydropyrrolyl). An "optional substituent" may be one of the foregoing substituent groups.

The term "side chain of a natural or non-natural alpha-amino acid" refers to the group R^Y in a natural or non-natural amino acid of formula NH_2-CH(R^Y)-COOH.

Examples of side chains of natural alpha amino acids include those of alanine, arginine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, histidine, 5-hydroxylysine, 4-hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, α-aminoadipic acid, α-amino-n-butyric acid, 3,4-dihydroxyphenylalanine, homoserine, α-methylserine, ornithine, pipecolic acid, and thyroxine.

Natural alpha-amino acids which contain functional substituents, for example amino, carboxyl, hydroxy, mercapto, guanidyl, imidazolyl, or indolyl groups in their characteristic side chains include arginine, lysine, glutamic acid, aspartic acid, tryptophan, histidine, serine, threonine, tyrosine, and cysteine. When R_5 or R^1_5 in the compounds of the invention is one of those side chains, the functional substituent may optionally be protected.

The term "protected" when used in relation to a functional substituent in a side chain of a natural alpha-amino acid means a derivative of such a substituent which is substantially non-functional. For example, carboxyl groups may be esterified (for example as a C_1-C_6 alkyl ester), amino groups may be converted to amides (for example as a NHCOC_1-C_6 alkyl amide) or carbamates (for example as an NHC(=O)OC_1-C_6 alkyl or NHC(=O)OCH_2Ph carbamate), hydroxyl groups may be converted to ethers (for example an OC_1-C_6 alkyl or a O(C_1-C_6 alkyl)phenyl ether) or esters (for example a OC(=O)C_1-C_6 alkyl ester) and thiol groups may be converted to thioethers (for example a tert-butyl or benzyl thioether) or thioesters (for example a SC(=O)C_1-C_6 alkyl thioester).

As used herein the term "salt" includes base addition, acid addition and quaternary salts. Compounds of the invention which are acidic can form salts, including pharmaceutically acceptable salts, with bases such as alkali metal hydroxides, e.g. sodium and potassium hydroxides; alkaline earth metal hydroxides e.g. calcium, barium and magnesium hydroxides; with organic bases e.g. N-methyl-D-glucamine, choline tris(hydroxymethyl)amino-methane, L-arginine, L-lysine, N-ethyl piperidine, dibenzylamine and the like. Those compounds (I) which are basic can form salts, including pharmaceutically acceptable salts with inorganic acids, e.g. with hydrohalic acids such as hydrochloric or hydrobromic acids, sulphuric acid, nitric acid or phosphoric acid and the like, and with organic acids e.g. with acetic, tartaric, succinic, fumaric,

maleic, malic, salicylic, citric, methanesulphonic, p-toluenesulphonic, benzoic, benzenesulphonic, glutamic, lactic, and mandelic acids and the like.

It is expected that compounds of the invention may be recovered in N-oxide, hydrate or solvate form, and such forms are expected to have the activity of the non-hydrated, non-solvated or non-N-oxidised forms. The term 'solvate' is used herein to describe a molecular complex comprising the compound of the invention and a stoichiometric amount of one or more pharmaceutically acceptable solvent molecules, for example, ethanol. The term 'hydrate' is employed when said solvent is water.

Compounds of the invention which contain one or more actual or potential chiral centres, because of the presence of asymmetric carbon atoms, can exist as a number of diastereoisomers with R or S stereochemistry at each chiral centre. The invention includes all such diastereoisomers and mixtures thereof.

The term "ester" or "esterified carboxyl group" in connection with substituent R_4 above means a group $R_{10}O(C=O)$- in which R_{10} is the group characterising the ester, notionally derived from the alcohol $R_{10}OH$.

The substituents R_1-R_3

R_1 is hydrogen, (C_1-C_6)alkyl, for example methyl, ethyl, n- or iso-propyl, (C_2-C_6)alkenyl, for example allyl, (C_2-C_6)alkynyl, for example -$CH_2C{\equiv}CH$ or (C_3-C_6)cycloalkyl, for example cyclopropyl, cyclopentyl or cyclohexyl. In one subclass of compounds of the invention R_1 is ethyl.

R_2 is hydrogen, (C_1-C_6)alkyl, for example methyl, ethyl,n- or iso-propyl, (C_2-C_6)alkenyl, for example allyl, (C_2-C_6)alkynyl, for example -$CH_2C{\equiv}CH$ or (C_3-C_6)cycloalkyl, for example cyclopropyl, cyclopentyl or cyclohexyl, or $C_{6\text{-}14}$ aryl for example phenyl or naphthyl. In one subclass of compounds of the invention R_2 is cyclopentyl.

R_3 is hydrogen, -CN, hydroxyl, halogen, (C_1-C_6)alkyl, for example methyl, ethyl,n- or iso-propyl, (C_2-C_6)alkenyl, for example allyl, (C_2-C_6)alkynyl, for example -$CH_2C{\equiv}CH$ or (C_3-C_6)cycloalkyl, for example cyclopropyl, cyclopentyl or cyclohexyl, -NR_6R_7 and (C_1-C_4)alkoxy, wherein R_6 and R_7 are independently hydrogen or optionally substituted (C_1-C_6)alkyl, for example methyl or ethyl. In one subclass of compounds of the invention R_3 is hydrogen.

The ring A

Ring **A** is a mono- or bi-cyclic carbocyclic or heterocyclic ring or a ring system having up to 12 ring atoms. Examples of such rings are piperidine, piperazine, pyridine, pyrimidine, pyrazoline, triazoline, furan, thiophene, pyrrole, thiazole, isothiazole, oxazole, isoxazole, and thiadiazole rings. Currently preferred rings A are phenyl, pyridinyl and pyrimidinyl.

Ring A may be substituted by any of the optional substituents referred to above, for example chloro, bromo or fluoro, trifluoromethyl, methoxy, and trifluoromethoxy.

The group R_4

R_4 is a carboxylic acid group or an ester group which is hydrolysable by one or more intracellular carboxylesterase enzymes to a carboxylic acid group. Intracellular carboxylesterase enzymes capable of hydrolysing the ester group of a compound of the invention to the corresponding acid include the three known human enzyme isotypes hCE-1, hCE-2 and hCE-3. Although these are considered to be the main enzymes, other enzymes such as biphenylhydrolase (BPH) may also have a role in hydrolysing the ester. In general, if the carboxylesterase hydrolyses the free amino acid ester to the parent acid it will also hydrolyse the ester motif when covalently conjugated to the PLK1 inhibitor. Hence, the broken cell assay described herein provides a straightforward, quick and simple first screen for esters which have the required hydrolysis profile. Ester motifs selected in that way may then be re-assayed in the same carboxylesterase assay when conjugated to the modulator *via* the chosen conjugation chemistry, to confirm that it is still a carboxylesterase substrate in that background.

Subject to the requirement that they be hydrolysable by intracellular carboxylesterase enzymes, examples of particular ester groups R_4 include those of formula $-(C=O)OR_{10}$ wherein R_{10} is $R_{11}R_{12}R_{13}C-$ wherein

(i) R_{11} is hydrogen, fluorine or optionally substituted $(C_1\text{-}C_3)$alkyl-$(Z^1)_a$-$[(C_1\text{-}C_3)\text{alkyl}]_b$- or $(C_2\text{-}C_3)$alkenyl-$(Z^1)_a$-$[(C_1\text{-}C_3)\text{alkyl}]_b$- wherein a and b are independently 0 or 1 and Z^1 is –O-, -S-, or $-NR_{14}$- wherein R_{14} is hydrogen or $(C_1\text{-}C_3)$alkyl; and R_{12} and R_{13} are independently hydrogen or $(C_1\text{-}C_3)$alkyl-;

(ii) R_{11} is hydrogen or optionally substituted $R_{15}R_{16}N$-$(C_1\text{-}C_3)$alkyl- wherein R_{15} is hydrogen or $(C_1\text{-}C_3)$alkyl and R_{16} is hydrogen or $(C_1\text{-}C_3)$alkyl; or R_{15} and R_{16} together with the nitrogen to which they are attached form an optionally substituted monocyclic heterocyclic ring of 5- or 6- ring atoms or bicyclic heterocyclic ring system of 8 to 10 ring atoms, and R_{12} and R_{13} are independently hydrogen or $(C_1\text{-}C_3)$alkyl-;or

(iii) R_{11} and R_{12} taken together with the carbon to which they are attached form an optionally substituted monocyclic carbocyclic ring of from 3 to 7 ring atoms or bicyclic carbocyclic ring system of 8 to 10 ring atoms, and R_{13} is hydrogen.

In cases (i), (ii) and (iii) above, "alkyl" includes fluoroalkyl.

Within these classes, R_{10} may be, for example, methyl, trifluoromethyl, ethyl, n- or iso-propyl, n-, sec- or tert-butyl, cyclohexyl, allyl, phenyl, benzyl, 2-, 3- or 4-pyridylmethyl, N-methylpiperidin-4-yl, tetrahydrofuran-3-yl, methoxyethyl, indanyl, norbonyl, dimethylaminoethyl, or morpholinoethyl. Currently preferred is where R_{10} is cyclopentyl.

Macrophages are known to play a key role in inflammatory disorders through the release of cytokines in particular TNFα and IL-1 (van Roon *et al.*, *Arthritis and Rheumatism* , 2003, 1229-1238). In rheumatoid arthritis they are major contributors to the maintenance of joint inflammation and joint destruction. Macrophages are also involved in tumour growth and development (Naldini and Carraro, *Curr Drug Targets Inflamm Allergy*, 2005, 3-8). Hence agents that selectively target macrophage cell proliferation and function could be of value in the treatment of cancer and autoimmune disease. Targeting specific cell types would be expected to lead to reduced side-effects. The inventors have discovered a method of targeting inhibitors to cells that express hCE-1, in particular macrophages and other cells derived from the myelo-monocytic lineage such as monocytes, osteoclasts and dendritic cells, This is based on the observation that the way in which the esterase motif is linked to the inhibitor determines whether it is hydrolysed by all three human carboxylesterases or just by hCE-1, and hence whether or not it accumulates in different cell types. Specifically it has been found that macrophages and other cells derived from the myelo-monocytic lineage, both normal and cancerous, contain the human carboxylesterase hCE-1 whereas other cell types do not. In the general formula (I) when the nitrogen of the esterase motif $R_1CH(R_2)NH$- is not directly linked to a carbonyl (-C(=O)-), ie when Y is not a –C(=O), -C(=O)O- or -C(=O)NR_3- radical, the ester will only be hydrolysed by hCE-1 and hence the inhibitors selectively accumulate in macrophage-related cells

The amino acid side chains ***R_5*** *and* R^1_5

Subject to the requirement that the ester group R_4 be hydrolysable by intracellular carboxylesterase enzymes, the identity of the side chain groups R_5 and R^1_5 are not critical.

For example, R_5 and R^1_5 may independently be phenyl, or heteroaryl such as pyridyl, or a group of formula $-CR_aR_bR_c$ in which:

each of R_a, R_b and R_c is independently hydrogen, $(C_1$-$C_6)$alkyl, $(C_2$-$C_6)$alkenyl, $(C_2$-$C_6)$alkynyl, phenyl$(C_1$-$C_6)$alkyl, $(C_3$-$C_8)$cycloalkyl; or

R_c is hydrogen and R_a and R_b are independently phenyl or heteroaryl such as pyridyl; or

R_c is hydrogen, $(C_1$-$C_6)$alkyl, $(C_2$-$C_6)$alkenyl, $(C_2$-$C_6)$alkynyl, phenyl$(C_1$-$C_6)$alkyl, or $(C_3$-$C_8)$cycloalkyl, and R_a and R_b together with the carbon atom to which they are attached form a 3 to 8 membered cycloalkyl or a 5- to 6-membered heterocyclic ring; or

R_a, R_b and R_c together with the carbon atom to which they are attached form a tricyclic ring (for example adamantyl); or

R_a and R_b are each independently $(C_1$-$C_6)$alkyl, $(C_2$-$C_6)$alkenyl, $(C_2$-$C_6)$alkynyl, phenyl$(C_1$-$C_6)$alkyl, or a group as defined for R_c below other than hydrogen, or R_a and R_b together with the carbon atom to which they are attached form a cycloalkyl or heterocyclic ring, and R_c is hydrogen, -OH, -SH, halogen, -CN, $-CO_2H$, $(C_1$-$C_4)$perfluoroalkyl, $-CH_2OH$, $-O(C_1$-$C_6)$alkyl, $-O(C_2$-$C_6)$alkenyl, $-S(C_1$-$C_6)$alkyl, $-SO(C_1$-$C_6)$alkyl, $-SO_2(C_1$-$C_6)$ alkyl, $-S(C_2$-$C_6)$alkenyl, $-SO(C_2$-$C_6)$alkenyl, $-SO_2(C_2$-$C_6)$alkenyl or a group -Q-W wherein Q represents a bond or -O-, -S-, -SO- or $-SO_2$- and W represents a phenyl, phenylalkyl, $(C_3$-$C_8)$cycloalkyl, $(C_3$-$C_8)$cycloalkylalkyl, $(C_4$-$C_8)$cycloalkenyl, $(C_4$-$C_8)$cycloalkenylalkyl, heteroaryl or heteroarylalkyl group, which group W may optionally be substituted by one or more substituents independently selected from, hydroxyl, halogen, -CN, $-CONH_2$, $-CONH(C_1$-$C_6)$alkyl, $-CONH(C_1$-C_6alkyl$)_2$, -CHO, $-CH_2OH$, $(C_1$-$C_4)$perfluoroalkyl, $-O(C_1$-$C_6)$alkyl, $-S(C_1$-$C_6)$alkyl, $-SO(C_1$-$C_6)$alkyl, $-SO_2(C_1$-$C_6)$alkyl, $-NO_2$, $-NH_2$, $-NH(C_1$-$C_6)$alkyl, $-N((C_1$-$C_6)$alkyl$)_2$, $-NHCO(C_1$-$C_6)$alkyl, $(C_1$-$C_6)$alkyl, $(C_2$-$C_6)$alkenyl, $(C_2$-$C_6)$alkynyl, $(C_3$-$C_8)$cycloalkyl, $(C_4$-$C_8)$cycloalkenyl, phenyl or benzyl.

In some cases, R_5 and $R^1{}_5$ are independently H-Alk^4-, phenyl, monocyclic heterocyclyl, C_3-C_7 cycloalkyl, phenyl(Alk^4)-, heterocyclyl(Alk^4)-, or C_3-C_7 cycloalkyl(Alk^4)-, wherein the heterocyclyl part is monocyclic heterocyclyl having 3-7 ring atoms, and wherein -Alk^4- is a straight or branched, divalent $(C_1$-$C_6)$alkylene, $(C_2$-$C_6)$alkenylene, or $(C_2$-$C_6)$alkynylene radical which may optionally be interrupted by, or terminate in, an ether (-O-), thioether (-S-) or amino ($-NR^A$-) link wherein R^A is hydrogen or optionally substituted $(C_1$-$C_3)$alkyl, and wherein the Alk^4-, or cyclic part is optionally substituted. For example, R_5 and $R^1{}_5$ independently may be C_1-C_6 alkyl substituent, for example methyl, ethyl, n-or iso-propyl, or n-, sec- or tert-butyl.

In a particular case, both at least one of R_5 and R^1_5 is methyl.

In a special case, R_5 and R^1_5 taken together with the carbon atom to which they are attached form a C_3-C_7 cycloalkyl ring, such as cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl ring.

For compounds of the invention which are to be administered systemically, esters with a slow rate of carboxylesterase cleavage are preferred, since they are less susceptible to pre-systemic metabolism. Their ability to reach their target tissue intact is therefore increased, and the ester can be converted inside the cells of the target tissue into the acid product. However, for local administration, where the ester is either directly applied to the target tissue or directed there by, for example, inhalation, it will often be desirable that the ester has a rapid rate of esterase cleavage, to minimise systemic exposure and consequent unwanted side effects. In the compounds of this invention, if the carbons adjacent to the alpha carbon of the alpha amino acid ester are monosubstituted, ie R_5 and R^1_5 are $-CH_2R^z$ (R^z being the mono-substituent) then the esters tend to be cleaved more rapidly than if that carbon is di- or tri-substituted, as in the case where R_5 and R^1_5 are for example, phenyl or cyclohexyl, or together form a ring.

The radical -Y-L^1-X^1 -

This radical (or bond) arises from the particular chemistry strategy chosen to link the amino acid ester motif $R_4CH(R_5)NH$- to the rest of the molecule. Clearly the chemistry strategy for that coupling may vary widely and thus many combinations of the variables Y, L^1, and X^1 are possible. However, when the inhibitor is bound to the enzyme at its active site, the ring A is located away from the enzyme, so by linking the amino acid ester motif to ring A it generally extends in a direction away from the enzyme, and thus minimises or avoids interference with the binding mode of the inhibitor. Hence the precise combination of variables making up the linking chemistry between the amino acid ester motif and the ring A will often be irrelevant to the primary binding mode of the compound as a whole. On the other hand, that linkage chemistry may in some cases pick up additional binding interactions with the enzyme, thereby enhancing binding.

It should also be noted that the benefits of the amino acid ester motif described above (facile entry into the cell, carboxylesterase hydrolysis within the cell, and accumulation within the cell of active carboxylic acid hydrolysis product) are best achieved when the linkage between the amino acid ester motif and the ring A is not a substrate for peptidase activity within the cell, which might result in cleavage of the amino acid from the molecule. Of course, stability to intracellular peptidases is easily tested by incubating the compound with disrupted cell contents, and analysing for any such cleavage.

With the foregoing general observations in mind, taking the variables making up the radical -Y-L^1-X^1- in turn:

specific preferred examples of Y are -(C=O)-, -(C=O)NH-, and -(C=O)O-. Y may also be a bond.

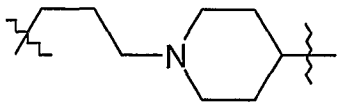
In the radical L^1, examples of Alk^1 and Alk^2 radicals, when present, include $-CH_2-$, $-CH_2CH_2-$, $-CH_2CH_2CH_2-$, $-CH_2CH_2CH_2CH_2-$, -CH=CH-, $-CH=CHCH_2-$, $-CH_2CH=CH-$, $CH_2CH=CHCH_2$--C≡C-, $-C\equiv CCH_2-$, $CH_2C\equiv C-$, and $CH_2C\equiv CCH_2$. Additional examples of Alk^1 and Alk^2 include $-CH_2W-$, $-CH_2CH_2W-$, $-CH_2CH_2WCH_2-$, $-CH_2CH_2WCH(CH_3)-$, $-CH_2WCH_2CH_2-$, $-CH_2WCH_2CH_2WCH_2-$, and $-WCH_2CH_2-$ where W is -O-, -S-, -NH-, $-N(CH_3)-$, or $-CH_2CH_2N(CH_2CH_2OH)CH_2-$. Further examples of Alk^1 and Alk^2 include divalent cyclopropyl, cyclopentyl and cyclohexyl radicals.

Alk^1 and Alk^2 when present may also be branched chain alkyl such as $-CH(CH_3)-$, $-C(CH_3)_2-$, or in either orientation $-CH_2CH(CH_3)-$, $-CH_2C(CH_3)_2-$.

In L^1, when n is 0, the radical is a hydrocarbon chain (optionally substituted and perhaps having an ether, thioether or amino linkage). Presently it is preferred that there be no optional substituents in L^1. When both m and p are 0, L^1 is a divalent mono- or bicyclic carbocyclic or heterocyclic radical with 5 - 13 ring atoms (optionally substituted). When n is 1 and at least one of m and p is 1, L^1 is a divalent radical including a hydrocarbon chain or chains and a mono- or bicyclic carbocyclic or heterocyclic radical with 5 - 13 ring atoms (optionally substituted).
When present, Q^1 may be, for example, a divalent phenyl, naphthyl, cyclopropyl, cyclopentyl, or cyclohexyl radical, or a mono-, or bi-cyclic heterocyclic radical having 5 to13 ring members, such as piperidinyl, piperazinyl, indolyl, pyridyl, thienyl, or pyrrolyl radical.

Specifically, in some embodiments of the invention, L^1, m and p may be 0 with n being 1. In other embodiments, n and p may be 0 with m being 1. In further embodiments, m, n and p may be all 0. In still further embodiments m may be 0, n may be 1 with Q^1 being a monocyclic heterocyclic radical, and p may be 0 or 1. Alk^1 and Alk^2, when present, may be selected from $-CH_2-$, $-CH_2CH_2-$, and $-CH_2CH_2CH_2-$ and Q^1 may be 1,4-phenylene.

In a specific example of the radical -Y-L^1-X^1-, Y is -C(=O)-, X^1 is -NHC(=O)- and L^1 has formula (III):



(III)

wherein the left hand valency is satisfied by Y and the right hand valency is satisfied by X^1.

As mentioned above, the compounds with which the invention is concerned are inhibitors of PLK1 kinase activity and are therefore of use for treatment of cell proliferative diseases such as cancer.

It will be understood that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing treatment. Optimum dose levels and frequency of dosing will be determined by clinical trial.

The compounds with which the invention is concerned may be prepared for administration by any route consistent with their pharmacokinetic properties. The orally administrable compositions may be in the form of tablets, capsules, powders, granules, lozenges, liquid or gel preparations, such as oral, topical, or sterile parenteral solutions or suspensions. Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrrolidone; fillers for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricant, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants for example potato starch, or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such

as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

For topical application to the skin, the drug may be made up into a cream, lotion or ointment. Cream or ointment formulations which may be used for the drug are conventional formulations well known in the art, for example as described in standard textbooks of pharmaceutics such as the British Pharmacopoeia.

For topical application by inhalation, the drug may be formulated for aerosol delivery for example, by pressure-driven jet atomizers or ultrasonic atomizers, or preferably by propellant-driven metered aerosols or propellant-free administration of micronized powders, for example, inhalation capsules or other "dry powder" delivery systems. Excipients, such as, for example, propellants (e.g. Frigen in the case of metered aerosols), surface-active substances, emulsifiers, stabilizers, preservatives, flavorings, and fillers (e.g. lactose in the case of powder inhalers) may be present in such inhaled formulations. For the purposes of inhalation, a large number of apparata are available with which aerosols of optimum particle size can be generated and administered, using an inhalation technique which is appropriate for the patient. In addition to the use of adaptors (spacers, expanders) and pear-shaped containers (e.g. Nebulator®, Volumatic®), and automatic devices emitting a puffer spray (Autohaler®), for metered aerosols, in particular in the case of powder inhalers, a number of technical solutions are available (e.g. Diskhaler®, Rotadisk®, Turbohaler® or the inhalers for example as described in European Patent Application EP 0 505 321).

For topical application to the eye, the drug may be made up into a solution or suspension in a suitable sterile aqueous or non aqueous vehicle. Additives, for instance buffers such as sodium metabisulphite or disodium edeate; preservatives including bactericidal and fungicidal agents such as phenyl mercuric acetate or nitrate, benzalkonium chloride or chlorhexidine, and thickening agents such as hypromellose may also be included.

The active ingredient may also be administered parenterally in a sterile medium. Depending on the vehicle and concentration used, the drug can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle.

The compounds of the invention may be used in conjunction with a number of known pharmaceutically active substances. For example, the compounds of the invention may be used with cytotoxics, HDAC inhibitors, kinase inhibitors, aminopeptidase inhibitors, protease

inhibitors, bcl-2 antagonists, inhibitors of mTor and monoclonal antibodies (for example those directed at growth factor receptors). Preferred cytotoxics include, for example, taxanes, platins, anti-metabolites such as 5-fluoracil, topoisomerase inhibitors and the like. The medicaments of the invention comprising amino acid derivatives of formula (I), tautomers thereof or pharmaceutically acceptable salts, N-oxides, hydrates or solvates thereof therefore typically further comprise a cytotoxic, an HDAC inhibitor, a kinase inhibitor, an aminopeptidase inhibitor and/or a monoclonal antibody.

Further, the present invention provides a pharmaceutical composition comprising:

(a) an amino acid derivative of formula (I), a tautomer thereof or a pharmaceutically acceptable salt, N-oxide, hydrate or solvate thereof;

(b) a cytotoxic agent, an HDAC inhibitor, a kinase inhibitor, an aminopeptidase inhibitor, a protease inhibitor, a bcl-2 antagonist, an inhibitor of mTor and/or a monoclonal antibody; and

(c) a pharmaceutically acceptable carrier or diluent.

Also provided is a product comprising:

(a) an amino acid derivative of formula (I), a tautomer thereof or a pharmaceutically acceptable salt, N-oxide, hydrate or solvate thereof; and

(b) a cytotoxic agent, an HDAC inhibitor, a kinase inhibitor, an aminopeptidase inhibitor, a protease inhibitor, a bcl-2 antagonist, an inhibitor of mTor and/or a monoclonal antibody,

for the separate, simultaneous or sequential use in the treatment of the human or animal body.

Synthesis

There are multiple synthetic strategies for the synthesis of the compounds (I) with which the present invention is concerned, but all rely on known chemistry, known to the synthetic organic chemist. Thus, compounds according to formula (I) can be synthesised according to procedures described in the standard literature and are well-known to those skilled in the art. Typical literature sources are "*Advanced organic chemistry*", 4th Edition (Wiley), J March; "*Comprehensive Organic Transformation*", 2nd Edition (Wiley), R.C. Larock , "*Handbook of Heterocyclic Chemistry*", 2nd Edition (Pergamon), A.R. Katritzky; review articles such as found in "*Synthesis*", "*Acc. Chem. Res.*", "*Chem. Rev*", or primary literature sources identified by standard literature searches online or from secondary sources such as "*Chemical Abstracts*" or "*Beilstein*".

The compounds of the invention may be prepared by a number of processes some of which are described specifically in the Examples below. In the reactions described below, it may be necessary to protect reactive functional groups, for example hydroxyl, amino and carboxy groups, where these are desired in the final product, to avoid their unwanted participation in the reactions [see for example, "*Protecting Groups in Organic Synthesis*", 3rd Edition, (Wiley), T.W. Greene]. Conventional protecting groups may be used in conjunction with standard practice. In some instances deprotection may be the final step in the synthesis of a compound of general formula (I), and the processes according to the invention described herein after are understood to extend to such removal of protecting groups.

Abbreviations

AcOH = acetic acid

Boc or boc = *tert*-butoxycarbonyl

BOC_2O = Di-*tert*-butyldicarbonate

Cbz = benzyloxycarbonyl

DCE = dichloroethane

DCM = dichloromethane

DIPEA = diisopropylethylamine

DMAP = dimethylamino pyridine

DMF = dimethylformamide

DMSO = dimethyl sulfoxide

EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

EtOAc = ethyl acetate

EtOH = ethanol

Et_2O = diethyl ether

Et_3N = triethylamine

HCl = hydrochloric acid

HOBt = N-hydroxybenzotriazole

K_2CO_3 = potassium carbonate

LiOH = lithium hydroxide

MeOH = methanol

$MgSO_4$ = magnesium sulphate

Na_2CO_3 = sodium carbonate

NaH = sodium hydride

$NaHCO_3$ = sodium hydrogen carbonate

NaI = sodium iodide

NaOH = sodium hydroxide

NBu_4Br = tetrabutylammonium bromide
$Pd(dppf)Cl_2$ = dichloro-(1,2-bis-(diphenylphosphino)ethane)-palladium(II)
Pd/C = palladium on carbon
PyBrOP = Bromo-tris-pyrrolidino phosphoniumhexafluorophosphate
STAB = sodium triacetoxyborohydride
TBTU = *O*-benzotriazol-1-yl-*N*,*N*,*N'*,*N'*-tetramethyluronium tetrafluoroborate
TFA = trifluoroacetic acid
THF = tetrahydrofuran

aq = aqueous
g = gram(s)
LCMS = high performance liquid chromatography/mass spectrometry
mg = milligram(s)
min = minutes
mL = milliliter(s)
µL = microlitre(s)
mol = mole(s)
mmol = millimole(s)
NMR = nuclear magnetic resonance
RT or rt = room temperature
sat = saturated

Commercially available reagents and solvents (HPLC grade) were used without further purification. Solvents were removed using a Buchi rotary evaporator. Microwave irradiation was carried out using a Biotage Initiator™ Eight microwave synthetiser. Purification of compounds by flash chromatography column was performed using silica gel, particle size 40–63µm (230-400 mesh) obtained from Fluorochem. Purification of compounds by preparative HPLC was performed on Gilson systems using reverse phase Axia™ prep Luna C18 columns (10µm, 100 x 21.2mm), gradient 0-100% B (A = water / 0.05% TFA, B = acetonitrile) over 10 min, flow = 25mL/min, UV detection at 254nm.

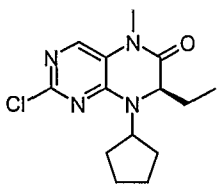
^1H NMR spectra were recorded on a Bruker 300 MHz AV spectrometer in deuterated solvents. Chemical shifts (δ) are in parts per million. Thin-layer chromatography (TLC) analysis was performed with Kieselgel 60 F_{254} (Merck) plates and visualized using UV light.

Analytical HPLC/MS was performed on an Agilent HP1100 LC system using reverse phase Luna C18 columns (3µm, 50 x 4.6mm), gradient 5-95% B (A = water / 0.1% Formic acid, B =

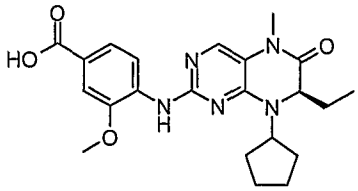
acetonitrile / 0.1% Formic acid) over 13.0 min, flow = 1.25mL/min. UV spectra were recorded at 220 and 254nm using a G1315B DAD detector. Mass spectra were obtained over the range m/z 150 to 800 on a LC/MSD SL G1956B detector. Data were integrated and reported using ChemStation and ChemStation Data Browser softwares.

Intermediates

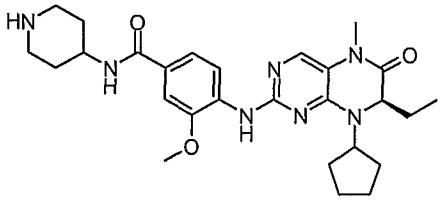
The intermediates for the preparation of the examples described herein are shown below (Figure 1):



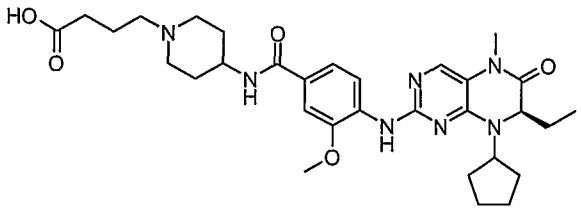
Intermediate A



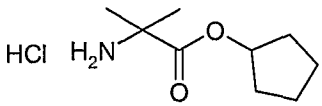
Intermediate B



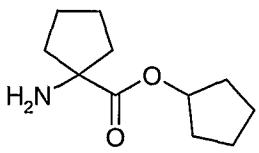
Intermediate C



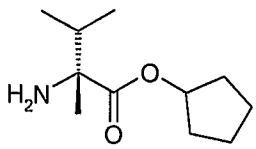
Intermediate D



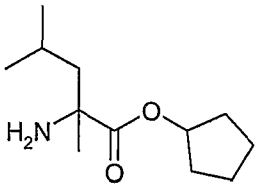
Intermediate E



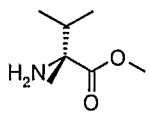
Intermediate F



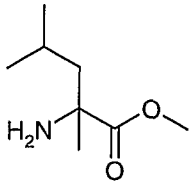
Intermediate G



Intermediate H



Intermediate I

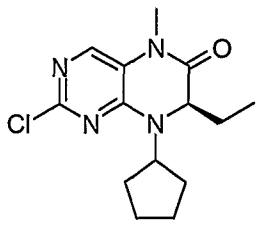


Intermediate J

Figure 1

Intermediate A:

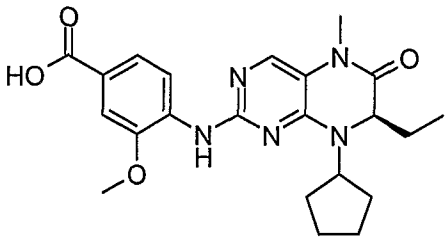
(7*R*)-2-Chloro-8-cyclopentyl-7-ethyl-5-methyl-7,8-dihydropteridin-6(5*H*)-one



The title compound was prepared using methodology described in WO2004076454.

Intermediate B:

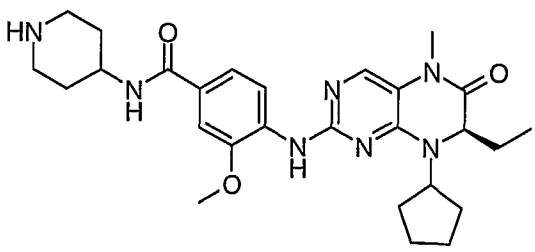
4-{[(7*R*)-8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxybenzoic acid



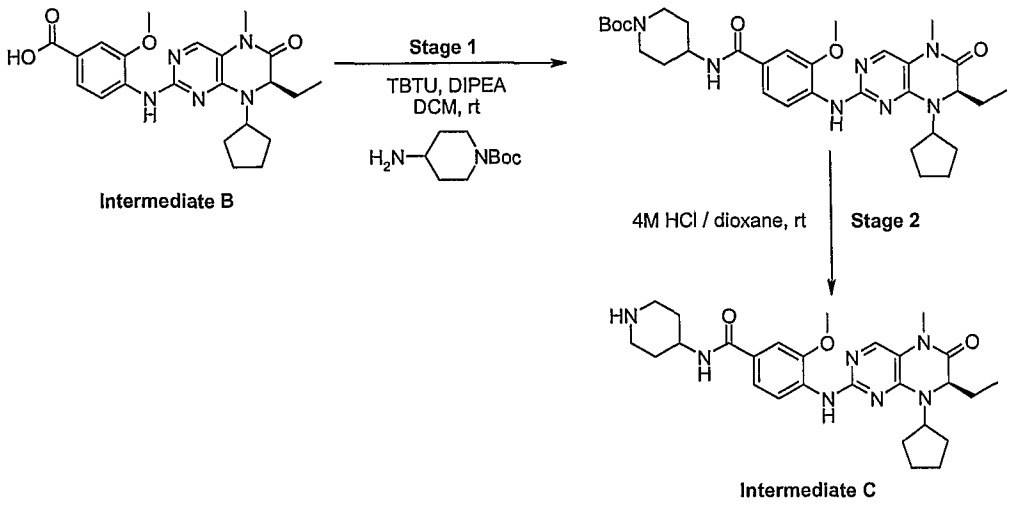
The title compound was prepared using methodology described in WO2004076454.

Intermediate C:

4-{[(7*R*)-8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxy-*N*-piperidin-4-ylbenzamide



The title compound was prepared from **Intermediate B** by the following methodology:



Scheme 1

Stage 1 - *tert*-butyl 4-[(4-{[(7*R*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxybenzoyl)amino]piperidine-1-carboxylate

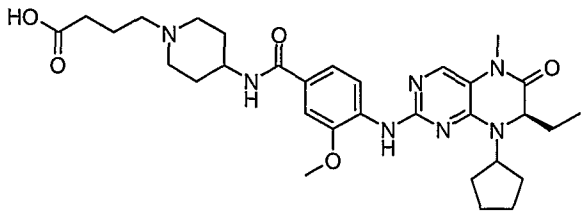
To a suspension of **Intermediate B** (500mg, 1.18mmol) in DCM (20mL) was added TBTU (415mg, 1.29mmol) and DIPEA (0.41mL, 2.35mmol). The reaction mixture was stirred at RT for 30 min and *tert*-butyl 4-aminopiperidine-1-carboxylate (282mg, 1.41mmol) was added. The reaction mixture was stirred at RT for 30 min, diluted with DCM (30mL), washed with water (2 x 30mL), dried ($MgSO_4$), and concentrated under reduced pressure to leave a thick brown oil. Trituration with Et_2O/heptane (1:3) afforded the title product as a beige solid (528mg, 74%). ESMS m/z: 608 $[M+H]^+$.

Stage 2 - 4-{[(7*R*)-8-Cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxy-*N*-piperidin-4-ylbenzamide

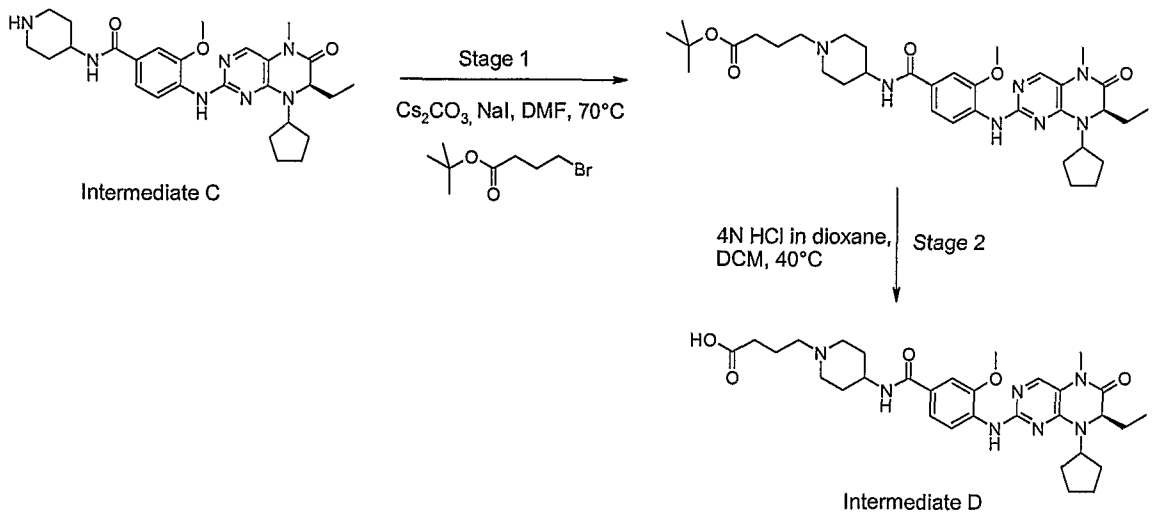
Stage 1 product (528mg, 0.87mmol) was suspended in 4N HCl in dioxane (10mL) and the reaction mixture was stirred at RT for 1 hour and then concentrated under reduced pressure. The residue was triturated with Et_2O and then partitioned between DCM (100mL) and sat Na_2CO_3 (50mL). The organic layer was separated, washed with sat Na_2CO_3 (50mL), dried ($MgSO_4$) and concentrated under reduced pressure to afford the title intermediate as a thick yellow oil, which solidified on standing (407mg, 92%). ESMS m/z 508 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.56 (1H, dd, J=8.4, 3.5 Hz), 7.57-7.76 (2H, m), 7.39-7.44 (1H, m), 4.53 (1H, br.s.), 4.08-4.34 (2H, m), 3.98 (3H, d, J=4.7Hz), 3.39-3.65 (2H, m), 3.29-3.38 (3H, m), 2.81-3.15 (2H, m), 1.41-2.44 (14H, m), 0.75-0.97 (3H, m).

Intermediate D:

4-{4-[(4-{[(7*R*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxybenzoyl)amino]piperidin-1-yl}butanoic acid



The title compound was prepared from **Intermediate C** by the following methodology:



Scheme 2

Stage 1- *tert*-butyl 4-{4-[(4-{[(7*R*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxybenzoyl)amino]piperidin-1-yl}butanoate

To a solution of **Intermediate C** (500mg, 0.98mmol) and *tert*-butyl 4-bromobutyrate (439mg, 1.97mmol) in DMF (8mL) was added sodium iodide (295mg, 1.97mmol) and caesium carbonate (802mg, 2.46mmol). The reaction mixture was stirred at 70°C overnight. Once cooled to room temperature, the reaction mixture was diluted with ethyl acetate (25mL). Water (25mL) was added and the product extracted into the organic layer. The aqueous layer was re-extracted with ethyl acetate (3 x 10mL) and the combined organic portions were washed with water (3 x 20mL). The crude product was concentrated onto silica. Purification on a 4g silica column using a CombiFlash® Companion® (Teledyne Isco Inc) (product eluted in 25% MeOH/DCM) gave the title compound as a yellow oil. (265mg, 41%). ESMS m/z 650 $[M+H]^+$. ^1H NMR (300 MHz,

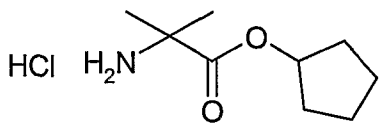
$CDCl_3$) δ: 8.48 (1H, d, J=8.5 Hz), 7.96 (1H, s), 7.65-7.50 (2H, m), 6.65 (1H, br. s.), 4.45 (1H, t, J=7.4 Hz), 4.16 (1H, dd, J=7.2, 3.0 Hz), 4.05 (1H, dd, J=7.1, 5.7 Hz), 3.91 (3H, s), 3.85-3.57 (2H, m), 3.27 (3H, s), 3.12-3.07 (4H, m), 2.60-2.46 (2H, m), 2.42-1.82 (12H, m), 1.72-1.57 (4H, m), 1.39 (9H, s), 0.82 (3H, t, J=7.4 Hz).

Stage 2- 4-{4-[(4-{[(7*R*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxybenzoyl)amino]piperidin-1-yl}butanoic acid

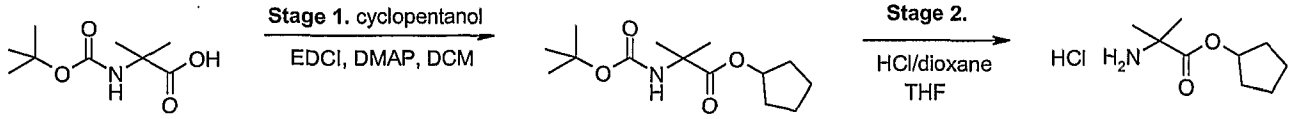
To a solution of **stage 1** product (265mg, 0.41mmol) in DCM (2mL) was added 4N HCl in dioxane (6mL). The reaction was stirred at 40°C for 1 hour. The reaction mixture was cooled to room temperature and the solvent removed in vacuo to afford the title intermediate as a yellow solid (242mg, 100%). ESMS m/z 594 $[M+H]^+$. ^1H NMR (300 MHz, MeO*D*) δ: 8.63 (1H, d, J=7.0 Hz), 7.99-7.82 (1H, m), 7.71-7.54 (2H, m), 4.52 (1H, dd, J=6.5, 3.1 Hz), 4.34 (1H, br. s.), 4.27-4.18 (1H, m), 4.06-3.95 (3H, m), 3.71 (2H, d, J=5.5 Hz), 3.33 (3H, s), 3.26-3.10 (4H, m), 2.50 (2H, t, J=6.6 Hz), 2.32-1.87 (12H, m), 1.78-1.58 (4H, m), 0.88 (3H, t, J=7.4 Hz).

Intermediate E:

Cyclopentyl 2-methylalaninate hydrochloride

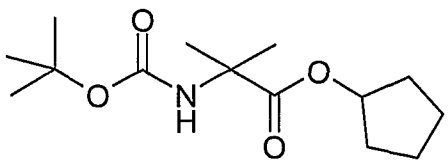


Intermediate E was synthesised using the route shown in Scheme 3 below.



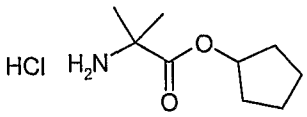
Scheme 3

Stage 1 – Cyclopentyl *N*-(*tert*-butoxycarbonyl)-2-methylalaninate



To a solution of *N*-(*tert*-butoxycarbonyl)-2-methylalanine (1.00 g, 4.92 mmol) in DCM (10mL) at 0 °C was added cyclopentanol (0.83mL, 9.84 mmol), EDCI (1.06 g, 5.42 mmol) and finally DMAP (60 mg, 0.49 mmol). The reaction mixture was warmed to RT and stirred for 18 hr. The DCM was removed *in vacuo* to give a clear oil. The crude residue was dissolved in EtOAc (100mL) and washed with water, 1N $NaHCO_3$ and brine. The organic phase was dried ($MgSO_4$) and concentrated *in vacuo*. The crude extract was purified by column chromatography (10% EtOAc in heptane) to yield the desired product as a clear oil (0.254 g, 20 %). ^1H NMR (300 MHz, $CDCl_3$) δ: 5.25-5.17 (1H, m), 5.04 (1H, br s), 1.93-1.54 (8H, m), 1.49 (6H, s), 1.45 (9H, s).

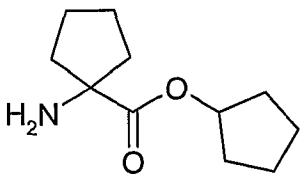
Stage 2 – Cyclopentyl 2-methylalaninate hydrochloride



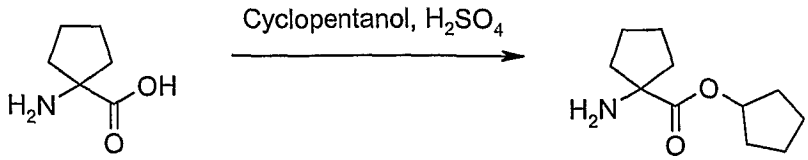
Cyclopentyl *N*-(*tert*-butoxycarbonyl)-2-methylalaninate (0.254 g, 0.93 mmol) was dissolved in THF (5mL) and treated with 4N HCl in dioxane (2mL) and the reaction mixture was stirred at RT for 24 hours. The crude mixture was concentrated under reduced pressure and triturated with diethyl ether to give a white precipitate. This was further washed with diethyl ether to give the desired product as a white powder (0.16 g, 82 %). ^1H NMR (300MHz, $CDCl_3$) δ: 4.97-4.93 (1H, m), 1.67-1.60 (2H, m), 1.58-1.30 (6H, m), 1.16 (6H, s).

Intermediate F:

Cyclopentyl 1-aminocyclopentanecarboxylate



Intermediate F was synthesised using the route shown in Scheme 4 below.

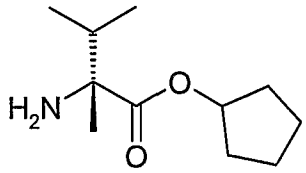


Scheme 4

To a solution of cycloleucine (2.58g, 20.0mmol) in cyclopentanol (20mL) was added concentrated H_2SO_4 (1.17mL, 22.0mmol). The reaction mixture was stirred at 70°C overnight. The solution was then cooled to RT and the cyclopentanol removed in vacuo. Ethyl acetate (30mL) and saturated $NaHCO_3$ (30mL) were added and the product extracted into the organic layer. The aqueous layer was re-extracted with ethyl acetate (3 x 10mL) and the combined organic portions were washed with water (3 x 10mL). The crude product was concentrated onto silica and purified by automated column chromatography (product eluted in 15% 0.2M NH_3 in MeOH in EtOAc) to afford the title intermediate as an off-white solid. (1.0g, 25%). ESMS m/z 198 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ: 4.93-4.87 (1H, m), 2.27-1.49 (16H, m).

Intermediate G:

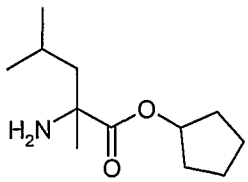
Cyclopentyl 3-methyl-D-isovalinate



Intermediate G was synthesised from (S)-α-methylvaline using the methodology as described for **Intermediate F.** ESMS m/z 200 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ: 5.17 (1H, t, J=5.6Hz), 3.63-2.89 (2H, m), 2.00 (1H, dt, J=13.8, 6.9Hz), 1.85-1.59 (8H, m), 1.30 (3H, s), 0.90 (6H, dd, J=13.4, 7.0Hz).

Intermediate H:

Cyclopentyl 3-methyl-D-isovalinate

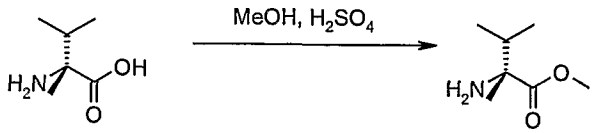


Intermediate H was synthesised from (R/S)-α-methylleucine using the methodology as described for **Intermediate F.** ESMS m/z 214 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ: 5.20-5.17 (1H, m),1.89-1.25 (11H, m) 1.38 (3H, s), 0.94 (3H, d J=6.4Hz), 0.88 (3H, d, J=6.7Hz).

Intermediate I:

Methyl 3-methyl-D-isovalinate

Intermediate I was synthesised using the route shown in Scheme 5 below.

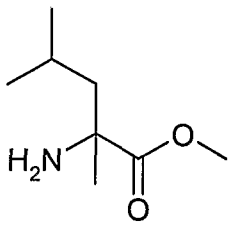


Scheme 5

To a solution of 3-methyl-D-isovaline (450 mg, 3.44mmol) in methanol (5mL) was added concentrated H_2SO_4 (0.5mL). The reaction mixture was stirred at reflux overnight. The solution was then cooled to RT and the solvent removed *in vacuo*. Ethyl acetate (30mL) and saturated $NaHCO_3$ (30mL) were added and the product extracted into the organic layer. The aqueous layer was re-extracted with ethyl acetate (3 x 10mL) and the combined organic portions were washed with water (3 x 10mL), dried ($MgSO_4$) and concentrated to afford the title compound (230mg, 46%) as a colourless oil. ESMS m/z 146 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ: 3.72 (3H, s), 2.00 (1H, dt, J=13.8, 6.9Hz), 1.27 (3H, s), 0.92 (3H, d J=6.9Hz), 0.88 (3H, d J=6.7Hz).

Intermediate J:

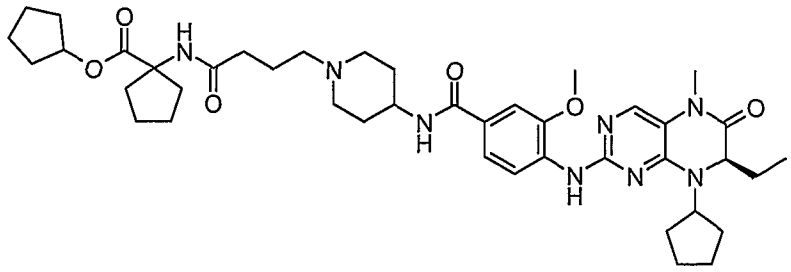
Methyl 2-methylleucinate



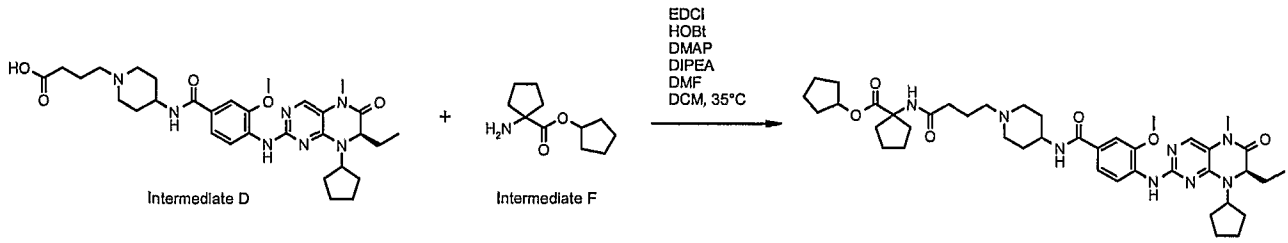
Intermediate J was synthesised from methyl 2-methylleucinate using the methodology as described for **Intermediate I.** ESMS m/z 160 $[M+H]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ: 3.70 (3H, s) 1.74-1.67 (1H, m), 1.64 (2H, s), 1.31 (3H, s), 0.92 (3H, d J=6.4Hz), 0.83 (3H, d, J=6.2Hz).

Example 1:

Cyclopentyl 1-[(4-{4-[(4-{[(7*R*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxybenzoyl)amino]piperidin-1-yl}butanoyl)amino]cyclopentanecarboxylate



The title compound was prepared using the route shown in Scheme 6 below:

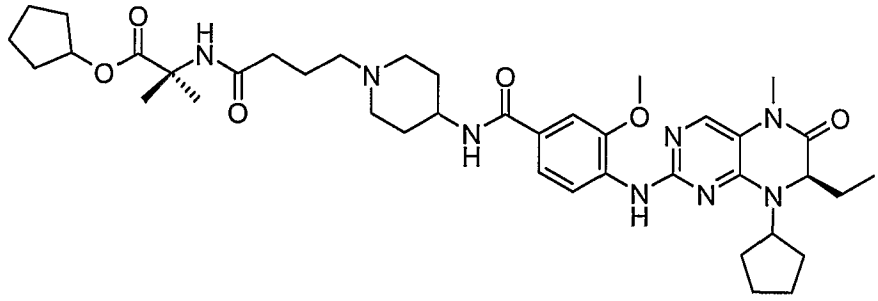


Scheme 6

To a solution of **Intermediate D** (242mg, 0.41mmol) and **Intermediate F** (201mg, 1.02mmol) in DMF (2mL) and DCM (10mL) was added HOBt (68.0mg, 0.49mmol), DMAP (5.0mg, 0.04mmol) and DIPEA (0.14mL, 0.82mmol). The reaction mixture was cooled to 0°C using an ice bath and EDCI (93.5mg, 0.49mmol) added. The reaction was stirred at 35°C overnight. After cooling to RT, DCM (20mL) and water (20mL) were added and the product extracted into the organic layer. The aqueous layer was re-extracted with DCM (3 x 10mL) and the combined organic fractions were dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the crude product as an orange oil (340mg). 150mg of this crude material was purified by preparative HPLC and dried on the freeze-drier to afford the title compound as a white solid (7.6mg, 5.4%). ESMS m/z 774 $[M+H]^+$. ^1H NMR (300 MHz, MeO*D*) δ: 7.91 (1H, d, J=8.3Hz), 7.68-7.53 (3H, m), 5.16 (1H, br.s.), 4.51 (1H, dd, J=6.4,3.0Hz), 4.39-4.27 (1H, m), 4.27-4.13 (1H, m), 4.00 (3H, s), 3.77-3.61 (2H, m), 3.34-3.33 (3H, m), 3.21 (4H, t, J=7.0Hz), 2.45 (2H, t, J=6.3Hz), 2.32-1.62 (32H, m), 0.88 (3H, t, J=7.3Hz).

Example 2:

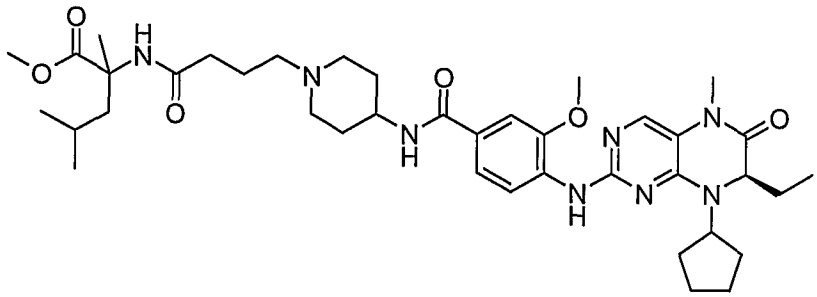
Cyclopentyl *N*-(4-{4-[(4-{[(7*R*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxybenzoyl)amino]piperidin-1-yl}butanoyl)-2-methyl-L-alaninate



This compound was prepared from **Intermediate E** using the same methodology described for **Example 1**. ESMS m/z 374 $[(M+2)/2]^+$. ^{1}H NMR (300 MHz, C*D*Cl$_3$) δ: 8.56 (1H, d, J=8.5Hz), 7.70 (1H, s), 7.63 (1H, s), 7.44 (1H, d, J=1.7Hz), 6.76 (1H, br.s), 6.15-6.01 (1H, m), 5.22 (1H, t, J=5.7Hz), 4.60-4.46 (1H, m), 4.24 (1H, dd, J=7.8, 3.7Hz), 3.99 (3H, s), 3.34 (3H, s), 3.14-3.00 (2H, m), 2.62-2.54 (2H, m), 2.28 (2H, t, J=7.1Hz), 2.15 (2H, br.s), 1.92-1.57 (24H, m), 1.54 (6H, s), 0.89 (3H, t, J=7.4Hz).

Example 3:

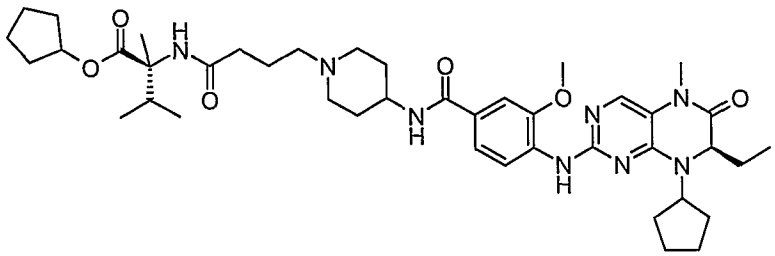
Methyl *N*-(4-{4-[(4-{[(7*R*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxybenzoyl)amino]piperidin-1-yl}butanoyl)-2-methylleucinate



This compound was prepared from **Intermediate J** using the same methodology described for **Example 1**. ESMS m/z 735 $[M+H]^+$. ^{1}H NMR (300 MHz, C*D*Cl$_3$) δ: 7.84 (1H, d, J=8.3 Hz), 7.47 (2H, br. s.), 7.39 (1H, d, J=8.5 Hz), 7.07 (1H, dd, J=15.8, 8.5 Hz), 4.38 (1H, dd, J=6.8, 3.0 Hz), 4.35-4.25 (2H, m), 3.93 (3H, s), 3.76 (3H, s), 3.31 (3H, s), 3.23-3.06 (2H, m), 2.97-2.78 (2H, m), 2.40 (1H, d, J=6.2 Hz), 2.47 (1H, t, J=6.6 Hz), 2.32-1.59 (20H, m), 1.57 (3H, s), 1.45-1.29 (1H, m), 1.00-0.80 (9H, m).

Example 4:

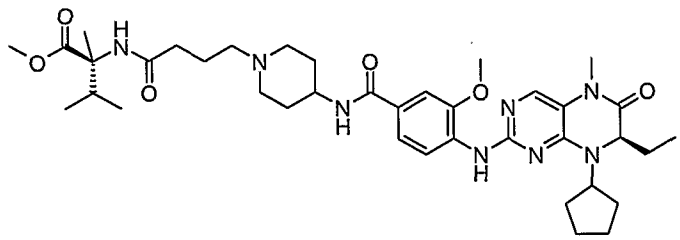
Cyclopentyl *N*-(4-{4-[(4-{[(7*R*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxybenzoyl)amino]piperidin-1-yl}butanoyl)-3-methyl-L-isovalinate



This compound was prepared from **Intermediate G** using the same methodology described for **Example 1**. ESMS m/z 775 $[M+H]^+$. ^1H NMR (300 MHz, MeO*D*), 7.92 (1H, d, J=8.3Hz), 7.64 (2H, s), 7.59 (1H, dd J=1.5, 8.2Hz), 5.15-5.19 (1H, m), 4.52 (1H, dd, J=3.6, 6.3Hz), 4.37-4.34 (1H, m), 4.30-4.20 (1H, m), 4.00 (3H, m), 3.69 (2H, m), 3.33 (3H, s), 3.26-3.20 (4H, m), 2.48 (2H, t, J=5.8Hz), 2.28 (2H, m), 2.12-1.56 (26H, m), 1.01 (3H, d J=6.8Hz), 0.94 (3H, d J=6.8Hz), 0.88 (3H, t, J=7.5Hz).

Example 5:

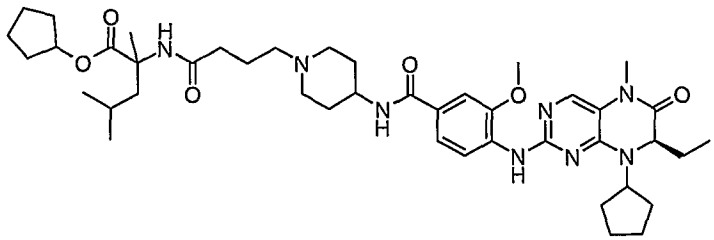
Methyl *N*-(4-{4-[(4-{[(7*R*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxybenzoyl)amino]piperidin-1-yl}butanoyl)-3-methyl-L-isovalinate



This compound was prepared from **Intermediate I** using the same methodology described for **Example 1**. ESMS m/z 721 $[M+H]^+$. ^1H NMR (300 MHz, MeO*D*), 7.92 (1 H, d, J=8.3 Hz), 7.64 (2 H, br. s.), 7.58 (1 H, d, J=7.9 Hz), 4.50 (1 H, dd, J=6.5, 3.1 Hz), 4.33 (1 H, t, J=8.9 Hz), 4.13 - 4.27 (1H, m), 4.00 (3 H, s), 3.71 (3 H, s), 3.31 (3 H, br. s.), 3.05 - 3.26 (4 H, m), 2.46 (2 H, br. s.), 2.27 (2H, br. s.), 1.81 - 2.17 (12 H, m), 1.56 - 1.74 (4 H, m), 1.45 (3 H, s), 1.31 (1 H, s), 1.02 (3 H, d, J=6.8 Hz), 0.93 (3 H, d, J=6.8 Hz), 0.88 (3 H, t, J=7.4 Hz).

Example 6:

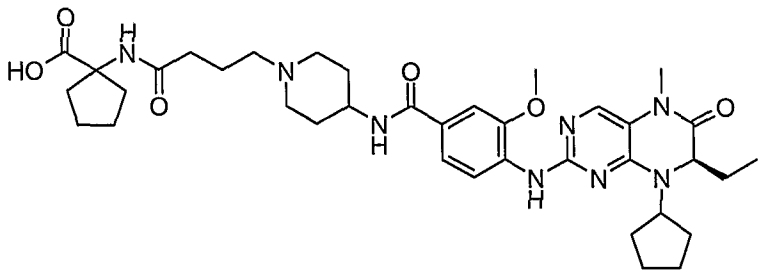
Cyclopentyl *N*-(4-{4-[(4-{[(7*R*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxybenzoyl)amino]piperidin-1-yl}butanoyl)-2-methylleucinate



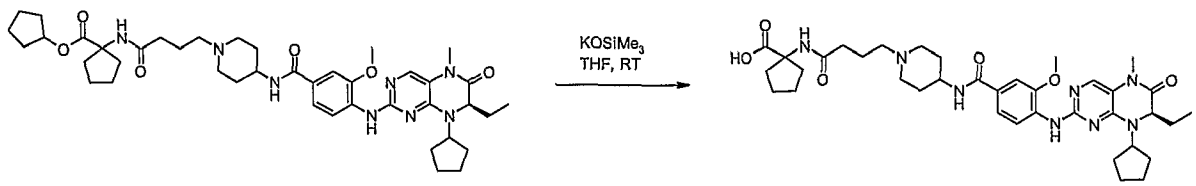
This compound was prepared from **Intermediate H** using the same methodology described for **Example 1**. ESMS m/z 789 $[M+H]^+$. ^1H NMR (300 MHz, MeO*D*), 7.93 (1H, d, J=8.9Hz), 7.64 (2H, d J=3.1Hz), 7.59 (1H, d J=8.2Hz), 5.19-5.15 (1H, m), 4.52-4.49 (1H, m), 4.34 (1H, t J=8.1), 4.30-4.27 (1H, m), 4.00 (3H, m), 3.69 (2H, m), 3.33 (3H, s), 3.20-3.16 (3H, m), 2.45 (2H, t, J=5.9Hz), 2.28 (2H, m), 2.12-1.56 (26H, m), 1.49 (3H, s), 0.94 (6H, d J=6.4Hz), 0.88 (3H, t, J=7.7Hz)

Example 7:

1-[(4-{4-[(4-{[(7*R*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxybenzoyl)amino]piperidin-1-yl}butanoyl)amino]cyclopentanecarboxylic acid



The title compound was prepared using the route shown in Scheme 7 below:



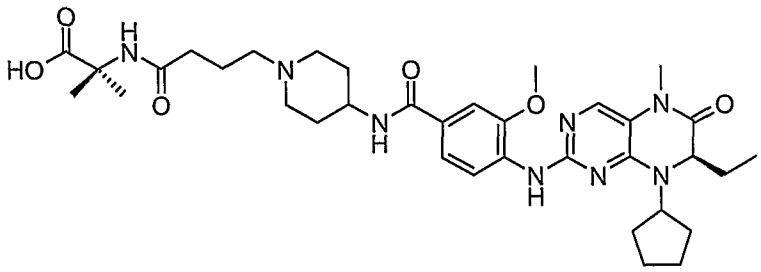
Scheme 7

To a solution of **Example 1** (170mg, 0.21mmol) in THF (6mL) was added potassium trimethyl silanoate (141mg, 1.10mmol). The reaction mixture was stirred at room temperature for 3 days. The solvent was removed under reduced pressure and the crude residue purified by preparative HPLC. The clean fractions were combined and dried on the freeze-drier to afford the title compound as a white solid (3.7mg, 2.4%). ESMS m/z 705 $[M+H]^+$. ^1H NMR (300 MHz, MeO*D*)

δ: 7.91 (1H, d, J=8.3Hz), 7.67-7.52 (3H, m), 4.51 (1H, dd, J=6.5,3.1Hz), 4.40-4.28 (1H, m), 4.28-4.16 (1H, m), 4.00 (3H, m), 3.70 (2H, d, J=12.4Hz), 3.33 (3H, br.s.), 3.26-3.06 (4H, m), 2.48 (2H, t, J=6.1Hz), 2.31-1.56 (24H, m), 0.87 (3H, t, J=7.4Hz).

Example 8:

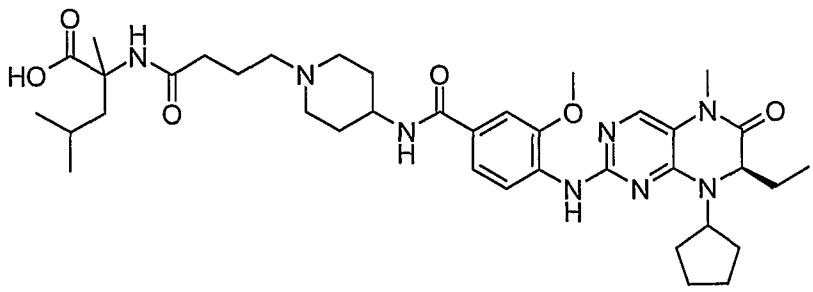
N-(4-{4-[(4-{[(7*R*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxybenzoyl)amino]piperidin-1-yl}butanoyl)-2-methyl-L-alanine



This compound was prepared from **Example 2** using the same methodology described for **Example 7**. ESMS m/z 340 $[(M+2)/2]^+$. ^{1}H NMR (300 MHz, MeO*D*) δ: 7.92 (1H, d, J=8.3Hz), 7.64 (2H, d, J=2.3Hz), 7.58 (1H, d, J=8.3Hz), 4.50 (1H, dd, J=6.5, 3.1 Hz), 4.35 (1H, d, J=8.3Hz), 4.28-4.16 (1H, m), 4.00 (3H, s), 3.80-3.59 (2H, m), 3.34 (3H, br.s.), 3.25-3.07 (4H, m), 2.47 (2H, t, J=6.2Hz), 2.35-2.20 (2H, m), 2.15-1.85 (10H, m), 1.77-1.58 (4H, m), 1.51 (6H, s), 0.88 (3H, t, J=7.4Hz).

Example 9:

N-(4-{4-[(4-{[(7*R*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxybenzoyl)amino]piperidin-1-yl}butanoyl)-2-methylleucine

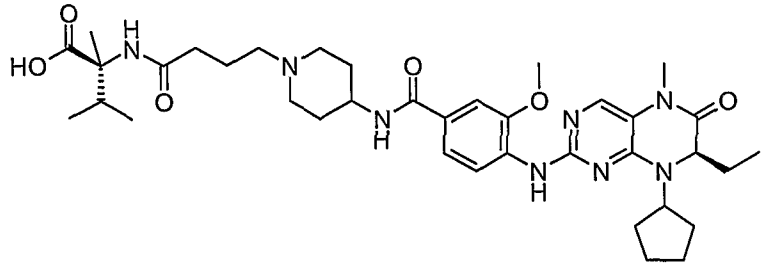


This compound was prepared from **Example 3** using the same methodology described for **Example 7**. ESMS m/z 721 $[M+H]^+$. ^{1}H NMR (300 MHz, MeO*D*) δ: 7.89 (1H, d, J=8.3 Hz), 7.64

(2H, s), 7.58(1H, dd, J=8.3, 1.7Hz), 4.51 (1H, dd, J=6.4, 3.2 Hz), 4.30 (1H, d, J=8.1 Hz), 4.22 (1H, t, J=11.9 Hz), 3.99 (3H, s), 3.69 (2H, d, J=12.8 Hz), 3.33 (3H, s), 3.26-3.03 (4H, m), 2.56-1.56 (21H, m), 1.53 (3H, s), 0.95 (6H, t, J=6.1 Hz), 0.87 (3H, t, J=7.4 Hz).

Example 10:

N-(4-{4-[(4-{[(7*R*)-8-cyclopentyl-7-ethyl-5-methyl-6-oxo-5,6,7,8-tetrahydropteridin-2-yl]amino}-3-methoxybenzoyl)amino]piperidin-1-yl}butanoyl)-3-methyl-L-isovaline



This compound was prepared from **Example 5** using the same methodology described for **Example 7**. ESMS m/z 707 $[M+H]^+$. ^1H NMR (300 MHz, MeO*D*), 7.72 (1H, s), 7.59-7.54 (3H, m), 4.41 (1H, dd, J=5.4, 8.5Hz), 4.26-4.22 (1H, m), 4.30-4.20 (1H, m), 4.00 (3H, m), 3.69 (2H, m), 3.33 (3H, s), 3.26-3.20 (4H, m), 2.52-2.48 (2H, m), 2.32-1.61 (20H, m), 1.04 (3H, d J=6.7Hz), 0.99 (3H, d J=6.9Hz), 0.88 (3H, t, J=7.5Hz).

Biological assays

PLK1 Enzyme Assay

The ability of compounds to inhibit PLK-1 kinase activity was measured in an assay performed by Invitrogen (Paisley, UK). The Z'-LYTE™ biochemical assay employs a fluorescence-based, coupled-enzyme format and is based on the differential sensitivity of phosphorylated and non-phosphorylated peptides to proteolytic cleavage. The peptide substrate is labelled with two fluorophores—one at each end—that make up a FRET pair. In the primary reaction, the kinase transfers the gamma-phosphate of ATP to a single serine or threonine residue in a synthetic FRET-peptide. In the secondary reaction, a site-specific protease recognizes and cleaves non-phosphorylated FRET-peptides. Phosphorylation of FRET-peptides suppresses cleavage by the Development Reagent. Cleavage disrupts FRET between the donor (i.e., coumarin) and acceptor (i.e., fluorescein) fluorophores on the FRET-peptide, whereas uncleaved, phosphorylated FRET-peptides maintain FRET. A radiometric method, which calculates the

ratio (the Emission Ratio) of donor emission to acceptor emission after excitation of the donor fluorophore at 400nm, is used to quantitate reaction progress.

The final 10µl Kinase Reaction consists of 2.8-25.3ng PLK1, 2µM Ser/Thr 16 Peptide substrate and ATP in 50mM HEPES pH 7.5, 0.01% BRIJ-35, 10mM MgCl2, 1mM EGTA. The assay is performed at an ATP concentration at, or close to, the Km. After the 60 minute Kinase Reaction incubation at room temperature, 5µL of a 1:8 dilution of Development Reagent is added. The assay plate is incubated for a further 60 minutes at room temperature and read on a fluorescence plate reader.

Duplicate data points are generated from a 1/3 log dilution series of a stock solution of test compound in DMSO. Nine dilutions steps are made from a top concentration of 10µM, and a "no compound" blank is included. Data is collected and analysed using XL*fit* software from IDBS. The dose response curve is curve fitted to model number 205 (sigmoidal dose-response model). From the curve generated, the concentration giving 50% inhibition is determined and reported.

IC50 results were allocated to one of 3 ranges as follows:
Range A: IC50<100nM,
Range B: IC50 from 100nM to 500nM;
and Range C: IC50 >500nM.
NT = Not tested

The results obtained for compounds of the Examples herein are given in the Table below (Table 1).

Cell inhibition Assay

Cells were seeded in 96W tissue culture plates (1 well = 30mm^2) in 50µl of the appropriate culture medium (see below). Seeding densities were cell-line dependent: HCT-116 = 1000 cells per well, Hut-78 = 2250 cells per well, U937 cells = 2000 cells per well. 24hrs later 50µL of the compound prepared in the same medium was added as 4 fold dilutions to give final concentrations in the range 0.15nM-2500nM (n=6 for each concentration). The plates were then incubated at 37°C, 5% CO_2 for 72hrs. Cell proliferation was assessed using WST-1 (a metabolic indicator dye, Roche Cat no. 1 644 807) according to the manufacturers instructions. The results were calculated as percentage of vehicle response and IC50 values represent the concentration of compound that inhibited the vehicle response by 50%.

HCT-116 Culture Medium - Dulbeccos MEM (Sigma D6546) plus 10% heat inactivated fetal calf serum (Hyclone SH30071 Thermo Fischer Scientific) containing 2mM Glutamine (Sigma cat no G-7513) and 50U/mL Penicillin and Streptomycin Sulphate (Sigma Cat no P-0781).

Hut-78 & U937 culture media: RPMI1640 (Sigma R0883) plus 10% heat inactivated fetal calf serum, as above and supplemented with 2mM Glutamine and 50U/mL Penicillin and Streptomycin Sulphate (details as above).

IC50 results were allocated to one of 3 ranges as follows:
Range A: IC50 <100nM,
Range B: IC50 from 100nM to 500nM;
and Range C: IC50 >500nM.
NT = Not tested

The results obtained for compounds of the Examples herein are given in the Table below (Table 1.

Example Number	Inhibitor Activity vs PLK1	Inhibitor Activity vs HCT 116 cell line	Inhibitor Activity vs Hut-78 cell line	Inhibitor Activity vs U937 cell line
1	A	A	A	A
2	A	A	A	A
3	A	A	A	A
4	A	B	B	B
5	A	B	A	A
6	A	B	B	A
7	A	NT	NT	NT
8	A	NT	NT	NT
9	A	NT	NT	NT
10	A	NT	NT	NT

Table 1

Broken Cell Carboxylesterase Assay

Any given compound of the present invention wherein R_4 is an ester group, may be tested to determine whether it meets the requirement that it be hydrolysed by intracellular esterases, by testing in the following assay.

Preparation of cell extract

U937 or HCT 116 tumour cells (~ 10^9) were washed in 4 volumes of Dulbeccos PBS (~ 1litre) and pelleted at 525g for 10min at 4°C. This was repeated twice and the final cell pellet was resuspended in 35mL of cold homogenising buffer (Trizma 10mM, NaCl 130mM, $CaCl_2$ 0.5mM pH 7.0 at 25°C). Homogenates were prepared by nitrogen cavitation (700psi for 50min at 4°C). The homogenate was kept on ice and supplemented with a cocktail of inhibitors at final concentrations of:

Leupeptin 1μM
Aprotinin 0.1μM
E64 8μM
Pepstatin 1.5μM
Bestatin 162μM
Chymostatin 33μM

After clarification of the cell homogenate by centrifugation at 525g for 10min, the resulting supernatant was used as a source of esterase activity and was stored at -80°C until required.

Measurement of ester cleavage

Hydrolysis of esters to the corresponding carboxylic acids can be measured using the cell extract, prepared as above. To this effect cell extract (~30μg / total assay volume of 0.5mL) was incubated at 37°C in a Tris- HCl 25mM, 125mM NaCl buffer, pH 7.5 at 25°C. At zero time the ester (substrate) was then added at a final concentration of 2.5 μM and the samples were incubated at 37°C for the appropriate time (usually 0 or 80min). Reactions were stopped by the addition of 3 x volumes of acetonitrile. For zero time samples the acetonitrile was added prior to the ester compound. After centrifugation at 12000 g for 5 min, samples were analysed for the ester and its corresponding carboxylic acid at RT by LCMS (Sciex API 3000, HP1100 binary pump, CTC PAL). Chromatography was based on an AceCN (75x2.1mm) column and a mobile phase of 5-95% acetonitrile in water /0.1% formic acid.

The table below (Table 2) presents data showing that several amino acid ester motifs, conjugated to various intracellular enzyme inhibitors by several different linker chemistries are all hydrolysed by intracellular carboxyesterases to the corresponding acid.

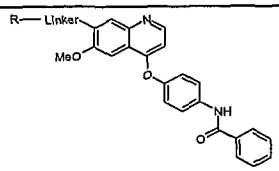
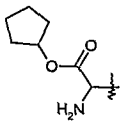
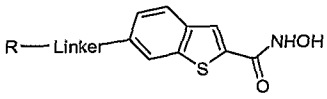
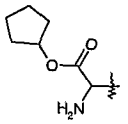
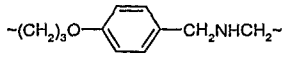
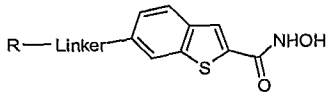
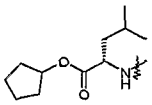
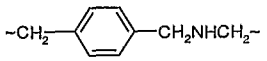
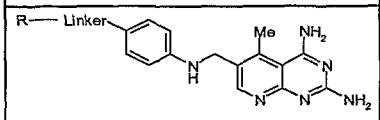
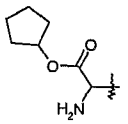
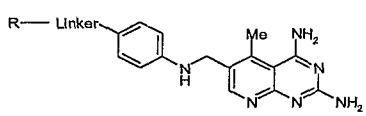
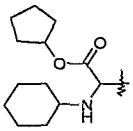
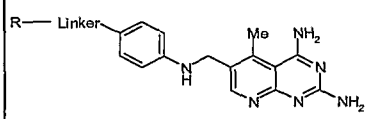
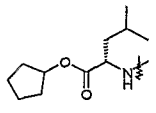
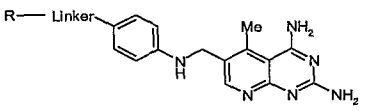
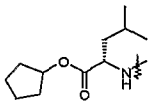
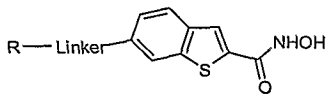
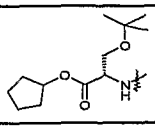
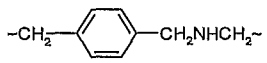
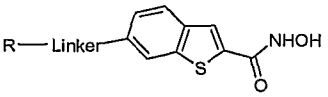
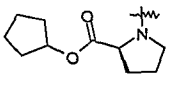
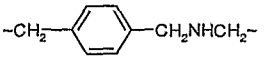
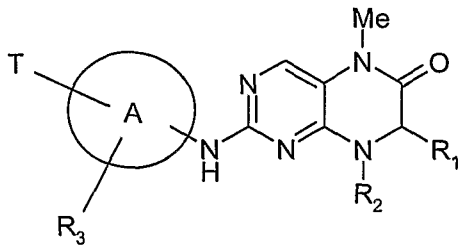
Structure of amino acid ester conjugate	R	Linker	Hydrolysis Rate Range U937Cells (pg/mL/min)	Preparation of amino ester conjugate
		~CH2CH2O~	100-1000	WO2006117552
			1000-50000	WO2006117548
			>50000	WO2006117549
		~CH2CH2O~	>50000	WO2006117567
		~CH2CH2O~	1000-50000	WO2006117567
		~CH2~	1000-50000	WO2006117567
		~CO~	>50000	WO2006117567
			>50000	WO2006117549
			>50000	WO2006117549

Table 2

Claims:

1. A compound of formula (I), or a salt:



(I)

wherein

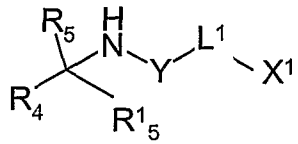
R_1 is hydrogen, or an optionally substituted (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl or (C_3-C_6)cycloalkyl group;

R_2 is hydrogen, or an optionally substituted (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl or (C_3-C_6)cycloalkyl group;

R_3 is hydrogen, -CN, hydroxyl, halogen, optionally substituted (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl or (C_3-C_6)cycloalkyl, -NR_6R_7 or C_1-C_4 alkoxy, wherein R_6 and R_7 are independently hydrogen or optionally substituted (C_1-C_6)alkyl;

ring **A** is an optionally substituted mono- or bi-cyclic carbocyclic or heterocyclic ring or a ring system having up to 12 ring atoms;

T is a radical of formula (II)



(II)

wherein

R_4 is a carboxylic acid group (-COOH), or an ester group which is hydrolysable by one or more intracellular esterase enzymes to a carboxylic acid group;

R_5 and **R^1_5** independently represent the side chain of a natural or non-natural alpha amino acid but neither of R_5 and R^1_5 is hydrogen, or **R_5** and **R^1_5** taken together with the carbon atom to which they are attached form a C_3-C_7 cycloalkyl ring;

Y is a bond, -C(=O)-, -S(=O)$_2$-, -C(=O)O-, -C(=O)NR$_6$-, -C(=S)-NR$_6$, -C(=NH)-NR$_6$ or -S(=O)$_2$NR$_6$- wherein R_6 is independently hydrogen or optionally substituted (C_1-C_6)alkyl;

L^1 is a divalent radical of formula -(Alk^1)$_m$(Q^1)$_n$(Alk^2)$_p$- wherein

m, **n** and **p** are independently 0 or 1,

Q^1 is (i) an optionally substituted divalent mono- or bicyclic carbocyclic or heterocyclic radical having 5 - 13 ring members, or (ii), in the case where p is 0, a divalent radical of formula -Q^2-X^2- wherein X^2 is -O-, -S- or NR^A- wherein R^A is hydrogen or optionally substituted C_1-C_3 alkyl, and Q^2 is an optionally substituted divalent mono- or bicyclic carbocyclic or heterocyclic radical having 5 - 13 ring members,

Alk^1 and **Alk^2** independently represent optionally substituted divalent (C_3-C_6)cycloalkyl radicals, or optionally substituted straight or branched, (C_1-C_6)alkylene, (C_2-C_6)alkenylene, or (C_2-C_6)alkynylene radicals which may optionally contain or terminate in an ether (-O-), thioether (-S-) or amino (-NR^A-) link wherein R^A is hydrogen or optionally substituted (C_1-C_3)alkyl;

X^1 represents a bond, -C(=O)-; or -S(=O)$_2$-; -NR$_6$C(=O)-, -C(=O)NR$_6$-, -NR$_6$C(=O)-NR$_7$- , -NR$_6$S(=O)$_2$-, or -S(=O)$_2$NR$_6$- wherein R_6 and R_7 are independently hydrogen or optionally substituted (C_1-C_6)alkyl.

2. A compound as claimed in claim 1 wherein R_1 is ethyl.

3. A compound as claimed in claim 1 or claim 2 wherein R_2 is cyclopentyl.

4. A compound as claimed in any of the preceding claims wherein ring A is a phenyl ring.

5. A compound as claimed in any of the preceding claims wherein R_4 is of formula -(C=O)OR$_{10}$ wherein R_{10} is $R_{11}R_{12}R_{13}C$- wherein

(i) R_{11} is hydrogen, fluorine or optionally substituted (C_1-C_3)alkyl-(Z^1)$_a$-[(C_1-C_3)alkyl]$_b$- or (C_2-C_3)alkenyl-(Z^1)$_a$-[(C_1-C_3)alkyl]$_b$- wherein a and b are independently 0 or 1 and Z^1

is –O-, -S-, or –NR_{14}- wherein R_{14} is hydrogen or (C_1-C_3)alkyl; and R_{12} and R_{13} are independently hydrogen or (C_1-C_3)alkyl-;

(ii) R_{11} is hydrogen or optionally substituted $R_{15}R_{16}N$-(C_1-C_3)alkyl- wherein R_{15} is hydrogen or (C_1-C_3)alkyl and R_{16} is hydrogen or (C_1-C_3)alkyl; or R_{15} and R_{16} together with the nitrogen to which they are attached form an optionally substituted monocyclic heterocyclic ring of 5- or 6- ring atoms or bicyclic heterocyclic ring system of 8 to 10 ring atoms, and R_{12} and R_{13} are independently hydrogen or (C_1-C_3)alkyl-;or

(iii) R_{11} and R_{12} taken together with the carbon to which they are attached form an optionally substituted monocyclic carbocyclic ring of from 3 to 7 ring atoms or bicyclic carbocyclic ring system of 8 to 10 ring atoms, and R_{13} is hydrogen.

and wherein in cases (i), (ii) and (iii) above, "alkyl" includes fluoroalkyl.

6. A compound as claimed in claim 5 wherein R_{10} is methyl, trifluoromethyl, ethyl, n- or iso-propyl, n-,sec- or tert-butyl, cyclohexyl, allyl, phenyl, benzyl, 2-, 3- or 4-pyridylmethyl, N-methylpiperidin-4-yl, tetrahydrofuran-3-yl, methoxyethyl, indanyl, norbonyl, dimethylaminoethyl, morpholinoethyl.

7. A compound as claimed in claim 5 wherein R_{10} is cyclopentyl.

8. A compound as claimed in any of the preceding claims wherein R_5 and R^1_5 are independently phenyl, or heteroaryl or a group of formula -$CR_aR_bR_c$ in which:

each of R_a, R_b and R_c is independently hydrogen, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, phenyl(C_1-C_6)alkyl, (C_3-C_8)cycloalkyl; or

R_c is hydrogen and R_a and R_b are independently phenyl or heteroaryl such as pyridyl; or

R_c is hydrogen, (C_1-C_6)alkyl, (C_2-C_6)alkenyl, (C_2-C_6)alkynyl, phenyl(C_1-C_6)alkyl, or (C_3-C_8)cycloalkyl, and R_a and R_b together with the carbon atom to which they are attached form a 3 to 8 membered cycloalkyl or a 5- to 6-membered heterocyclic ring; or

R_a, R_b and R_c together with the carbon atom to which they are attached form a tricyclic ring (for example adamantyl); or

R_a and R_b are each independently $(C_1$-$C_6)$alkyl, $(C_2$-$C_6)$alkenyl, $(C_2$-$C_6)$alkynyl, phenyl$(C_1$-$C_6)$alkyl, or a group as defined for R_c below other than hydrogen, or R_a and R_b together with the carbon atom to which they are attached form a cycloalkyl or heterocyclic ring, and R_c is hydrogen, -OH, -SH, halogen, -CN, $-CO_2H$, $(C_1$-$C_4)$perfluoroalkyl, $-CH_2OH$, $-O(C_1$-$C_6)$alkyl, $-O(C_2$-$C_6)$alkenyl, $-S(C_1$-$C_6)$alkyl, $-SO(C_1$-$C_6)$alkyl, $-SO_2(C_1$-$C_6)$ alkyl, $-S(C_2$-$C_6)$alkenyl, $-SO(C_2$-$C_6)$alkenyl, $-SO_2(C_2$-$C_6)$alkenyl or a group -Q-W wherein Q represents a bond or -O-, -S-, -SO- or $-SO_2$- and W represents a phenyl, phenylalkyl, $(C_3$-$C_8)$cycloalkyl, $(C_3$-$C_8)$cycloalkylalkyl, $(C_4$-$C_8)$cycloalkenyl, $(C_4$-$C_8)$cycloalkenylalkyl, heteroaryl or heteroarylalkyl group, which group W may optionally be substituted by one or more substituents independently selected from, hydroxyl, halogen, -CN, $-CONH_2$, $-CONH(C_1$-$C_6)$alkyl, $-CONH(C_1$-C_6alkyl$)_2$, -CHO, $-CH_2OH$, $(C_1$-$C_4)$perfluoroalkyl, $-O(C_1$-$C_6)$alkyl, $-S(C_1$-$C_6)$alkyl, $-SO(C_1$-$C_6)$alkyl, $-SO_2(C_1$-$C_6)$alkyl, $-NO_2$, $-NH_2$, $-NH(C_1$-$C_6)$alkyl, $-N((C_1$-$C_6)$alkyl$)_2$, $-NHCO(C_1$-$C_6)$alkyl, $(C_1$-$C_6)$alkyl, $(C_2$-$C_6)$alkenyl, $(C_2$-$C_6)$alkynyl, $(C_3$-$C_8)$cycloalkyl, $(C_4$-$C_8)$cycloalkenyl, phenyl or benzyl.

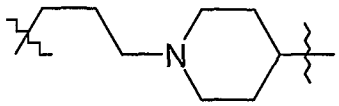
9. A compound as claimed in any of claims 1 to 7 wherein R_5 and $R^1{}_5$ are independently H-Alk^4-, phenyl, monocyclic heterocyclyl, C_3-C_7 cycloalkyl, phenyl(Alk^4)-, heterocyclyl(Alk^4)-, or C_3-C_7 cycloalkyl(Alk^4)-, wherein the heterocyclyl part is monocyclic heterocyclyl having 3-7 ring atoms, and wherein -Alk^4- is a straight or branched, divalent $(C_1$-$C_6)$alkylene, $(C_2$-$C_6)$alkenylene, or $(C_2$-$C_6)$alkynylene radical which may optionally be interrupted by, or terminate in, an ether (-O-), thioether (-S-) or amino (-NR^A-) link wherein R^A is hydrogen or optionally substituted $(C_1$-$C_3)$alkyl, and wherein the Alk^4-, or cyclic part is optionally substituted.

10. A compound as claimed in any of claims 1 to 7 wherein R_5 and $R^1{}_5$ are independently methyl, ethyl, or n-or iso-propyl, or n, sec or tert-butyl.

11. A compound as claimed in any of the preceding claims wherein at least one of R_5 and $R^1{}_5$ is methyl.

12. A compound as claimed in any of claims 1 to 7 wherein R_5 and $R^1{}_5$ taken together with the carbon atom to which they are attached form a C_3-C_7 cycloalkyl ring, such as cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl ring.

13. A compound as claimed in any of the preceding claims wherein the radical -Y-L^1-X^1-, Y is -C(=O)-, X^1 is -NHC(=O)- and L^1 has formula (III):



(III)

wherein the left hand valency is satisfied by Y and the right hand valency is satisfied by X^1.

14. A compound as claimed in claim 1 which is the subject of any of the Examples herein.

15. A pharmaceutical composition comprising a compound as claimed in any of the preceding claims, together with a pharmaceutically acceptable carrier.

16. The use of a compound as claimed in any of claims 1 to 14 in the preparation of a composition for inhibition of PLK1 activity *in vitro* or *in vivo*.

17. A method of treatment of conditions mediated by PLK1 activity, which comprises administering to a subject suffering such disease an effective amount of a compound of formula (I) as claimed in any of claims 1 to 14.

18. The use as claimed in claim 16 or a method as claimed in claim 17 for treatment of cell proliferative diseases.

19 The use as claimed in claim 16 or a method as claimed in claim 17 for treatment of solid tumours

20. The use as claimed in claim 16 or a method as claimed in claim 17 for treatment of haemato-oncological tumours.

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2009/001035

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D475/00 A61K31/519 A61P35/00

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, WPI Data, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X,Y	WO 2004/076454 A (BOEHRINGER INGELHEIM PHARMA [DE]; HOFFMANN MATTHIAS [DE]; GRAUERT MATT) 10 September 2004 (2004-09-10) cited in the application Abstract; claims; examples.	1-20
X,Y	WO 03/020722 A (BOEHRINGER INGELHEIM PHARMA [DE]; HOFFMANN MATTHIAS [DE]; GRAUERT MATT) 13 March 2003 (2003-03-13) Abstract; claims; examples.	1-20

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

☒ See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 July 2009	14/07/2009

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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