PYRAZOLE DERIVATIVES FOR THE INHIBITION OF CDK'S AND GSK'S

This invention relates to pyrazole compounds that inhibit or modulate the activity of Cyclin Dependent Kinases (CDK) and Glycogen Synthase Kinases (GSK) kinases, to the use of the compounds in the treatment or prophylaxis of disease states or conditions mediated by the kinases, and to novel compounds having kinase inhibitory or modulating activity. Also provided are pharmaceutical compositions containing the compounds and novel chemical intermediates.

Background of the Invention

Protein kinases constitute a large family of structurally related enzymes that are responsible for the control of a wide variety of signal transduction processes within the cell (Hardie, G. and Hanks, S. (1995) *The Protein Kinase Facts Book. I and II,* Academic Press, San Diego, CA). The kinases may be categorized into families by the substrates they phosphorylate (e.g., protein-tyrosine, protein-serine/threonine, lipids, etc.). Sequence motifs have been identified that generally correspond to each of these kinase families (e.g., Hanks, S.K., Hunter, T., *FASEB J.,* 9:576-596 (1995); Knighton, *et al.*, *Science,* 253:407-414 (1991); Hiles, *et al.*, *Cell,* 70:419-429 (1992); Kunz, *et al.*, *Cell,* 73:585-596 (1993); Garcia-Bustos, *et al.*, *EMBO J.,* 13:2352-2361 (1994)).

Protein kinases may be characterized by their regulation mechanisms. These mechanisms include, for example, autophosphorylation, transphosphorylation by other kinases, protein-protein interactions, protein-lipid interactions, and protein-polynucleotide interactions. An individual protein kinase may be regulated by more than one mechanism.

Kinases regulate many different cell processes including, but not limited to, proliferation, differentiation, apoptosis, motility, transcription, translation and other signalling processes, by adding phosphate groups to target proteins. These phosphorylation events act as molecular on/off switches that can modulate or regulate the target protein biological function. Phosphorylation of target proteins occurs in response to a variety of extracellular signals (hormones,

neurotransmitters, growth and differentiation factors, etc.), cell cycle events, environmental or nutritional stresses, etc. The appropriate protein kinase functions in signalling pathways to activate or inactivate (either directly or indirectly), for example, a metabolic enzyme, regulatory protein, receptor, cytoskeletal protein, ion channel or pump, or transcription factor. Uncontrolled signalling due to defective control of protein phosphorylation has been implicated in a number of diseases, including, for example, inflammation, cancer, allergy/asthma, disease and conditions of the immune system, disease and conditions of the central nervous system, and angiogenesis.

Cyclin Dependent Kinases

The process of eukaryotic cell division may be broadly divided into a series of sequential phases termed G1, S, G2 and M. Correct progression through the various phases of the cell cycle has been shown to be critically dependent upon the spatial and temporal regulation of a family of proteins known as cyclin dependent kinases (cdks) and a diverse set of their cognate protein partners termed cyclins. Cdks are cdc2 (also known as cdk1) homologous serine-threonine kinase proteins that are able to utilise ATP as a substrate in the phosphorylation of diverse polypeptides in a sequence dependent context. Cyclins are a family of proteins characterised by a homology region, containing approximately 100 amino acids, termed the "cyclin box" which is used in binding to, and defining selectivity for, specific cdk partner proteins.

Modulation of the expression levels, degradation rates, and activation levels of various cdks and cyclins throughout the cell cycle leads to the cyclical formation of a series of cdk/cyclin complexes, in which the cdks are enzymatically active. The formation of these complexes controls passage through discrete cell cycle checkpoints and thereby enables the process of cell division to continue. Failure to satisfy the pre-requisite biochemical criteria at a given cell cycle checkpoint, *i.e.* failure to form a required cdk/cyclin complex, can lead to cell cycle arrest and/or cellular apoptosis. Aberrant cellular proliferation, as manifested in cancer, can often be attributed to loss of correct cell cycle control. Inhibition of cdk enzymatic

activity therefore provides a means by which abnormally dividing cells can have their division arrested and/or be killed. The diversity of cdks, and cdk complexes, and their critical roles in mediating the cell cycle, provides a broad spectrum of potential therapeutic targets selected on the basis of a defined biochemical rationale.

Progression from the G1 phase to the S phase of the cell cycle is primarily regulated by cdk2, cdk3, cdk4 and cdk6 via association with members of the D and E type cyclins. The D-type cyclins appear instrumental in enabling passage beyond the G1 restriction point, where as the cdk2/cyclin E complex is key to the transition from the G1 to S phase. Subsequent progression through S phase and entry into G2 is thought to require the cdk2/cyclin A complex. Both mitosis, and the G2 to M phase transition which triggers it, are regulated by complexes of cdk1 and the A and B type cyclins.

During G1 phase Retinoblastoma protein (Rb), and related pocket proteins such as p130, are substrates for cdk(2, 4, & 6)/cyclin complexes. Progression through G1 is in part facilitated by hyperphosphorylation, and thus inactivation, of Rb and p130 by the cdk(4/6)/cyclin-D complexes. Hyperphosphorylation of Rb and p130 causes the release of transcription factors, such as E2F, and thus the expression of genes necessary for progression through G1 and for entry into S-phase, such as the gene for cyclin E. Expression of cyclin E facilitates formation of the cdk2/cyclin E complex which amplifies, or maintains, E2F levels via further phosphorylation of Rb. The cdk2/cyclin E complex also phosphorylates other proteins necessary for DNA replication, such as NPAT, which has been implicated in histone biosynthesis. G1 progression and the G1/S transition are also regulated via the mitogen stimulated Myc pathway, which feeds into the cdk2/cyclin E pathway. Cdk2 is also connected to the p53 mediated DNA damage response pathway via p53 regulation of p21 levels. p21 is a protein inhibitor of cdk2/cyclin E and is thus capable of blocking, or delaying, the G1/S transition. The cdk2/cyclin E complex may thus represent a point at which biochemical stimuli from the Rb, Myc and p53 pathways are to some degree integrated. Cdk2 and/or the cdk2/cyclin E complex therefore

represent good targets for therapeutics designed at arresting, or recovering control of, the cell cycle in aberrantly dividing cells.

The exact role of cdk3 in the cell cycle is not clear. As yet no cognate cyclin partner has been identified, but a dominant negative form of cdk3 delayed cells in G1, thereby suggesting that cdk3 has a role in regulating the G1/S transition.

Although most cdks have been implicated in regulation of the cell cycle there is evidence that certain members of the cdk family are involved in other biochemical processes. This is exemplified by cdk5 which is necessary for correct neuronal development and which has also been implicated in the phosphorylation of several neuronal proteins such as Tau, NUDE-1, synapsin1, DARPP32 and the Munc18/Syntaxin1A complex. Neuronal cdk5 is conventionally activated by binding to the p35/p39 proteins. Cdk5 activity can, however, be deregulated by the binding of p25, a truncated version of p35. Conversion of p35 to p25, and subsequent deregulation of cdk5 activity, can be induced by ischemia, excitotoxicity, and β-amyloid peptide. Consequently p25 has been implicated in the pathogenesis of neurodegenerative diseases, such as Alzheimer's, and is therefore of interest as a target for therapeutics directed against these diseases.

Cdk7 is a nuclear protein that has cdc2 CAK activity and binds to cyclin H. Cdk7 has been identified as component of the TFIIH transcriptional complex which has RNA polymerase II C-terminal domain (CTD) activity. This has been associated with the regulation of HIV-1 transcription via a Tat-mediated biochemical pathway. Cdk8 binds cyclin C and has been implicated in the phosphorylation of the CTD of RNA polymerase II. Similarly the cdk9/cyclin-T1 complex (P-TEFb complex) has been implicated in elongation control of RNA polymerase II. PTEF-b is also required for activation of transcription of the HIV-1 genome by the viral transactivator Tat through its interaction with cyclin T1. Cdk7, cdk8, cdk9 and the P-TEFb complex are therefore potential targets for anti-viral therapeutics.

At a molecular level mediation of cdk/cyclin complex activity requires a series of stimulatory and inhibitory phosphorylation, or dephosphorylation, events. Cdk

phosphorylation is performed by a group of cdk activating kinases (CAKs) and/or kinases such as wee1, Myt1 and Mik1. Dephosphorylation is performed by phosphatases such as cdc25(a & c), pp2a, or KAP.

Cdk/cyclin complex activity may be further regulated by two families of endogenous cellular proteinaceous inhibitors: the Kip/Cip family, or the INK family. The INK proteins specifically bind cdk4 and cdk6. $p16^{ink4}$ (also known as MTS1) is a potential tumour suppressor gene that is mutated, or deleted, in a large number of primary cancers. The Kip/Cip family contains proteins such as $p21^{Cip1,Waf1}$, $p27^{Kip1}$ and $p57^{kip2}$. As discussed previously p21 is induced by p53 and is able to inactivate the cdk2/cyclin(E/A) and cdk4/cyclin(D1/D2/D3) complexes. Atypically low levels of p27 expression have been observed in breast, colon and prostate cancers. Conversely over expression of cyclin E in solid tumours has been shown to correlate with poor patient prognosis. Over expression of cyclin D1 has been associated with oesophageal, breast, squamous, and non-small cell lung carcinomas.

The pivotal roles of cdks, and their associated proteins, in co-ordinating and driving the cell cycle in proliferating cells have been outlined above. Some of the biochemical pathways in which cdks play a key role have also been described. The development of monotherapies for the treatment of proliferative disorders, such as cancers, using therapeutics targeted generically at cdks, or at specific cdks, is therefore potentially highly desirable. Cdk inhibitors could conceivably also be used to treat other conditions such as viral infections, autoimmune diseases and neuro-degenerative diseases, amongst others. Cdk targeted therapeutics may also provide clinical benefits in the treatment of the previously described diseases when used in combination therapy with either existing, or new, therapeutic agents. Cdk targeted anticancer therapies could potentially have advantages over many current antitumour agents as they would not directly interact with DNA and should therefore reduce the risk of secondary tumour development.

Glycogen Synthase Kinase

Glycogen Synthase Kinase-3 (GSK3) is a serine-threonine kinase that occurs as two ubiquitously expressed isoforms in humans (GSK3α & beta GSK3β). GSK3 has been implicated as having roles in embryonic development, protein synthesis, cell proliferation, cell differentiation, microtubule dynamics, cell motility and cellular apoptosis. As such GSK3 has been implicated in the progression of disease states such as diabetes, cancer, Alzheimer's disease, stroke, epilepsy, motor neuron disease and/or head trauma. Phylogenetically GSK3 is most closely related to the cyclin dependent kinases (CDKs).

The consensus peptide substrate sequence recognised by GSK3 is (Ser/Thr)-X-X-X-(pSer/pThr), where X is any amino acid (at positions (n+1), (n+2), (n+3)) and pSer and pThr are phospho-serine and phospho-threonine respectively (n+4). GSK3 phosphorylates the first serine, or threonine, at position (n). Phospho-serine, or phospho-threonine, at the (n+4) position appear necessary for priming GSK3 to give maximal substrate turnover. Phosphorylation of GSK3α at Ser21, or GSK3β at Ser9, leads to inhibition of GSK3. Mutagenesis and peptide competition studies have led to the model that the phosphorylated N-terminus of GSK3 is able to compete with phospho-peptide substrate (S/TXXXpS/pT) via an autoinhibitory mechanism. There are also data suggesting that GSK3α and GSKβ may be subtly regulated by phosphorylation of tyrosines 279 and 216 respectively. Mutation of these residues to a Phe caused a reduction in *in vivo* kinase activity. The X-ray crystallographic structure of GSK3β has helped to shed light on all aspects of GSK3 activation and regulation.

GSK3 forms part of the mammalian insulin response pathway and is able to phosphorylate, and thereby inactivate, glycogen synthase. Upregulation of glycogen synthase activity, and thereby glycogen synthesis, through inhibition of GSK3, has thus been considered a potential means of combating type II, or non-insulin-dependent diabetes mellitus (NIDDM): a condition in which body tissues become resistant to insulin stimulation. The cellular insulin response in liver, adipose, or muscle tissues, is triggered by insulin binding to an extracellular insulin receptor. This causes the phosphorylation, and subsequent recruitment to the

plasma membrane, of the insulin receptor substrate (IRS) proteins. Further phosphorylation of the IRS proteins initiates recruitment of phosphoinositide-3 kinase (PI3K) to the plasma membrane where it is able to liberate the second messenger phosphatidylinosityl 3,4,5-trisphosphate (PIP3). This facilitates co-localisation of 3-phosphoinositide-dedependent protein kinase 1 (PDK1) and protein kinase B (PKB or Akt) to the membrane, where PDK1 activates PKB. PKB is able to phosphorylate, and thereby inhibit, GSK3α and/or GSKβ through phosphorylation of Ser9, or ser21, respectively. The inhibition of GSK3 then triggers upregulation of glycogen synthase activity. Therapeutic agents able to inhibit GSK3 may thus be able to induce cellular responses akin to those seen on insulin stimulation. A further *in vivo* substrate of GSK3 is the eukaryotic protein synthesis initiation factor 2B (eIF2B). eIF2B is inactivated via phosphorylation and is thus able to suppress protein biosynthesis. Inhibition of GSK3, e.g. by inactivation of the "mammalian target of rapamycin" protein (mTOR), can thus upregulate protein biosynthesis. Finally there is some evidence for regulation of GSK3 activity via the mitogen activated protein kinase (MAPK) pathway through phosphorylation of GSK3 by kinases such as mitogen activated protein kinase activated protein kinase 1 (MAPKAP-K1 or RSK). These data suggest that GSK3 activity may be modulated by mitogenic, insulin and/or amino acid stimulii.

It has also been shown that GSK3β is a key component in the vertebrate Wnt signalling pathway. This biochemical pathway has been shown to be critical for normal embryonic development and regulates cell proliferation in normal tissues. GSK3 becomes inhibited in response to Wnt stimulii. This can lead to the de-phosphorylation of GSK3 substrates such as Axin, the adenomatous polyposis coli (APC) gene product and β-catenin. Aberrant regulation of the Wnt pathway has been associated with many cancers. Mutations in APC, and/or β-catenin, are common in colorectal cancer and other tumours. β-catenin has also been shown to be of importance in cell adhesion. Thus GSK3 may also modulate cellular adhesion processes to some degree. Apart from the biochemical pathways already described there are also data implicating GSK3 in the regulation of cell division via phosphorylation of cyclin-D1, in the phosphorylation of transcription factors such

as c-Jun, CCAAT/enhancer binding protein α (C/EBPα), c-Myc and/or other substrates such as Nuclear Factor of Activated T-cells (NFATc), Heat Shock Factor-1 (HSF-1) and the c-AMP response element binding protein (CREB). GSK3 also appears to play a role, albeit tissue specific, in regulating cellular apoptosis. The role of GSK3 in modulating cellular apoptosis, via a pro-apoptotic mechanism, may be of particular relevance to medical conditions in which neuronal apoptosis can occur. Examples of these are head trauma, stroke, epilepsy, Alzheimer's and motor neuron diseases, progressive supranuclear palsy, corticobasal degeneration, and Pick's disease. *In vitro* it has been shown that GSK3 is able to hyper-phosphorylate the microtubule associated protein Tau. Hyperphosphorylation of Tau disrupts its normal binding to microtubules and may also lead to the formation of intra-cellular Tau filaments. It is believed that the progressive accumulation of these filaments leads to eventual neuronal dysfunction and degeneration. Inhbition of Tau phosphorylation, through inhibition of GSK3, may thus provide a means of limiting and/or preventing neurodegenerative effects.

Diffuse Large B-cell Lymphomas (DLBCL)

Cell cycle progression is regulated by the combined action of cyclins, cyclin-dependent kinases (CDKs), and CDK-inhibitors (CDKi), which are negative cell cycle regulators. p27KIP1 is a CDKi key in cell cycle regulation, whose degradation is required for G1/S transition. In spite of the absence of p27KIP1 expression in proliferating lymphocytes, some aggressive B-cell lymphomas have been reported to show an anomalous p27KIP1 staining. An abnormally high expression of p27KIP1 was found in lymphomas of this type. Analysis of the clinical relevance of these findings showed that a high level of p27KIP1 expression in this type of tumour is an adverse prognostic marker, in both univariate and multivariate analysis. These results show that there is abnormal p27KIP1 expression in Diffuse Large B-cell Lymphomas (DLBCL), with adverse clinical significance, suggesting that this anomalous p27KIP1 protein may be rendered non-functional through interaction with other cell cycle regulator proteins. (Br. J. Cancer. 1999 Jul;80(9):1427-34. p27KIP1 is abnormally expressed in Diffuse Large

B-cell Lymphomas and is associated with an adverse clinical outcome. Saez A, Sanchez E, Sanchez-Beato M, Cruz MA, Chacon I, Munoz E, Camacho FI, Martinez-Montero JC, Mollejo M, Garcia JF, Piris MA. Department of Pathology, Virgen de la Salud Hospital, Toledo, Spain.)

Chronic Lymphocytic Leukemia

B-Cell chronic lymphocytic leukaemia (CLL) is the most common leukaemia in the Western hemisphere, with approximately 10,000 new cases diagnosed each year (Parker SL, Tong T, Bolden S, Wingo PA: Cancer statistics, 1997. Ca. Cancer. J. Clin. 47:5, (1997)). Relative to other forms of leukaemia, the overall prognosis of CLL is good, with even the most advanced stage patients having a median survival of 3 years.

The addition of fludarabine as initial therapy for symptomatic CLL patients has led to a higher rate of complete responses (27% *v* 3%) and duration of progression-free survival (33 *v* 17 months) as compared with previously used alkylator-based therapies. Although attaining a complete clinical response after therapy is the initial step toward improving survival in CLL, the majority of patients either do not attain complete remission or fail to respond to fludarabine. Furthermore, all patients with CLL treated with fludarabine eventually relapse, making its role as a single agent purely palliative (Rai KR, Peterson B, Elias L, Shepherd L, Hines J, Nelson D, Cheson B, Kolitz J, Schiffer CA: A randomized comparison of fludarabine and chlorambucil for patients with previously untreated chronic lymphocytic leukemia. A CALGB SWOG, CTG/NCI-C and ECOG Inter-Group Study. Blood 88:141a, 1996 (abstr 552, suppl 1). Therefore, identifying new agents with novel mechanisms of action that complement fludarabine's cytotoxicity and abrogate the resistance induced by intrinsic CLL drug-resistance factors will be necessary if further advances in the therapy of this disease are to be realized.

The most extensively studied, uniformly predictive factor for poor response to therapy and inferior survival in CLL patients is aberrant p53 function, as characterized by point mutations or chromosome 17p13 deletions. Indeed, virtually

no responses to either alkylator or purine analog therapy have been documented in multiple single institution case series for those CLL patients with abnormal p53 function. Introduction of a therapeutic agent that has the ability to overcome the drug resistance associated with p53 mutation in CLL would potentially be a major advance for the treatment of the disease.

Flavopiridol and CYC 202, inhibitors of cyclin-dependent kinases induce in vitro apoptosis of malignant cells from B-cell chronic lymphocytic leukemia (B-CLL).

Flavopiridol exposure results in the stimulation of caspase 3 activity and in caspase-dependent cleavage of p27(kip1), a negative regulator of the cell cycle, which is overexpressed in B-CLL (Blood. 1998 Nov 15;92(10):3804-16 Flavopiridol induces apoptosis in chronic lymphocytic leukemia cells via activation of caspase-3 without evidence of bcl-2 modulation or dependence on functional p53. Byrd JC, Shinn C, Waselenko JK, Fuchs EJ, Lehman TA, Nguyen PL, Flinn IW, Diehl LF, Sausville E, Grever MR).

Prior Art

WO 02/34721 from Du Pont discloses a class of indeno [1,2-c]pyrazol-4-ones as inhibitors of cyclin dependent kinases.

WO 01/81348 from Bristol Myers Squibb describes the use of 5-thio-, sulphinyl- and sulphonylpyrazolo[3,4-b]-pyridines as cyclin dependent kinase inhibitors.

WO 00/62778 also from Bristol Myers Squibb discloses a class of protein tyrosine kinase inhibitors.

WO 01/72745A1 from Cyclacel describes 2-substituted 4-heteroaryl-pyrimidines and their preparation, pharmaceutical compositions containing them and their use as inhibitors of cyclin-dependant kinases (CDKs) and hence their use in the treatment of proliferative disorders such as cancer, leukaemia, psoriasis and the like.

WO 99/21845 from Agouron describes 4-aminothiazole derivatives for inhibiting cyclin-dependent kinases (CDKs), such as CDK1, CDK2, CDK4, and CDK6. The

invention is also directed to the therapeutic or prophylactic use of pharmaceutical compositions containing such compounds and to methods of treating malignancies and other disorders by administering effective amounts of such compounds.

WO 01/53274 from Agouron discloses as CDK kinase inhibitors a class of compounds which can comprise an amide-substituted benzene ring linked to an N-containing heterocyclic group.

WO 01/98290 (Pharmacia & Upjohn) discloses a class of 3-aminocarbonyl-2-carboxamido thiophene derivatives as protein kinase inhibitors.

WO 01/53268 and WO 01/02369 from Agouron disclose compounds that mediate or inhibit cell proliferation through the inhibition of protein kinases such as cyclin dependent kinase or tyrosine kinase. The Agouron compounds have an aryl or heteroaryl ring attached directly or though a CH=CH or CH=N group to the 3-position of an indazole ring.

WO 00/39108 and WO 02/00651 (both to Du Pont Pharmaceuticals) describe heterocyclic compounds that are inhibitors of trypsin-like serine protease enzymes, especially factor Xa and thrombin. The compounds are stated to be useful as anticoagulants or for the prevention of thromboembolic disorders.

US 2002/0091116 (Zhu *et al.*), WO 01/19798 and WO 01/64642 each disclose diverse groups of heterocyclic compounds as inhibitors of Factor Xa. Some 1-substituted pyrazole carboxamides are disclosed and exemplified.

US 6,127,382, WO 01/70668, WO 00/68191, WO 97/48672, WO 97/19052 and WO 97/19062 (all to Allergan) each describe compounds having retinoid-like activity for use in the treatment of various hyperproliferative diseases including cancers.

WO 02/070510 (Bayer) describes a class of amino-dicarboxylic acid compounds for use in the treatment of cardiovascular diseases. Although pyrazoles are mentioned generically, there are no specific examples of pyrazoles in this document.

WO 97/03071 (Knoll AG) discloses a class of heterocyclyl-carboxamide derivatives for use in the treatment of central nervous system disorders. Pyrazoles are mentioned generally as examples of heterocyclic groups but no specific pyrazole compounds are disclosed or exemplified.

WO 97/40017 (Novo Nordisk) describes compounds that are modulators of protein tyrosine phosphatases.

WO 03/020217 (Univ. Connecticut) discloses a class of pyrazole 3-carboxamides as cannabinoid receptor modulators for treating neurological conditions. It is stated (page 15) that the compounds can be used in cancer chemotherapy but it is not made clear whether the compounds are active as anti-cancer agents or whether they are administered for other purposes.

WO 01/58869 (Bristol Myers Squibb) discloses cannabinoid receptor modulators that can be used *inter alia* to treat a variety of diseases. The main use envisaged is the treatment of respiratory diseases, although reference is made to the treatment of cancer.

WO 01/02385 (Aventis Crop Science) discloses 1-(quinoline-4-yl)-1H-pyrazole derivatives as fungicides. 1-Unsubsituted pyrazoles are disclosed as synthetic intermediates.

WO 2004/039795 (Fujisawa) discloses amides containing a 1-substituted pyrazole group as inhibitors of apolipoprotein B secretion. The compounds are stated to be useful in treating such conditions as hyperlipidemia.

WO 2004/000318 (Cellular Genomics) discloses various amino-substituted monocycles as kinase modulators. None of the exemplified compounds are pyrazoles.

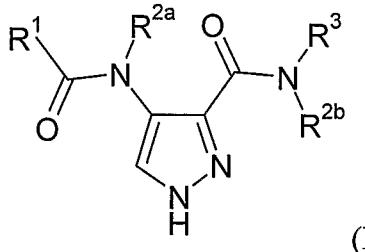
Our earlier co-pending application WO 2005/012256, which was published after the priority date of the present application, discloses 3,4-disubstituted pyrazole compounds as inhibitors of CDK and GSK-3 kinases.

Summary of the Invention

The invention provides compounds that have cyclin dependent kinase inhibiting or modulating activity and glycogen synthase kinase-3 (GSK3) inhibiting or modulating activity, and which it is envisaged will be useful in preventing or treating disease states or conditions mediated by the kinases.

Thus, for example, it is envisaged that the compounds of the invention will be useful in alleviating or reducing the incidence of cancer.

In a first aspect, the invention provides a compound of the formula (I):



(I)

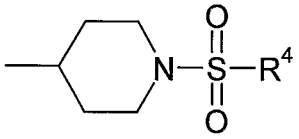
or salts, tautomers, solvates and N-oxides thereof;

wherein:

R^1 is 2,6-dichlorophenyl;

R^{2a} and R^{2b} are both hydrogen;

and R^3 is a group:



where R^4 is C_{1-4} alkyl.

The term "alkyl" covers both straight chain and branched chain alkyl groups.

The C_{1-4} alkyl group can be a C_1, C_2, C_3 or C_4 alkyl group.

Within the group of C_{1-4} alkyl groups are the sub-groups of:

- C_{1-3} alkyl groups;
- C_{1-2} alkyl groups;

- C_{2-3} alkyl groups; and
- C_{2-4} alkyl groups.

One particular sub-group is C_{1-3} alkyl.

Particular C_{1-4} alkyl groups are methyl, ethyl, *i*-propyl, *n*-butyl, *i*-butyl and *tert*-butyl groups.

Another sub-group of C_{1-4} alkyl groups consists of methyl, ethyl, *i*-propyl and *n*-propyl groups.

One preferred group is a methyl group.

Other particular groups R^4 are ethyl and isopropyl.

Accordingly, a preferred compound of the invention is 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid (1-methanesulphonyl-piperidin-4-yl)-amide.

The invention also provides *inter alia*:

- A compound of the formula (I) or any sub-groups or examples thereof as defined herein for use in the prophylaxis or treatment of a disease state or condition mediated by a cyclin dependent kinase or glycogen synthase kinase-3.

- A method for the prophylaxis or treatment of a disease state or condition mediated by a cyclin dependent kinase or glycogen synthase kinase-3, which method comprises administering to a subject in need thereof a compound of the formula (I) or any sub-groups or examples thereof as defined herein.

- A method for alleviating or reducing the incidence of a disease state or condition mediated by a cyclin dependent kinase or glycogen synthase kinase-3, which method comprises administering to a subject in need thereof

a compound of the formula (I) or any sub-groups or examples thereof as defined herein.

- A method for treating a disease or condition comprising or arising from abnormal cell growth in a mammal, which method comprises administering to the mammal a compound of the formula (I) or any sub-groups or examples thereof as defined herein in an amount effective in inhibiting abnormal cell growth.

- A method for alleviating or reducing the incidence of a disease or condition comprising or arising from abnormal cell growth in a mammal, which method comprises administering to the mammal a compound of the formula (I) or any sub-groups or examples thereof as defined herein in an amount effective in inhibiting abnormal cell growth.

- A method for treating a disease or condition comprising or arising from abnormal cell growth in a mammal, the method comprising administering to the mammal a compound of the formula (I) or any sub-groups or examples thereof as defined herein in an amount effective to inhibit a cdk kinase (such as cdk1 or cdk2) or glycogen synthase kinase-3 activity.

- A method for alleviating or reducing the incidence of a disease or condition comprising or arising from abnormal cell growth in a mammal, the method comprising administering to the mammal a compound of the formula (I) or any sub-groups or examples thereof as defined herein in an amount effective to inhibit a cdk kinase (such as cdk1 or cdk2) or glycogen synthase kinase-3 activity.

- A method of inhibiting a cyclin dependent kinase or glycogen synthase kinase-3, which method comprises contacting the kinase with a kinase-inhibiting compound of the formula (I) or any sub-groups or examples thereof as defined herein.

- A method of modulating a cellular process (for example cell division) by inhibiting the activity of a cyclin dependent kinase or glycogen synthase kinase-3 using a compound of the formula (I) or any sub-groups or examples thereof as defined herein.

- A compound of the formula (I) or any sub-groups or examples thereof as defined herein for use in the prophylaxis or treatment of a disease state as described herein.

- The use of a compound of the formula (I) or any sub-groups or examples thereof as defined herein for the manufacture of a medicament, wherein the medicament is for any one or more of the uses defined herein.

- A pharmaceutical composition comprising a compound of the formula (I) or any sub-groups or examples thereof as defined herein and a pharmaceutically acceptable carrier.

- A pharmaceutical composition comprising a compound of the formula (I) or any sub-groups or examples thereof as defined herein and a pharmaceutically acceptable carrier in a form suitable for oral administration.

- A compound of the formula (I) or any sub-groups or examples thereof as defined herein for use in medicine.

- A method for the diagnosis and treatment of a disease state or condition mediated by a cyclin dependent kinase, which method comprises (i) screening a patient to determine whether a disease or condition from which the patient is or may be suffering is one which would be susceptible to treatment with a compound having activity against cyclin dependent kinases; and (ii) where it is indicated that the disease or condition from which the patient is thus susceptible, thereafter administering to the patient a compound of the formula (I) or any sub-groups or examples thereof as defined herein.

- The use of a compound of the formula (I) or any sub-groups or examples thereof as defined herein for the manufacture of a medicament for the treatment or prophylaxis of a disease state or condition in a patient who has been screened and has been determined as suffering from, or being at risk of suffering from, a disease or condition which would be susceptible to treatment with a compound having activity against cyclin dependent kinase.

- A compound of the formula (I) or any sub-groups or examples thereof as defined herein for use in inhibiting tumour growth in a mammal.

- A compound of the formula (I) or any sub-groups or examples thereof as defined herein for use in inhibiting the growth of tumour cells (e.g. in a mammal).

- A method of inhibiting tumour growth in a mammal (e.g. a human), which method comprises administering to the mammal (e.g. a human) an effective tumour growth-inhibiting amount of a compound of the formula (I) or any sub-groups or examples thereof as defined herein.

- A method of inhibiting the growth of tumour cells (e.g. tumour cells present in a mammal such as a human), which method comprises contacting the tumour cells with an effective tumour cell growth-inhibiting amount of a compound of the formula (I) or any sub-groups or examples thereof as defined herein.

- A compound as defined herein for any of the uses and methods set forth above, and as described elsewhere herein.

General Preferences and Definitions

In this application, unless the context indicates otherwise, references to a compound of formula (I) includes all subgroups of formula (I) as defined herein and the term 'subgroups' includes all preferences, embodiments, examples and particular compounds defined herein.

Any references to formula (I) herein shall also be taken to refer to and any sub-group of compounds within formula (I) and any preferences and examples thereof unless the context requires otherwise.

Salts, Solvates, Tautomers, Isomers, N-Oxides, Esters, Prodrugs and Isotopes

A reference to a compound of the formulae (I) and sub-groups thereof also includes ionic forms, salts, solvates, isomers, tautomers, N-oxides, esters, prodrugs, isotopes and protected forms thereof, for example, as discussed below; preferably, the salts or tautomers or isomers or N-oxides or solvates thereof; and more preferably, the salts or tautomers or N-oxides or solvates thereof

Many compounds of the formula (I) can exist in the form of salts, for example acid addition salts or, in certain cases salts of organic and inorganic bases such as carboxylate, sulphonate and phosphate salts. All such salts are within the scope of this invention, and references to compounds of the formula (I) include the salt forms of the compounds. .

The salts of the present invention can be synthesized from the parent compound that contains a basic or acidic moiety by conventional chemical methods such as methods described in *Pharmaceutical Salts: Properties, Selection, and Use*, P. Heinrich Stahl (Editor), Camille G. Wermuth (Editor), ISBN: 3-90639-026-8, Hardcover, 388 pages, August 2002. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media such as ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are used.

Acid addition salts may be formed with a wide variety of acids, both inorganic and organic. Examples of acid addition salts include salts formed with an acid selected from the group consisting of acetic, 2,2-dichloroacetic, adipic, alginic, ascorbic (e.g. L-ascorbic), L-aspartic, benzenesulphonic, benzoic, 4-acetamidobenzoic, butanoic, (+) camphoric, camphor-sulphonic, (+)-(1*S*)-camphor-10-sulphonic, capric, caproic, caprylic, cinnamic, citric, cyclamic, dodecylsulphuric, ethane-1,2-

disulphonic, ethanesulphonic, 2-hydroxyethanesulphonic, formic, fumaric, galactaric, gentisic, glucoheptonic, D-gluconic, glucuronic (e.g. D-glucuronic), glutamic (e.g. L-glutamic), α-oxoglutaric, glycolic, hippuric, hydrobromic, hydrochloric, hydriodic, isethionic, (+)-L-lactic, (±)-DL-lactic, lactobionic, maleic, malic, (-)-L-malic, malonic, (±)-DL-mandelic, methanesulphonic, naphthalene-2-sulphonic, naphthalene-1,5-disulphonic, 1-hydroxy-2-naphthoic, nicotinic, nitric, oleic, orotic, oxalic, palmitic, pamoic, phosphoric, propionic, L-pyroglutamic, salicylic, 4-amino-salicylic, sebacic, stearic, succinic, sulphuric, tannic, (+)-L-tartaric, thiocyanic, *p*-toluenesulphonic, undecylenic and valeric acids, as well as acylated amino acids and cation exchange resins.

One particular group of salts consists of salts formed from acetic, hydrochloric, hydriodic, phosphoric, nitric, sulphuric, citric, lactic, succinic, maleic, malic, isethionic, fumaric, benzenesulphonic, toluenesulphonic, methanesulphonic (mesylate), ethanesulphonic, naphthalenesulphonic, valeric, acetic, propanoic, butanoic, malonic, glucuronic and lactobionic acids.

One sub-group of salts consists of salts formed from hydrochloric, acetic, methanesulphonic, adipic, L-aspartic and DL-lactic acids.

Another sub-group of salts consists of the acetate, mesylate, ethanesulphonate, DL-lactate, adipate, D-glucuronate, D-gluconate and hydrochloride salts.

Particular salts for use in the preparation of liquid (e.g. aqueous) compositions of the compounds of formulae (I) and sub-groups and examples thereof as described herein are salts having a solubility in a given liquid carrier (e.g. water) of greater than 10 μg/ml of the liquid carrier (e.g. water), more typically greater than 0.5 mg/ml and preferably greater than 1 mg/ml.

In one embodiment of the invention, there is provided a pharmaceutical composition comprising an aqueous solution containing a compound of the formula (I) and sub-groups and examples thereof as described herein in the form of a salt in a concentration of greater than greater than 10 μg/ml of the liquid carrier (e.g. water), more typically greater than 0.5 mg/ml and preferably greater than 1 mg/ml.

If the compound is anionic, or has a functional group which may be anionic (e.g., -COOH may be -COO^-), then a salt may be formed with a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal ions such as Na^+ and K^+, alkaline earth metal cations such as Ca^{2+} and Mg^{2+}, and other cations such as Al^{3+}. Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e., NH_4^+) and substituted ammonium ions (e.g., NH_3R^+, $NH_2R_2^+$, NHR_3^+, NR_4^+). Examples of some suitable substituted ammonium ions are those derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is $N(CH_3)_4^+$.

Where the compounds of the formula (I) contain an amine function, these may form quaternary ammonium salts, for example by reaction with an alkylating agent according to methods well known to the skilled person. Such quaternary ammonium compounds are within the scope of formula (I).

The salt forms of the compounds of the invention are typically pharmaceutically acceptable salts, and examples of pharmaceutically acceptable salts are discussed in Berge *et al.*, 1977, "Pharmaceutically Acceptable Salts," *J. Pharm. Sci.,* Vol. 66, pp. 1-19. However, salts that are not pharmaceutically acceptable may also be prepared as intermediate forms which may then be converted into pharmaceutically acceptable salts. Such non-pharmaceutically acceptable salts forms, which may be useful, for example, in the purification or separation of the compounds of the invention, also form part of the invention.

Compounds of the formula (I) containing an amine function may also form N-oxides. A reference herein to a compound of the formula (I) that contains an amine function also includes the N-oxide.

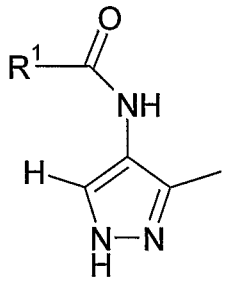
Where a compound contains several amine functions, one or more than one nitrogen atom may be oxidised to form an N-oxide. Particular examples of N-

oxides are the N-oxides of a tertiary amine or a nitrogen atom of a nitrogen-containing heterocycle.

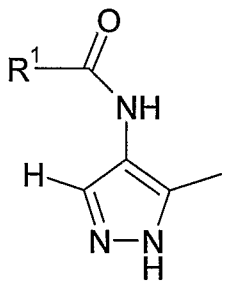
N-Oxides can be formed by treatment of the corresponding amine with an oxidizing agent such as hydrogen peroxide or a per-acid (e.g. a peroxycarboxylic acid), see for example *Advanced Organic Chemistry*, by Jerry March, 4th Edition, Wiley Interscience, pages. More particularly, N-oxides can be made by the procedure of L. W. Deady (*Syn. Comm.* 1977, 7, 509-514) in which the amine compound is reacted with *m*-chloroperoxybenzoic acid (MCPBA), for example, in an inert solvent such as dichloromethane.

Compounds of the formula (I) may exist in a number of different geometric isomeric, and tautomeric forms and references to compounds of the formula (I) include all such forms. For the avoidance of doubt, where a compound can exist in one of several geometric isomeric or tautomeric forms and only one is specifically described or shown, all others are nevertheless embraced by formula (I).

For example, in compounds of the formula (I) the pyrazole ring can exist in the two tautomeric forms A and B below. For simplicity, the general formula (I) illustrates form A but the formula is to be taken as embracing both tautomeric forms.



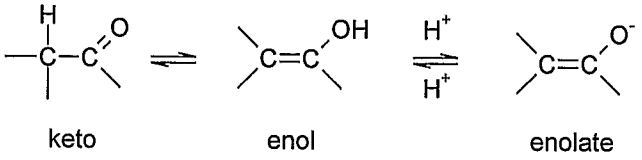
A



B

Other examples of tautomeric forms include, for example, keto-, enol-, and enolate-forms, as in, for example, the following tautomeric pairs: keto/enol (illustrated below), imine/enamine, amide/imino alcohol, amidine/amidine, nitroso/oxime,

thioketone/enethiol, and nitro/aci-nitro.



Where compounds of the formula (I) contain one or more chiral centres (e.g. as in the case of the compounds wherein R^4 is 2-butyl), and can exist in the form of two or more optical isomers, references to compounds of the formula (I) include all optical isomeric forms thereof (e.g. enantiomers, epimers and diastereoisomers), either as individual optical isomers, or mixtures (e.g. racemic mixtures) or two or more optical isomers, unless the context requires otherwise.

The optical isomers may be characterised and identified by their optical activity (i.e. as + and – isomers, or *d* and *l* isomers) or they may be characterised in terms of their absolute stereochemistry using the "R and S" nomenclature developed by Cahn, Ingold and Prelog, see *Advanced Organic Chemistry* by Jerry March, 4th Edition, John Wiley & Sons, New York, 1992, pages 109-114, and see also Cahn, Ingold & Prelog, *Angew. Chem. Int. Ed. Engl.*, 1966, 5, 385-415.

Optical isomers can be separated by a number of techniques including chiral chromatography (chromatography on a chiral support) and such techniques are well known to the person skilled in the art.

As an alternative to chiral chromatography, optical isomers can be separated by forming diastereoisomeric salts with chiral acids such as (+)-tartaric acid, (-)-pyroglutamic acid, (-)-di-toluoyl-L-tartaric acid, (+)-mandelic acid, (-)-malic acid, and (-)-camphorsulphonic, separating the diastereoisomers by preferential crystallisation, and then dissociating the salts to give the individual enantiomer of the free base.

Where compounds of the formula (I) exist as two or more optical isomeric forms, one enantiomer in a pair of enantiomers may exhibit advantages over the other enantiomer, for example, in terms of biological activity. Thus, in certain

circumstances, it may be desirable to use as a therapeutic agent only one of a pair of enantiomers, or only one of a plurality of diastereoisomers. Accordingly, the invention provides compositions containing a compound of the formula (I) having one or more chiral centres, wherein at least 55% (e.g. at least 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95%) of the compound of the formula (I) is present as a single optical isomer (e.g. enantiomer or diastereoisomer). In one general embodiment, 99% or more (e.g. substantially all) of the total amount of the compound of the formula (I) may be present as a single optical isomer (e.g. enantiomer or diastereoisomer).

The compounds of the invention include compounds with one or more isotopic substitutions, and a reference to a particular element includes within its scope all isotopes of the element. For example, a reference to hydrogen includes within its scope ^{1}H, ^{2}H (D), and ^{3}H (T). Similarly, references to carbon and oxygen include within their scope respectively ^{12}C, ^{13}C and ^{14}C and ^{16}O and ^{18}O.

The isotopes may be radioactive or non-radioactive. In one embodiment of the invention, the compounds contain no radioactive isotopes. Such compounds are preferred for therapeutic use. In another embodiment, however, the compound may contain one or more radioisotopes. Compounds containing such radioisotopes may be useful in a diagnostic context.

Esters such as carboxylic acid esters and acyloxy esters of the compounds of formula (I) bearing a carboxylic acid group or a hydroxyl group are also embraced by Formula (I). Examples of esters are compounds containing the group -C(=O)OR, wherein R is an ester substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Particular examples of ester groups include, but are not limited to, $-C(=O)OCH_3$, $-C(=O)OCH_2CH_3$, $-C(=O)OC(CH_3)_3$, and -C(=O)OPh. Examples of acyloxy (reverse ester) groups are represented by -OC(=O)R, wherein R is an acyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Particular examples of acyloxy groups

include, but are not limited to, -OC(=O)CH_3 (acetoxy), -OC(=O)CH_2CH_3, -OC(=O)$C(CH_3)_3$, -OC(=O)Ph, and -OC(=O)CH_2Ph.

Also encompassed by formula (I) are any polymorphic forms of the compounds, solvates (e.g. hydrates), complexes (e.g. inclusion complexes or clathrates with compounds such as cyclodextrins, or complexes with metals) of the compounds, and pro-drugs of the compounds. By "prodrugs" is meant for example any compound that is converted *in vivo* into a biologically active compound of the formula (I).

For example, some prodrugs are esters of the active compound (e.g., a physiologically acceptable metabolically labile ester). During metabolism, the ester group (-C(=O)OR) is cleaved to yield the active drug. Such esters may be formed by esterification, for example, of any of the carboxylic acid groups (-C(=O)OH) in the parent compound, with, where appropriate, prior protection of any other reactive groups present in the parent compound, followed by deprotection if required.

Examples of such metabolically labile esters include those of the formula -C(=O)OR wherein R is:

C_{1-7}alkyl

(e.g., -Me, -Et, -nPr, -iPr, -nBu, -sBu, -iBu, -tBu);

C_{1-7}aminoalkyl

(e.g., aminoethyl; 2-(N,N-diethylamino)ethyl; 2-(4-morpholino)ethyl); and

acyloxy-C_{1-7}alkyl

(e.g., acyloxymethyl;

acyloxyethyl;

pivaloyloxymethyl;

acetoxymethyl;

1-acetoxyethyl;

1-(1-methoxy-1-methyl)ethyl-carbonxyloxyethyl;

1-(benzoyloxy)ethyl; isopropoxy-carbonyloxymethyl;

1-isopropoxy-carbonyloxyethyl; cyclohexyl-carbonyloxymethyl;

1-cyclohexyl-carbonyloxyethyl;

cyclohexyloxy-carbonyloxymethyl;
1-cyclohexyloxy-carbonyloxyethyl;
(4-tetrahydropyranyloxy) carbonyloxymethyl;
1-(4-tetrahydropyranyloxy)carbonyloxyethyl;
(4-tetrahydropyranyl)carbonyloxymethyl; and
1-(4-tetrahydropyranyl)carbonyloxyethyl).

Also, some prodrugs are activated enzymatically to yield the active compound, or a compound which, upon further chemical reaction, yields the active compound (for example, as in ADEPT, GDEPT, LIDEPT, etc.). For example, the prodrug may be a sugar derivative or other glycoside conjugate, or may be an amino acid ester derivative.

Biological Activity

The compounds of the formulae (I) and sub-groups thereof are inhibitors of cyclin dependent kinases. For example, compounds of the invention are inhibitors of cyclin dependent kinases, and in particular cyclin dependent kinases selected from CDK1, CDK2, CDK3, CDK4, CDK5, CDK6 and CDK9, and more particularly selected from CDK1, CDK2, CDK3, CDK4, CDK5 and CDK9.

Preferred compounds are compounds that inhibit one or more CDK kinases selected from CDK1, CDK2, CDK4 and CDK9, for example CDK1 and/or CDK2.

Compounds of the invention also have activity against glycogen synthase kinase-3 (GSK-3).

As a consequence of their activity in modulating or inhibiting CDK and glycogen synthase kinase, they are expected to be useful in providing a means of arresting, or recovering control of, the cell cycle in abnormally dividing cells. It is therefore anticipated that the compounds will prove useful in treating or preventing proliferative disorders such as cancers. It is also envisaged that the compounds of the invention will be useful in treating conditions such as viral infections, type II or non-insulin dependent diabetes mellitus, autoimmune diseases, head trauma, stroke,

epilepsy, neurodegenerative diseases such as Alzheimer's, motor neurone disease, progressive supranuclear palsy, corticobasal degeneration and Pick's disease, for example autoimmune diseases and neurodegenerative diseases.

One sub-group of disease states and conditions where it is envisaged that the compounds of the invention will be useful consists of viral infections, autoimmune diseases and neurodegenerative diseases.

CDKs play a role in the regulation of the cell cycle, apoptosis, transcription, differentiation and CNS function. Therefore, CDK inhibitors could be useful in the treatment of diseases in which there is a disorder of proliferation, apoptosis or differentiation such as cancer. In particular RB+ve tumours may be particularly sensitive to CDK inhibitors. RB-ve tumours may also be sensitive to CDK inhibitors.

Examples of cancers which may be inhibited include, but are not limited to, a carcinoma, for example a carcinoma of the bladder, breast, colon (e.g. colorectal carcinomas such as colon adenocarcinoma and colon adenoma), kidney, epidermis, liver, lung, for example adenocarcinoma, small cell lung cancer and non-small cell lung carcinomas, oesophagus, gall bladder, ovary, pancreas e.g. exocrine pancreatic carcinoma, stomach, cervix, thyroid, prostate, or skin, for example squamous cell carcinoma; a hematopoietic tumour of lymphoid lineage, for example leukemia, acute lymphocytic leukemia, chronic lymphocytic leukaemia, B-cell lymphoma (such as diffuse large B cell lymphoma), T-cell lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, hairy cell lymphoma, or Burkett's lymphoma; a hematopoietic tumour of myeloid lineage, for example acute and chronic myelogenous leukemias, myelodysplastic syndrome, or promyelocytic leukemia; thyroid follicular cancer; a tumour of mesenchymal origin, for example fibrosarcoma or habdomyosarcoma; a tumour of the central or peripheral nervous system, for example astrocytoma, neuroblastoma, glioma or schwannoma; melanoma; seminoma; teratocarcinoma; osteosarcoma; xeroderma pigmentosum; keratoctanthoma; thyroid follicular cancer; or Kaposi's sarcoma.

The cancers may be cancers which are sensitive to inhibition of any one or more cyclin dependent kinases selected from CDK1, CDK2, CDK3, CDK4, CDK5 and CDK6, for example, one or more CDK kinases selected from CDK1, CDK2, CDK4 and CDK5, e.g. CDK1 and/or CDK2.

Whether or not a particular cancer is one which is sensitive to inhibition by a cyclin dependent kinase may be determined by means of a cell growth assay as set out in the examples below or by a method as set out in the section headed "Methods of Diagnosis".

CDKs are also known to play a role in apoptosis, proliferation, differentiation and transcription and therefore CDK inhibitors could also be useful in the treatment of the following diseases other than cancer; viral infections, for example herpes virus, pox virus, Epstein-Barr virus, Sindbis virus, adenovirus, HIV, HPV, HCV and HCMV; prevention of AIDS development in HIV-infected individuals; chronic inflammatory diseases, for example systemic lupus erythematosus, autoimmune mediated glomerulonephritis, rheumatoid arthritis, psoriasis, inflammatory bowel disease, and autoimmune diabetes mellitus; cardiovascular diseases for example cardiac hypertrophy, restenosis, atherosclerosis; neurodegenerative disorders, for example Alzheimer's disease, AIDS-related dementia, Parkinson's disease, amyotropic lateral sclerosis, retinitis pigmentosa, spinal muscular atropy and cerebellar degeneration; glomerulonephritis; myelodysplastic syndromes, ischemic injury associated myocardial infarctions, stroke and reperfusion injury, arrhythmia, atherosclerosis, toxin-induced or alcohol related liver diseases, haematological diseases, for example, chronic anemia and aplastic anemia; degenerative diseases of the musculoskeletal system, for example, osteoporosis and arthritis, aspirin-senstive rhinosinusitis, cystic fibrosis, multiple sclerosis, kidney diseases and cancer pain.

It has also been discovered that some cyclin-dependent kinase inhibitors can be used in combination with other anticancer agents. For example, the cyclin-dependent kinase inhibitor flavopiridol has been used with other anticancer agents in combination therapy.

Thus, in the pharmaceutical compositions, uses or methods of this invention for treating a disease or condition comprising abnormal cell growth, the disease or condition comprising abnormal cell growth in one embodiment is a cancer.

One group of cancers includes human breast cancers (e.g. primary breast tumours, node-negative breast cancer, invasive duct adenocarcinomas of the breast, non-endometrioid breast cancers); and mantle cell lymphomas. In addition, other cancers are colorectal and endometrial cancers.

Another sub-set of cancers includes hematopoietic tumours of lymphoid lineage, for example leukemia, chronic lymphocytic leukaemia, mantle cell lymphoma and B-cell lymphoma (such as diffuse large B cell lymphoma).

One particular cancer is chronic lymphocytic leukaemia.

Another particular cancer is mantle cell lymphoma.

Another particular cancer is diffuse large B cell lymphoma

Another sub-set of cancers includes breast cancer, ovarian cancer, colon cancer, prostate cancer, oesophageal cancer, squamous cancer and non-small cell lung carcinomas.

The activity of the compounds of the invention as inhibitors of cyclin dependent kinases and glycogen synthase kinase-3 can be measured using the assays set forth in the examples below and the level of activity exhibited by a given compound can be defined in terms of the IC_{50} value. Preferred compounds of the present invention are compounds having an IC_{50} value of less than 1 micromolar, more preferably less than 0.1 micromolar.

Advantages of the Compounds of the Invention

Compounds of the formulae (I) and sub-groups thereof as defined herein, for example the compound 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic

acid (1-methanesulphonyl-piperidin-4-yl)-amide, have advantages over prior art compounds.

Potentially the compounds of the invention have physiochemical properties suitable for oral exposure.

Compounds of the invention have a higher IC_{50} for transcription than IC_{50} for proliferation in HCT-116 cells: thus, for example, the IC_{50} for transcription is ~100-fold higher than the IC_{50} for proliferation. This is advantageous as the compound could be better tolerated thus allowing it to be dosed at higher levels and for longer doses.

In particular, compounds of the formula (I) exhibit improved oral bioavailability relative to prior art compounds. Oral bioavailability can be defined as the ratio (F) of the plasma exposure of a compound when dosed by the oral route to the plasma exposure of the compound when dosed by the intravenous (i.v.) route, expressed as a percentage.

Compounds having an oral bioavailability (F value) of greater than 30%, more preferably greater than 40%, are particularly advantageous in that they may be adminstered orally rather than, or as well as, by parenteral administration.

The compound 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid (1-methanesulphonyl-piperidin-4-yl)-amide, for example, has 40-50% bioavailability when administered to mice by the oral route.

The compounds of the invention, for example the compound 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid (1-methanesulphonyl-piperidin-4-yl)-amide, have greater in vitro kinase (CDK2) inhibitory activity and more potent anti-proliferative effects on cancer cell lines. In addition, the compounds have lower activity versus GSK3β and are more selective for CDK2 over GSK3β. Therefore the action of the compounds is dominated by cell cycle effects via the CDK inhibition and not complicated by the additional consequences of GSK3beta inhibition on, for example, insulin sensitivity, growth factor action. The

compounds there have a cleaner cell cycle inhibition profile and fewer side effects from the additional effects via GSK3 beta. A comparison of the biological properties of the compound 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid (1-methanesulphonyl-piperidin-4-yl)-amide with the properties of its 2,6-difluorobenzoylamino analogue is set out in Example 11 below.

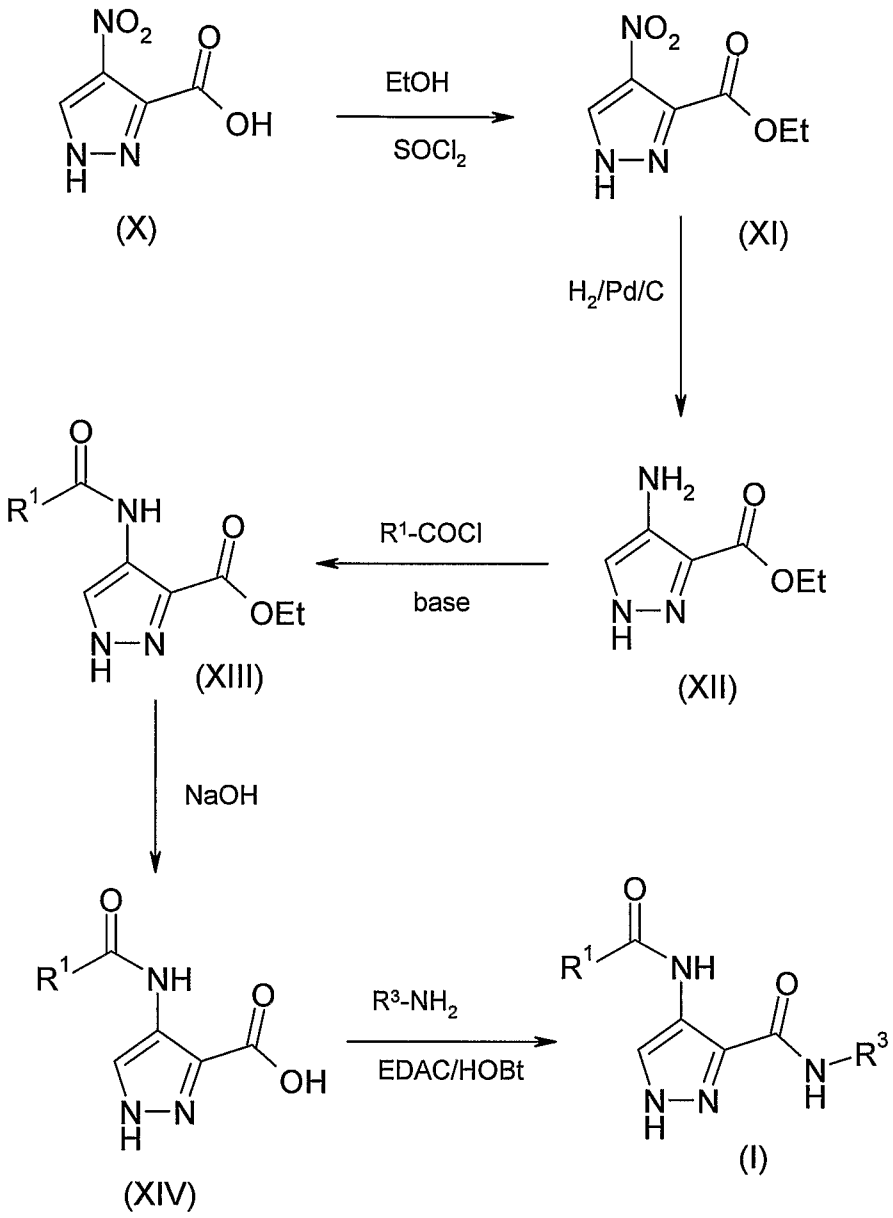
Methods for the Preparation of Compounds of the Formula (I)

In this section, as in all other sections of this application unless the context indicates otherwise, references to Formula (I) also include all sub-groups and examples therof as defined herein. Where a reference is made to a group R^1 and R^3 or any other "R" group, the definition of the group in question is as set out above and as set out in the following sections of this application unless the context requires otherwise.

Compounds of the formula (I) can be prepared in accordance with synthetic methods well known to the skilled person, and by methods set out below and as described in our application PCT/GB2004/003179 (WO 2005/012256), the contents of which are incorporated herein by reference.

For example, compounds of the formula (I) can be prepared by the sequence of reactions shown in Scheme 1.

The starting material for the synthetic route shown in Scheme 1 is the 4-nitro-pyrazole-3-carboxylic acid (X) which can either be obtained commercially or can be prepared by nitration of the corresponding 4-unsubstituted pyrazole carboxy compound.



Scheme 1

The nitro-pyrazole carboxylic acid (X) is converted to the corresponding ester (XI), for example the methyl or ethyl ester (of which the ethyl ester is shown), by reaction with the appropriate alcohol such as ethanol in the presence of an acid catalyst or thionyl chloride. The reaction may be carried out at ambient temperature using the esterifying alcohol as the solvent.

The nitro-ester (XI) can be reduced to the corresponding amine (XII) by standard methods for converting a nitro group to an amino group. Thus, for example, the nitro group can be reduced to the amine by hydrogenation over a palladium on charcoal catalyst. The hydrogenation reaction can be carried out in a solvent such as ethanol at ambient temperature.

The resulting amine (XII) can be converted to the amide (XIII) by reaction with an acid chloride of the formula R^1COCl in the presence of a non-interfering base such as triethylamine. The reaction may be carried out at around room temperature in a polar solvent such as dioxan. The acid chloride can be prepared by treatment of the carboxylic acid R^1CO_2H with thionyl chloride, or by reaction with oxalyl chloride in the presence of a catalytic amount of dimethyl formamide, or by reaction of a potassium salt of the acid with oxalyl chloride.

As an alternative to using the acid chloride method described above, the amine (XII) can be converted to the amide (XIII) by reaction with the carboxylic acid R^1CO_2H in the presence of amide coupling reagents of the type commonly used in the formation of peptide linkages. Examples of such reagents include 1,3-dicyclohexylcarbodiimide (DCC) (Sheehan *et al, J. Amer. Chem Soc.* 1955, 77, 1067), 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide (referred to herein either as EDC or EDAC but also known in the art as EDCI and WSCDI) (Sheehan *et al, J. Org. Chem., 1961, 26, 2525*), uronium-based coupling agents such as *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) and phosphonium-based coupling agents such as 1-benzo-triazolyloxytris-(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) (Castro *et al, Tetrahedron Letters*, 1990, 31, 205). Carbodiimide-based coupling agents are advantageously used in combination with 1-hydroxy-7-azabenzotriazole (HOAt) (L. A. Carpino, *J. Amer. Chem. Soc.*, 1993, 115, 4397) or 1-hydroxybenzotriazole (HOBt) (Konig *et al, Chem. Ber.*, 103, 708, 2024-2034). Preferred coupling reagents include EDC (EDAC) and DCC in combination with HOAt or HOBt.

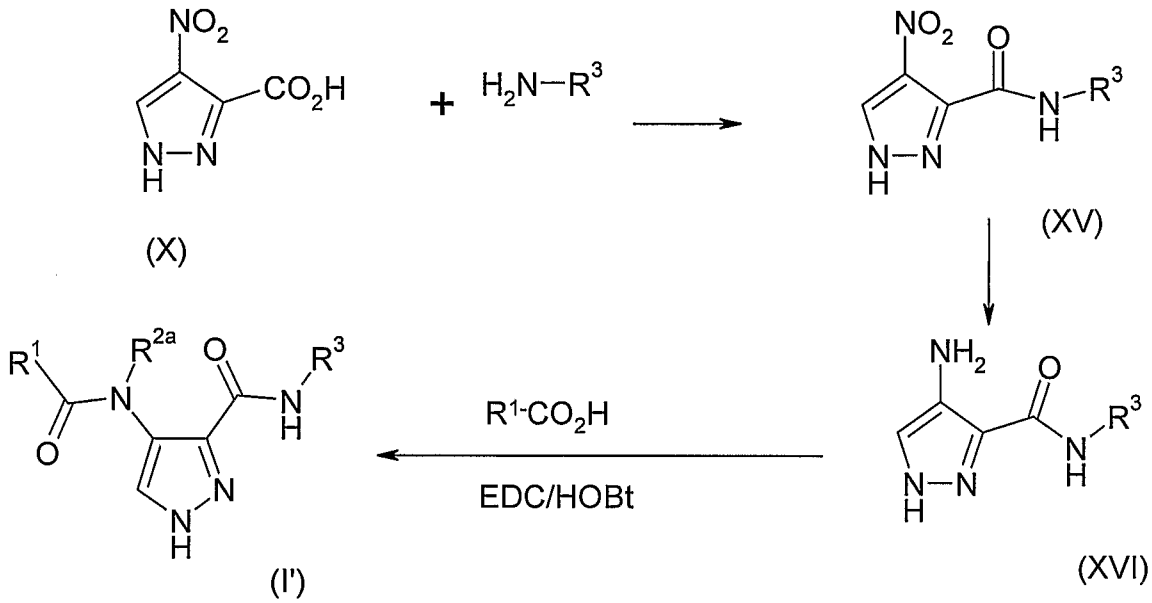
The coupling reaction is typically carried out in a non-aqueous, non-protic solvent such as acetonitrile, dioxan, dimethylsulphoxide, dichloromethane,

dimethylformamide or N-methylpyrrolidine, or in an aqueous solvent optionally together with one or more miscible co-solvents. The reaction can be carried out at room temperature or, where the reactants are less reactive (for example in the case of electron-poor anilines bearing electron withdrawing groups such as sulphonamide groups) at an appropriately elevated temperature. The reaction may be carried out in the presence of a non-interfering base, for example a tertiary amine such as triethylamine or *N,N*-diisopropylethylamine.

The amide (XIII) is subsequently hydrolysed to the carboxylic acid (XIV) by treatment with an aqueous alkali metal hydroxide such sodium hydroxide. The saponification reaction may be carried out using an organic co-solvent such as an alcohol (e.g. methanol) and the reaction mixture is typically heated to a non-extreme temperature, for example up to about 50-60 °C.

The carboxylic acid (XIV) can then be converted to a compound of the formula (I) by reaction with an amine R^3-NH_2 using the amide forming conditions described above. Thus, for example, the amide coupling reaction may be carried out in the presence of EDC and HOBt in a polar solvent such as DMF.

An alternative general route to compounds of the formula (I) wherein R^{2b} is hydrogen is shown in Scheme 2.

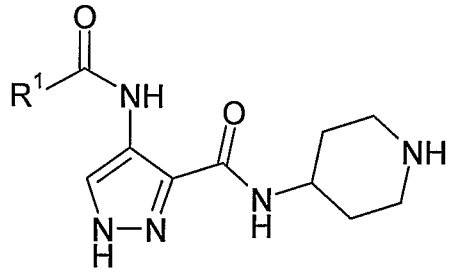


Scheme 2

In Scheme 2, the nitro-pyrazole-carboxylic acid (X), or an activated derivative thereof such as an acid chloride, is reacted with amine R^3-NH_2 using the amide forming conditions described above to give the nitro-pyrazole-amide (XV) which is then reduced to the corresponding amino compound (XVI) using a standard method of reducing nitro groups, for example the method involving hydrogenation over a Pd/C catalyst as described above.

The amine (XVI) is then coupled with a carboxylic acid of the formula R^1-CO_2H or an activated derivative thereof such as an acid chloride or anhydride under the amide-forming conditions described above in relation to Scheme 1. Thus, for example, as an alternative to using an acid chloride, the coupling reaction can be carried out in the presence of EDAC (EDC) and HOBt in a solvent such as DMF to give a compound of the formula (I') which corresponds to a compound of the formula (I) wherein R^{2b} is hydrogen.

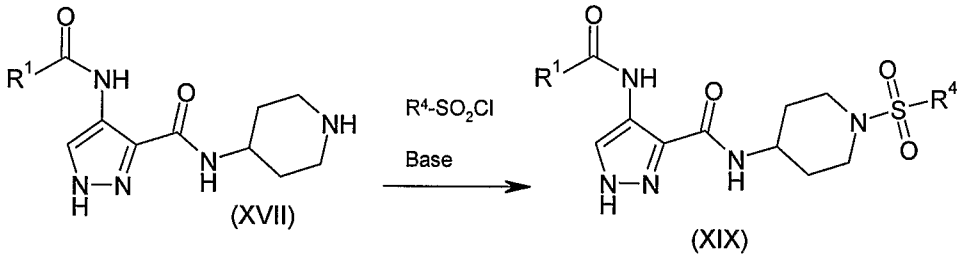
Compounds of the formula (I) can also be prepared from a compound of the formula (XVII):



(XVII)

by reaction with an appropriate sulphonylating agent, for example a sulphonyl chloride such as methanesulphonyl chloride.

An illustrative reaction sequence showing the conversion of a compound of the formula (XVII) into sulphonyl derivatives of the formula (I) is set out in Scheme 3.



Scheme 3

As shown in Scheme 3, a compound of the formula (I) in which R^3 is a piperidine ring bearing a sulphonyl group $-SO_2R^4$ (i.e. a compound of the formula (XIX)) can be prepared by reacting the compound of the formula (XVII) with a sulphonyl chloride R^4SO_2Cl (such as methane sulphonyl chloride) in the presence of a non-interfering base such as diisopropylethylamine. The reaction is typically carried out at room temperature in a non-aqueous non-protic solvent such as dioxane and dichloromethane.

The sulphonyl chlorides of the formula R^4SO_2Cl may be obtained from commercial sources, or can be prepared by a number of procedures. For example, alkylsulphonyl chlorides can be prepared by reacting an alkyl halide with sodium sulphite with heating in an aqueous organic solvent such as water/dioxane to form the corresponding sulphonic acid followed by treatment with thionyl chloride in the presence of DMF to give the sulphonyl chloride.

In an alternative preparation, a thiol R^4SH/ $R^{4a}SH$ can be reacted with potassium nitrate and sulphuryl chloride to give the required sulphonyl chloride.

In many of the reactions described above, it may be necessary to protect one or more groups to prevent reaction from taking place at an undesirable location on the molecule. Examples of protecting groups, and methods of protecting and deprotecting functional groups, can be found in *Protective Groups in Organic Synthesis* (T. Green and P. Wuts; 3rd Edition; John Wiley and Sons, 1999).
For example, an amine group may be protected as an amide (-NRCO-R) or a urethane (-NRCO-OR), for example, as: a methyl amide (-NHCO-CH_3); a benzyloxy amide (-NHCO-$OCH_2C_6H_5$, -NH-Cbz); as a t-butoxy amide (-NHCO-$OC(CH_3)_3$, -NH-Boc); a 2-biphenyl-2-propoxy amide (-NHCO-$OC(CH_3)_2C_6H_4C_6H_5$, -NH-Bpoc), as a 9-fluorenylmethoxy amide (-NH-Fmoc), as a 6-nitroveratryloxy amide (-NH-Nvoc), as a 2-trimethylsilylethyloxy amide (-NH-Teoc), as a 2,2,2-trichloroethyloxy amide (-NH-Troc), as an allyloxy amide (-NH-Alloc), or as a 2(-phenylsulphonyl)ethyloxy amide (-NH-Psec). Other protecting groups for amines, such as cyclic amines and heterocyclic N-H groups, include toluenesulphonyl (tosyl) and methanesulphonyl (mesyl) groups and benzyl groups such as a *para*-methoxybenzyl (PMB) group. A carboxylic acid group may be protected as an ester for example, as: a C_{1-7} alkyl ester (e.g., a methyl ester; a t-butyl ester); a C_{1-7} haloalkyl ester (e.g., a C_{1-7} trihaloalkyl ester); a tri-C_{1-7} alkylsilyl-C_{1-7}alkyl ester; or a C_{5-20} aryl-C_{1-7} alkyl ester (e.g., a benzyl ester; a nitrobenzyl ester); or as an amide, for example, as a methyl amide. A thiol group may be protected, for example, as a thioether (-SR), for example, as: a benzyl thioether; an acetamidomethyl ether (-S-$CH_2NHC(=O)CH_3$).

Novel chemical intermediates (for example novel compounds of the formulae (XIII), (XIV), (XV) and (XVI)) used in the processes set forth above and in the examples represent a further aspect of the invention.

Methods of Purification

The compounds may be isolated and purified by a number of methods well known to those skilled in the art and examples of such methods include chromatographic techniques such as column chromatography (e.g. flash chromatography) and HPLC. Preparative LC-MS is a standard and effective method used for the purification of small organic molecules such as the compounds described herein. The methods for the liquid chromatography (LC) and mass spectrometry (MS) can be varied to provide better separation of the crude materials and improved detection of the samples by MS. Optimisation of the preparative gradient LC method will involve varying columns, volatile eluents and modifiers, and gradients. Methods are well known in the art for optimising preparative LC-MS methods and then using them to purify compounds. Such methods are described in Rosentreter U, Huber U.; Optimal fraction collecting in preparative LC/MS; *J Comb Chem.*; 2004; 6(2), 159-64 and Leister W, Strauss K, Wisnoski D, Zhao Z, Lindsley C., Development of a custom high-throughput preparative liquid chromatography/mass spectrometer platform for the preparative purification and analytical analysis of compound libraries; *J Comb Chem.*; 2003; 5(3); 322-9.

One such system for purifying compounds via preparative LC-MS is described in the experimental section below although a person skilled in the art will appreciate that alternative systems and methods to those described could be used. In particular, normal phase preparative LC based methods might be used in place of the reverse phase methods described here. Most preparative LC-MS systems utilise reverse phase LC and volatile acidic modifiers, since the approach is very effective for the purification of small molecules and because the eluents are compatible with positive ion electrospray mass spectrometry. Employing other chromatographic solutions e.g. normal phase LC, alternatively buffered mobile phase, basic modifiers etc as outlined in the analytical methods described above could alternatively be used to purify the compounds.

Pharmaceutical Formulations

While it is possible for the active compound to be administered alone, it is preferable to present it as a pharmaceutical composition (e.g. formulation)

comprising at least one active compound of the invention together with one or more pharmaceutically acceptable carriers, adjuvants, excipients, diluents, fillers, buffers, stabilisers, preservatives, lubricants, or other materials well known to those skilled in the art and optionally other therapeutic or prophylactic agents; for example agents that reduce or alleviate some of the side effects associated with chemotherapy. Particular examples of such agents include anti-emetic agents and agents that prevent or decrease the duration of chemotherapy-associated neutropenia and prevent complications that arise from reduced levels of red blood cells or white blood cells, for example erythropoietin (EPO), granulocyte macrophage-colony stimulating factor (GM-CSF), and granulocyte-colony stimulating factor (G-CSF).

Thus, the present invention further provides pharmaceutical compositions, as defined above, and methods of making a pharmaceutical composition comprising admixing at least one active compound, as defined above, together with one or more pharmaceutically acceptable carriers, excipients, buffers, adjuvants, stabilizers, or other materials, as described herein.

The term "pharmaceutically acceptable" as used herein pertains to compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of a subject (e.g. human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, excipient, etc. must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation.

Accordingly, in a further aspect, the invention provides compounds of the formula (I) and sub-groups thereof as defined herein in the form of pharmaceutical compositions.

The pharmaceutical compositions can be in any form suitable for oral, parenteral, topical, intranasal, ophthalmic, otic, rectal, intra-vaginal, or transdermal administration. Where the compositions are intended for parenteral administration,

they can be formulated for intravenous, intramuscular, intraperitoneal, subcutaneous administration or for direct delivery into a target organ or tissue by injection, infusion or other means of delivery. The delivery can be by bolus injection, short term infusion or longer term infusion and can be via passive delivery or through the utilisation of a suitable infusion pump.

Pharmaceutical formulations adapted for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats, co-solvents, organic solvent mixtures, cyclodextrin complexation agents, emulsifying agents (for forming and stabilizing emulsion formulations), liposome components for forming liposomes, gellable polymers for forming polymeric gels, lyophilisation protectants and combinations of agents for, *inter alia*, stabilising the active ingredient in a soluble form and rendering the formulation isotonic with the blood of the intended recipient. Pharmaceutical formulations for parenteral administration may also take the form of aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents (R. G. Strickly, Solubilizing Excipients in oral and injectable formulations, Pharmaceutical Research, Vol 21(2) 2004, p 201-230).

A drug molecule that is ionizable can be solubilized to the desired concentration by pH adjustment if the drug's pK_a is sufficiently away from the formulation pH value. The acceptable range is pH 2-12 for intravenous and intramuscular administration, but subcutaneously the range is pH 2.7-9.0. The solution pH is controlled by either the salt form of the drug, strong acids/bases such as hydrochloric acid or sodium hydroxide, or by solutions of buffers which include but are not limited to buffering solutions formed from glycine, citrate, acetate, maleate, succinate, histidine, phosphate, tris(hydroxymethyl)aminomethane (TRIS), or carbonate.

The combination of an aqueous solution and a water-soluble organic solvent/surfactant (i.e., a cosolvent) is often used in injectable formulations. The water-soluble organic solvents and surfactants used in injectable formulations include but are not limited to propylene glycol, ethanol, polyethylene glycol 300, polyethylene glycol 400, glycerin, dimethylacetamide (DMA), N-methyl-2-pyrrolidone (NMP; Pharmasolve),

dimethylsulphoxide (DMSO), Solutol HS 15, Cremophor EL, Cremophor RH 60, and polysorbate 80. Such formulations can usually be, but are not always, diluted prior to injection.

Propylene glycol, PEG 300, ethanol, Cremophor EL, Cremophor RH 60, and polysorbate 80 are the entirely organic water-miscible solvents and surfactants used in commercially available injectable formulations and can be used in combinations with each other. The resulting organic formulations are usually diluted at least 2-fold prior to IV bolus or IV infusion.

Alternatively increased water solubility can be achieved through molecular complexation with cyclodextrins

Liposomes are closed spherical vesicles composed of outer lipid bilayer membranes and an inner aqueous core and with an overall diameter of <100 μm. Depending on the level of hydrophobicity, moderately hydrophobic drugs can be solubilized by liposomes if the drug becomes encapsulated or intercalated within the liposome. Hydrophobic drugs can also be solubilized by liposomes if the drug molecule becomes an integral part of the lipid bilayer membrane, and in this case, the hydrophobic drug is dissolved in the lipid portion of the lipid bilayer. A typical liposome formulation contains water with phospholipid at ~5-20 mg/ml, an isotonicifier, a pH 5-8 buffer, and optionally cholesterol.

The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use.

The pharmaceutical formulation can be prepared by lyophilising a compound of Formula (I) or acid addition salt thereof. Lyophilisation refers to the procedure of freeze-drying a composition. Freeze-drying and lyophilisation are therefore used herein as synonyms. A typical process is to solubilise the compound and the resulting formulation is clarified, sterile filtered and aseptically transferred to

containers appropriate for lyophilisation (e.g. vials). In the case of vials, they are partially stoppered with lyo-stoppers. The formulation can be cooled to freezing and subjected to lyophilisation under standard conditions and then hermetically capped forming a stable, dry lyophile formulation. The composition will typically have a low residual water content, e.g. less than 5% e.g. less than 1% by weight based on weight of the lyophile.

The lyophilisation formulation may contain other excipients for example, thickening agents, dispersing agents, buffers, antioxidants, preservatives, and tonicity adjusters. Typical buffers include phosphate, acetate, citrate and glycine. Examples of antioxidants include ascorbic acid, sodium bisulphite, sodium metabisulphite, monothioglycerol, thiourea, butylated hydroxytoluene, butylated hydroxyl anisole, and ethylenediamietetraacetic acid salts. Preservatives may include benzoic acid and its salts, sorbic acid and its salts, alkyl esters of *para*-hydroxybenzoic acid, phenol, chlorobutanol, benzyl alcohol, thimerosal, benzalkonium chloride and cetylpyridinium chloride. The buffers mentioned previously, as well as dextrose and sodium chloride, can be used for tonicity adjustment if necessary.

Bulking agents are generally used in lyophilisation technology for facilitating the process and/or providing bulk and/or mechanical integrity to the lyophilized cake. Bulking agent means a freely water soluble, solid particulate diluent that when co-lyophilised with the compound or salt thereof, provides a physically stable lyophilized cake, a more optimal freeze-drying process and rapid and complete reconstitution. The bulking agent may also be utilised to make the solution isotonic.

The water-soluble bulking agent can be any of the pharmaceutically acceptable inert solid materials typically used for lyophilisation. Such bulking agents include, for example, sugars such as glucose, maltose, sucrose, and lactose; polyalcohols such as sorbitol or mannitol; amino acids such as glycine; polymers such as polyvinylpyrrolidine; and polysaccharides such as dextran.

The ratio of the weight of the bulking agent to the weight of active compound is typically within the range from about 1 to about 5, for example of about 1 to about 3, e.g. in the range of about 1 to 2.

Alternatively they can be provided in a solution form which may be concentrated and sealed in a suitable vial. Sterilisation of dosage forms may be via filtration or by autoclaving of the vials and their contents at appropriate stages of the formulation process. The supplied formulation may require further dilution or preparation before delivery for example dilution into suitable sterile infusion packs.

Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

In one preferred embodiment of the invention, the pharmaceutical composition is in a form suitable for i.v. administration, for example by injection or infusion.

Pharmaceutical compositions of the present invention for parenteral injection can also comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions as well as sterile powders for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), carboxymethylcellulose and suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

The compositions of the present invention may also contain adjuvants such as preservatives, wetting agents, emulsifying agents, and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents such as sugars, sodium chloride, and the like. Prolonged absorption of the injectable

pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

If a compound is not stable in aqueous media or has low solubility in aqueous media, it can be formulated as a concentrate in organic solvents. The concentrate can then be diluted to a lower concentration in an aqueous system, and can be sufficiently stable for the short period of time during dosing. Therefore in another aspect, there is provided a pharmaceutical composition comprising a non aqueous solution composed entirely of one or more organic solvents, which can be dosed as is or more commonly diluted with a suitable IV excipient (saline, dextrose; buffered or not buffered) before administration (Solubilizing excipients in oral and injectable formulations, Pharmaceutical Research, 21(2), 2004, p201-230). Examples of solvents and surfactants are propylene glycol, PEG300, PEG400, ethanol, dimethylacetamide (DMA), N-methyl-2-pyrrolidone (NMP, Pharmasolve), Glycerin, Cremophor EL, Cremophor RH 60 and polysorbate. Particular non aqueous solutions are composed of 70-80% propylene glycol, and 20-30% ethanol. One particular non aqueous solution is composed of 70% propylene glycol, and 30% ethanol. Another is 80% propylene glycol and 20% ethanol.Normally these solvents are used in combination and usually diluted at least 2-fold before IV bolus or IV infusion. The typical amounts for bolus IV formulations are ~50% for Glycerin, propylene glycol, PEG300, PEG400, and ~20% for ethanol. The typical amounts for IV infusion formulations are ~15% for Glycerin, 3% for DMA, and ~10% for propylene glycol, PEG300, PEG400 and ethanol.

In one preferred embodiment of the invention, the pharmaceutical composition is in a form suitable for i.v. administration, for example by injection or infusion. For intravenous administration, the solution can be dosed as is, or can be injected into an infusion bag (containing a pharmaceutically acceptable excipient, such as 0.9% saline or 5% dextrose), before administration.

In another preferred embodiment, the pharmaceutical composition is in a form suitable for sub-cutaneous (s.c.) administration.

Pharmaceutical dosage forms suitable for oral administration include tablets, capsules, caplets, pills, lozenges, syrups, solutions, powders, granules, elixirs and suspensions, sublingual tablets, wafers or patches and buccal patches.

Pharmaceutical compositions containing compounds of the formula (I) can be formulated in accordance with known techniques, see for example, Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA, USA.

Thus, tablet compositions can contain a unit dosage of active compound together with an inert diluent or carrier such as a sugar or sugar alcohol, eg; lactose, sucrose, sorbitol or mannitol; and/or a non-sugar derived diluent such as sodium carbonate, calcium phosphate, calcium carbonate, or a cellulose or derivative thereof such as methyl cellulose, ethyl cellulose, hydroxypropyl methyl cellulose, and starches such as corn starch. Tablets may also contain such standard ingredients as binding and granulating agents such as polyvinylpyrrolidone, disintegrants (e.g. swellable crosslinked polymers such as crosslinked carboxymethylcellulose), lubricating agents (e.g. stearates), preservatives (e.g. parabens), antioxidants (e.g. BHT), buffering agents (for example phosphate or citrate buffers), and effervescent agents such as citrate/bicarbonate mixtures. Such excipients are well known and do not need to be discussed in detail here.

Capsule formulations may be of the hard gelatin or soft gelatin variety and can contain the active component in solid, semi-solid, or liquid form. Gelatin capsules can be formed from animal gelatin or synthetic or plant derived equivalents thereof.

The solid dosage forms (eg; tablets, capsules etc.) can be coated or un-coated, but typically have a coating, for example a protective film coating (e.g. a wax or varnish) or a release controlling coating. The coating (e.g. a Eudragit ™ type polymer) can be designed to release the active component at a desired location within the gastro-intestinal tract. Thus, the coating can be selected so as to degrade under certain pH conditions within the gastrointestinal tract, thereby selectively release the compound in the stomach or in the ileum or duodenum.

Instead of, or in addition to, a coating, the drug can be presented in a solid matrix comprising a release controlling agent, for example a release delaying agent which may be adapted to selectively release the compound under conditions of varying acidity or alkalinity in the gastrointestinal tract. Alternatively, the matrix material or release retarding coating can take the form of an erodible polymer (e.g. a maleic anhydride polymer) which is substantially continuously eroded as the dosage form passes through the gastrointestinal tract. As a further alternative, the active compound can be formulated in a delivery system that provides osmotic control of the release of the compound. Osmotic release and other delayed release or sustained release formulations may be prepared in accordance with methods well known to those skilled in the art.

The pharmaceutical compositions comprise from approximately 1% to approximately 95%, preferably from approximately 20% to approximately 90%, active ingredient. Pharmaceutical compositions according to the invention may be, for example, in unit dose form, such as in the form of ampoules, vials, suppositories, dragées, tablets or capsules.

Pharmaceutical compositions for oral administration can be obtained by combining the active ingredient with solid carriers, if desired granulating a resulting mixture, and processing the mixture, if desired or necessary, after the addition of appropriate excipients, into tablets, dragee cores or capsules. It is also possible for them to be incorporated into plastics carriers that allow the active ingredients to diffuse or be released in measured amounts.

The compounds of the invention can also be formulated as solid dispersions. Solid dispersions are homogeneous extremely fine disperse phases of two or more solids. Solid solutions (molecularly disperse systems), one type of solid dispersion, are well known for use in pharmaceutical technology (see (Chiou and Riegelman, J. Pharm. Sci., 60, 1281-1300 (1971)) and are useful in increasing dissolution rates and increasing the bioavailability of poorly water-soluble drugs.

Solid dispersions of drugs are generally produced by melt or solvent evaporation methods. For melt processing, the materials (excipients) which are usually semisolid and waxy in nature, are heated to cause melting and dissolution of the drug substance, followed by hardening by cooling to very low temperatures. The solid dispersion can then be pulverized, sieved, mixed with excipients, and encapsulated into hard gelatin capsules or compressed into tablets. Alternatively the use of surface-active and self-emulsifying carriers allows the encapsulation of solid dispersions directly into hard gelatin capsules as melts. Solid plugs are formed inside the capsules when the melts are cooled to room temperature.

Solid solutions can also be manufactured by dissolving the drug and the required excipient in either an aqueous solution or a pharmaceutically acceptable organic solvent, followed by removal of the solvent, using a pharmaceutically acceptable method, such as spray drying. The resulting solid can be particle sized if required, optionally mixed with exipients and either made into tablets or filled into capsules.

A particularly suitable polymeric auxiliary for producing such solid dispersions or solid solutions is polyvinylpyrrolidone (PVP).

The present invention provides a pharmaceutical composition comprising a substantially amorphous solid solution, said solid solution comprising

(a) a compound of the formula (I), for example the compound of Example 1; and

(b) a polymer selected from the group consisting of:

polyvinylpyrrolidone (povidone), crosslinked polyvinylpyrrolidone (crospovidone), hydroxypropyl methylcellulose, hydroxypropylcellulose, polyethylene oxide, gelatin, crosslinked polyacrylic acid (carbomer), carboxymethylcellulose, crosslinked carboxymethylcellulose (croscarmellose), methylcellulose, methacrylic acid copolymer, methacrylate copolymer, and water soluble salts such as sodium and ammonium salts of methacrylic acid and methacrylate copolymers, cellulose acetate phthalate, hydroxypropylmethylcellulose phthalate and propylene glycol alginate;

wherein the ratio of said compound to said polymer is about 1:1 to about 1:6, for example a 1:3 ratio, spray dried from a mixture of one of chloroform or dichloromethane and one of methanol or ethanol, preferably dichloromethane/ethanol in a 1:1 ratio.

This invention also provides solid dosage forms comprising the solid solution described above. Solid dosage forms include tablets, capsules and chewable tablets. Known excipients can be blended with the solid solution to provide the desired dosage form. For example, a capsule can contain the solid solution blended with (a) a disintegrant and a lubricant, or (b) a disintegrant, a lubricant and a surfactant. A tablet can contain the solid solution blended with at least one disintegrant, a lubricant, a surfactant, and a glidant. The chewable tablet can contain the solid solution blended with a bulking agent, a lubricant, and if desired an additional sweetening agent (such as an artificial sweetener), and suitable flavours.

The pharmaceutical formulations may be presented to a patient in "patient packs" containing an entire course of treatment in a single package, usually a blister pack. Patient packs have an advantage over traditional prescriptions, where a pharmacist divides a patient's supply of a pharmaceutical from a bulk supply, in that the patient always has access to the package insert contained in the patient pack, normally missing in patient prescriptions. The inclusion of a package insert has been shown to improve patient compliance with the physician's instructions.

Compositions for topical use include ointments, creams, sprays, patches, gels, liquid drops and inserts (for example intraocular inserts). Such compositions can be formulated in accordance with known methods.

Compositions for parenteral administration are typically presented as sterile aqueous or oily solutions or fine suspensions, or may be provided in finely divided sterile powder form for making up extemporaneously with sterile water for injection.

Examples of formulations for rectal or intra-vaginal administration include pessaries and suppositories which may be, for example, formed from a shaped moldable or waxy material containing the active compound.

Compositions for administration by inhalation may take the form of inhalable powder compositions or liquid or powder sprays, and can be administrated in standard form using powder inhaler devices or aerosol dispensing devices. Such devices are well known. For administration by inhalation, the powdered formulations typically comprise the active compound together with an inert solid powdered diluent such as lactose.

The compounds of the formula (I) will generally be presented in unit dosage form and, as such, will typically contain sufficient compound to provide a desired level of biological activity. For example, a formulation may contain from 1 nanogram to 2 grams of active ingredient, e.g. from 1 nanogram to 2 milligrams of active ingredient. Within this range, particular sub-ranges of compound are 0.1 milligrams to 2 grams of active ingredient (more usually from 10 milligrams to 1 gram, e.g. 50 milligrams to 500 milligrams), or 1 microgram to 20 milligrams (for example 1 microgram to 10 milligrams, e.g. 0.1 milligrams to 2 milligrams of active ingredient).

For oral compositions, a unit dosage form may contain from 1 milligram to 2 grams, more typically 10 milligrams to 1 gram, for example 50 milligrams to 1 gram, e.g. 100 miligrams to 1 gram, of active compound.

The active compound will be administered to a patient in need thereof (for example a human or animal patient) in an amount sufficient to achieve the desired therapeutic effect.

Methods of Treatment

It is envisaged that the compounds of the formula (I) and sub-groups as defined herein will be useful in the prophylaxis or treatment of a range of disease states or

conditions mediated by cyclin dependent kinases and glycogen synthase kinase-3. Examples of such disease states and conditions are set out above.

The compounds are generally administered to a subject in need of such administration, for example a human or animal patient, preferably a human.

The compounds will typically be administered in amounts that are therapeutically or prophylactically useful and which generally are non-toxic. However, in certain situations (for example in the case of life threatening diseases), the benefits of administering a compound of the formula (I) may outweigh the disadvantages of any toxic effects or side effects, in which case it may be considered desirable to administer compounds in amounts that are associated with a degree of toxicity.

The compounds may be administered over a prolonged term to maintain beneficial therapeutic effects or may be administered for a short period only. Alternatively they may be administered in a pulsatile or continuous manner.

A typical daily dose of the compound of formula (I) can be in the range from 100 picograms to 100 milligrams per kilogram of body weight, more typically 5 nanograms to 25 milligrams per kilogram of bodyweight, and more usually 10 nanograms to 15 milligrams per kilogram (e.g. 10 nanograms to 10 milligrams, and more typically 1 microgram per kilogram to 20 milligrams per kilogram, for example 1 microgram to 10 milligrams per kilogram) per kilogram of bodyweight although higher or lower doses may be administered where required. The compound of the formula (I) can be administered on a daily basis or on a repeat basis every 2, or 3, or 4, or 5, or 6, or 7, or 10 or 14, or 21, or 28 days for example.

The compounds of the invention may be administered orally in a range of doses, for example 1 to 1500 mg, 2 to 800 mg, or 5 to 500 mg, e.g. 2 to 200 mg or 10 to 1000 mg, particular examples of doses including 10, 20, 50 and 80 mg. The compound may be administered once or more than once each day. The compound can be administered continuously (i.e. taken every day without a break for the duration of the treatment regimen). Alternatively, the compound can be administered intermittently, i.e. taken continuously for a given period such as a week, then

discontinued for a period such as a week and then taken continuously for another period such as a week and so on throughout the duration of the treatment regimen. Examples of treatment regimens involving intermittent administration include regimens wherein administration is in cycles of one week on, one week off; or two weeks on, one week off; or three weeks on, one week off; or two weeks on, two weeks off; or four weeks on two weeks off; or one week on three weeks off - for one or more cycles, e.g. 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more cycles.

An example of a dosage for i.v administration for a 60 kilogram person comprises administering a compound of the formula (I) as defined herein at a starting dosage of 4.5-10.8 mg/60 kg/day (equivalent to 75-180 μg/kg/day) and subsequently by an efficacious dose of 44-97 mg/60 kg/day (equivalent to 0.7-1.6 mg/kg/day) or an efficacious dose of 72-274 mg/60 kg/day (equivalent to 1.2-4.6 mg/kg/day) although higher or lower doses may be administered where required. The mg/kg dose would scale pro-rata for any given body weight.

In one particular dosing schedule, a patient will be given an infusion of a compound of the formula (I) for periods of one hour daily for up to ten days in particular up to five days for one week, and the treatment repeated at a desired interval such as two to four weeks, in particular every three weeks.

More particularly, a patient may be given an infusion of a compound of the formula (I) for periods of one hour daily for 5 days and the treatment repeated every three weeks.

In another particular dosing schedule, a patient is given an infusion over 30 minutes to 1 hour followed by maintenance infusions of variable duration, for example 1 to 5 hours, e.g. 3 hours.

In a further particular dosing schedule, a patient is given a continuous infusion for a period of 12 hours to 5 days, an in particular a continuous infusion of 24 hours to 72 hours.

Ultimately, however, the quantity of compound administered and the type of composition used will be commensurate with the nature of the disease or physiological condition being treated and will be at the discretion of the physician.

The compounds of formula (I) and sub-groups as defined herein can be administered as the sole therapeutic agent or they can be administered in combination therapy with one of more other compounds for treatment of a particular disease state, for example a neoplastic disease such as a cancer as hereinbefore defined. Examples of other therapeutic agents or therapies that may be administered or used together (whether concurrently or at different time intervals) with the compounds of the invention include but are not limited to topoisomerase inhibitors, alkylating agents, antimetabolites, DNA binders, microtubule inhibitors (tubulin targeting agents), monoclonal antibodies and signal transduction inhibitors, particular examples being cisplatin, cyclophosphamide, doxorubicin, irinotecan, fludarabine, 5FU, taxanes, mitomycin C and radiotherapy.

For the case of CDK inhibitors combined with other therapies, the two or more treatments may be given in individually varying dose schedules and via different routes.

Where the compound of the formula (I) is administered in combination therapy with one, two, three, four or more other therapeutic agents (preferably one or two, more preferably one), the compounds can be administered simultaneously or sequentially. When administered sequentially, they can be administered at closely spaced intervals (for example over a period of 5-10 minutes) or at longer intervals (for example 1, 2, 3, 4 or more hours apart, or even longer periods apart where required), the precise dosage regimen being commensurate with the properties of the therapeutic agent(s).

The compounds of the invention may also be administered in conjunction with non-chemotherapeutic treatments such as radiotherapy, photodynamic therapy, gene therapy; surgery and controlled diets.

For use in combination therapy with another chemotherapeutic agent, the compound of the formula (I) and one, two, three, four or more other therapeutic agents can be, for example, formulated together in a dosage form containing two, three, four or more therapeutic agents. In an alternative, the individual therapeutic agents may be formulated separately and presented together in the form of a kit, optionally with instructions for their use.

A person skilled in the art would know through his or her common general knowledge the dosing regimes and combination therapies to use.

Methods of Diagnosis

Prior to administration of a compound of the formula (I), a patient may be screened to determine whether a disease or condition from which the patient is or may be suffering is one which would be susceptible to treatment with a compound having activity against cyclin dependent kinases.

For example, a biological sample taken from a patient may be analysed to determine whether a condition or disease, such as cancer, that the patient is or may be suffering from is one which is characterised by a genetic abnormality or abnormal protein expression which leads to over-activation of CDKs or to sensitisation of a pathway to normal CDK activity. Examples of such abnormalities that result in activation or sensitisation of the CDK2 signal include up-regulation of cyclin E, (Harwell RM, Mull BB, Porter DC, Keyomarsi K.; J Biol Chem. 2004 Mar 26;279(13):12695-705) or loss of p21 or p27, or presence of CDC4 variants (Rajagopalan H, Jallepalli PV, Rago C, Velculescu VE, Kinzler KW, Vogelstein B, Lengauer C.; Nature. 2004 Mar 4;428(6978):77-81). Tumours with mutants of CDC4 or up-regulation, in particular over-expression, of cyclin E or loss of p21 or p27 may be particularly sensitive to CDK inhibitors. The term up-regulation includes elevated expression or over-expression, including gene amplification (i.e. multiple gene copies) and increased expression by a transcriptional effect, and hyperactivity and activation, including activation by mutations.

Thus, the patient may be subjected to a diagnostic test to detect a marker characteristic of up-regulation of cyclin E, or loss of p21 or p27, or presence of CDC4 variants. The term diagnosis includes screening. By marker we include genetic markers including, for example, the measurement of DNA composition to identify mutations of CDC4. The term marker also includes markers which are characteristic of up regulation of cyclin E, including enzyme activity, enzyme levels, enzyme state (e.g. phosphorylated or not) and mRNA levels of the aforementioned proteins. Tumours with upregulation of cyclin E, or loss of p21 or p27 may be particularly sensitive to CDK inhibitors. Tumours may preferentially be screened for upregulation of cyclin E, or loss of p21 or p27 prior to treatment. Thus, the patient may be subjected to a diagnostic test to detect a marker characteristic of up-regulation of cyclin E, or loss of p21 or p27.

The diagnostic tests are typically conducted on a biological sample selected from tumour biopsy samples, blood samples (isolation and enrichment of shed tumour cells), stool biopsies, sputum, chromosome analysis, pleural fluid, peritoneal fluid, or urine.

It has been found, Rajagopalan et al (Nature. 2004 Mar 4;428(6978):77-81), that there were mutations present in CDC4 (also known as Fbw7 or Archipelago) in human colorectal cancers and endometrial cancers (Spruck et al, Cancer Res. 2002 Aug 15;62(16):4535-9). Identification of individual carrying a mutation in CDC4 may mean that the patient would be particularly suitable for treatment with a CDK inhibitor. Tumours may preferentially be screened for presence of a CDC4 variant prior to treatment. The screening process will typically involve direct sequencing, oligonucleotide microarray analysis, or a mutant specific antibody.

Methods of identification and analysis of mutations and up-regulation of proteins are well known to a person skilled in the art. Screening methods could include, but are not limited to, standard methods such as reverse-transcriptase polymerase chain reaction (RT-PCR) or in-situ hybridisation.

In screening by RT-PCR, the level of mRNA in the tumour is assessed by creating a cDNA copy of the mRNA followed by amplification of the cDNA by PCR. Methods of PCR amplification, the selection of primers, and conditions for amplification, are known to a person skilled in the art. Nucleic acid manipulations and PCR are carried out by standard methods, as described for example in Ausubel, F.M. et al., eds. Current Protocols in Molecular Biology, 2004, John Wiley & Sons Inc., or Innis, M.A. et-al., eds. PCR Protocols: a guide to methods and applications, 1990, Academic Press, San Diego. Reactions and manipulations involving nucleic acid techniques are also described in Sambrook et al., 2001, 3rd Ed, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press. Alternatively a commercially available kit for RT-PCR (for example Roche Molecular Biochemicals) may be used, or methodology as set forth in United States patents 4,666,828; 4,683,202; 4,801,531; 5,192,659, 5,272,057, 5,882,864, and 6,218,529 and incorporated herein by reference.

An example of an in-situ hybridisation technique for assessing mRNA expression would be fluorescence in-situ hybridisation (FISH) (see Angerer, 1987 Meth. Enzymol., 152: 649).

Generally, in situ hybridization comprises the following major steps: (1) fixation of tissue to be analyzed; (2) prehybridization treatment of the sample to increase accessibility of target nucleic acid, and to reduce nonspecific binding; (3) hybridization of the mixture of nucleic acids to the nucleic acid in the biological structure or tissue; (4) post-hybridization washes to remove nucleic acid fragments not bound in the hybridization, and (5) detection of the hybridized nucleic acid fragments. The probes used in such applications are typically labeled, for example, with radioisotopes or fluorescent reporters. Preferred probes are sufficiently long, for example, from about 50, 100, or 200 nucleotides to about 1000 or more nucleotides, to enable specific hybridization with the target nucleic acid(s) under stringent conditions. Standard methods for carrying out FISH are described in Ausubel, F.M. et al., eds. Current Protocols in Molecular Biology, 2004, John Wiley & Sons Inc and Fluorescence In Situ Hybridization: Technical Overview by

John M. S. Bartlett in Molecular Diagnosis of Cancer, Methods and Protocols, 2nd ed.; ISBN: 1-59259-760-2; March 2004, pps. 077-088; Series: Methods in Molecular Medicine.

Alternatively, the protein products expressed from the mRNAs may be assayed by immunohistochemistry of tumour samples, solid phase immunoassay with microtiter plates, Western blotting, 2-dimensional SDS-polyacrylamide gel electrophoresis, ELISA, flow cytometry and other methods known in the art for detection of specific proteins. Detection methods would include the use of site specific antibodies. The skilled person will recognize that all such well-known techniques for detection of upregulation of cyclin E, or loss of p21 or p27, or detection of CDC4 variants could be applicable in the present case.

Therefore, all of these techniques could also be used to identify tumours particularly suitable for treatment with the compounds of the invention.

Tumours with mutants of CDC4 or up-regulation, in particular over-expression, of cyclin E or loss of p21 or p27 may be particularly sensitive to CDK inhibitors. Tumours may preferentially be screened for up-regulation, in particular over-expression, of cyclin E (Harwell RM, Mull BB, Porter DC, Keyomarsi K.; J Biol Chem. 2004 Mar 26;279(13):12695-705) or loss of p21 or p27 or for CDC4 variants prior to treatment (Rajagopalan H, Jallepalli PV, Rago C, Velculescu VE, Kinzler KW, Vogelstein B, Lengauer C.; Nature. 2004 Mar 4;428(6978):77-81).

Patients with mantle cell lymphoma (MCL) could be selected for treatment with a compound of the invention using diagnostic tests outlined herein. MCL is a distinct clinicopathologic entity of non-Hodgkin's lymphoma, characterized by proliferation of small to medium-sized lymphocytes with co-expression of CD5 and CD20, an aggressive and incurable clinical course, and frequent t(11;14)(q13;q32) translocation. Over-expression of cyclin D1 mRNA, found in mantle cell lymphoma (MCL), is a critical diagnostic marker. Yatabe et al (Blood. 2000 Apr 1;95(7):2253-61) proposed that cyclin D1-positivity should be included as one of the standard criteria for MCL, and that innovative therapies for this incurable

disease should be explored on the basis of the new criteria. Jones et al (J Mol Diagn. 2004 May;6(2):84-9) developed a real-time, quantitative, reverse transcription PCR assay for cyclin D1 (CCND1) expression to aid in the diagnosis of mantle cell lymphoma (MCL). Howe et al (Clin Chem. 2004 Jan;50(1):80-7) used real-time quantitative RT-PCR to evaluate cyclin D1 mRNA expression and found that quantitative RT-PCR for cyclin D1 mRNA normalized to CD19 mRNA can be used in the diagnosis of MCL in blood, marrow, and tissue. Alternatively, patients with breast cancer could be selected for treatment with a CDK inhibitor using diagnostic tests outline above. Tumour cells commonly overexpress cyclin E and it has been shown that cyclin E is over-expressed in breast cancer (Harwell et al, Cancer Res, 2000, 60, 481-489). Therefore breast cancer may in particular be treated with a CDK inhibitor as provided herein.

Antifungal Use

In a further aspect, the invention provides the use of the compounds of the formula (I) and sub-groups thereof as defined herein as antifungal agents.

The compounds of the formula (I) and sub-groups thereof as defined herein may be used in animal medicine (for example in the treatment of mammals such as humans), or in the treatment of plants (e.g. in agriculture and horticulture), or as general antifungal agents, for example as preservatives and disinfectants.

In one embodiment, the invention provides a compound of the formula (I) and sub-groups thereof as defined herein for use in the prophylaxis or treatment of a fungal infection in a mammal such as a human.

Also provided is the use of a compound of the formula (I) and sub-groups thereof as defined herein for the manufacture of a medicament for use in the prophylaxis or treatment of a fungal infection in a mammal such as a human.

For example, compounds of the invention may be administered to human patients suffering from, or at risk of infection by, topical fungal infections caused by among other organisms, species of Candida, Trichophyton, Microsporum or

Epidermophyton, or in mucosal infections caused by Candida albicans (e.g. thrush and vaginal candidiasis). The compounds of the invention can also be administered for the treatment or prophylaxis of systemic fungal infections caused by, for example, Candida albicans, Cryptococcus neoformans, Aspergillus flavus, Aspergillus fumigatus, Coccidiodies, Paracoccidioides, Histoplasma or Blastomyces.

In another aspect, the invention provides an antifungal composition for agricultural (including horticultural) use, comprising a compound of the formulae (I) and sub-groups thereof as defined herein together with an agriculturally acceptable diluent or carrier.

The invention further provides a method of treating an animal (including a mammal such as a human), plant or seed having a fungal infection, which comprises treating said animal, plant or seed, or the locus of said plant or seed, with an effective amount of a compound of the formula (I) and sub-groups thereof as defined herein.

The invention also provides a method of treating a fungal infection in a plant or seed which comprises treating the plant or seed with an antifungally effective amount of a fungicidal composition containing a compound of the formula (I) and sub-groups thereof as defined herein.

Differential screening assays may be used to select for those compounds of the present invention with specificity for non-human CDK enzymes. Compounds which act specifically on the CDK enzymes of eukaryotic pathogens can be used as anti-fungal or anti-parasitic agents. Inhibitors of the Candida CDK kinase, CKSI, can be used in the treatment of candidiasis. Antifungal agents can be used against infections of the type hereinbefore defined, or opportunistic infections that commonly occur in debilitated and immunosuppressed patients such as patients with leukemias and lymphomas, people who are receiving immunosuppressive therapy, and patients with predisposing conditions such as diabetes mellitus or AIDS, as well as for non-immunosuppressed patients.

Assays described in the art can be used to screen for agents which may be useful for inhibiting at least one fungus implicated in mycosis such as candidiasis, aspergillosis, mucormycosis, blastomycosis, geotrichosis, cryptococcosis, chromoblastomycosis, coccidiodomycosis, conidiosporosis, histoplasmosis, maduromycosis, rhinosporidosis, nocardiosis, para-actinomycosis, penicilliosis, monoliasis, or sporotrichosis. The differential screening assays can be used to identify anti-fungal agents which may have therapeutic value in the treatment of aspergillosis by making use of the CDK genes cloned from yeast such as Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Aspergillus nidulans, or Aspergillus terreus, or where the mycotic infection is mucon-nycosis, the CDK assay can be derived from yeast such as Rhizopus arrhizus, Rhizopus oryzae, Absidia corymbifera, Absidia ramosa, or Mucorpusillus. Sources of other CDK enzymes include the pathogen Pneumocystis carinii.

By way of example, *in vitro* evaluation of the antifungal activity of the compounds can be performed by determining the minimum inhibitory concentration (M.I.C.) which is the concentration of the test compounds, in a suitable medium, at which growth of the particular microorganism fails to occur. In practice, a series of agar plates, each having the test compound incorporated at a particular concentration is inoculated with a standard culture of, for example, Candida albicans and each plate is then incubated for an appropriate period at 37 °C. The plates are then examined for the presence or absence of growth of the fungus and the appropriate M.I.C. value is noted. Alternatively, a turbidity assay in liquid cultures can be performed and a protocol outlining an example of this assay can be found in the Examples below.

The *in vivo* evaluation of the compounds can be carried out at a series of dose levels by intraperitoneal or intravenous injection or by oral administration, to mice that have been inoculated with a fungus, e.g., a strain of Candida albicans or Aspergillus flavus. The activity of the compounds can be assessed by monitoring the growth of the fungal infection in groups of treated and untreated mice (by histology or by retrieving fungi from the infection). The activity may be measured in terms of the

dose level at which the compound provides 50% protection against the lethal effect of the infection (PD_{50}).

For human antifungal use, the compounds of the formula (I) and sub-groups thereof as defined herein can be administered alone or in admixture with a pharmaceutical carrier selected in accordance with the intended route of administration and standard pharmaceutical practice. Thus, for example, they may be administered orally, parenterally, intravenously, intramuscularly or subcutaneously by means of the formulations described above in the section headed "Pharmaceutical Formulations".

For oral and parenteral administration to human patients, the daily dosage level of the antifungal compounds of the invention can be from 0.01 to 10 mg/kg (in divided doses), depending on *inter alia* the potency of the compounds when administered by either the oral or parenteral route. Tablets or capsules of the compounds may contain, for example, from 5 mg to 0.5 g of active compound for administration singly or two or more at a time as appropriate. The physician in any event will determine the actual dosage (effective amount) which will be most suitable for an individual patient and it will vary with the age, weight and response of the particular patient.

Alternatively, the antifungal compounds can be administered in the form of a suppository or pessary, or they may be applied topically in the form of a lotion, solution, cream, ointment or dusting powder. For example, they can be incorporated into a cream consisting of an aqueous emulsion of polyethylene glycols or liquid paraffin; or they can be incorporated, at a concentration between 1 and 10%, into an ointment consisting of a white wax or white soft paraffin base together with such stabilizers and preservatives as may be required.

In addition to the therapeutic uses described above, anti-fungal agents developed with such differential screening assays can be used, for example, as preservatives in foodstuff, feed supplement for promoting weight gain in livestock, or in disinfectant formulations for treatment of non-living matter, e.g., for decontaminating hospital

equipment and rooms. In similar fashion, side by side comparison of inhibition of a mammalian CDK and an insect CDK, such as the Drosophilia CDK5 gene (Hellmich et al. (1994) FEBS Lett 356:317-21), will permit selection amongst the compounds herein of inhibitors which discriminate between the human/mammalian and insect enzymes. Accordingly, the present invention expressly contemplates the use and formulation of the compounds of the invention in insecticides, such as for use in management of insects like the fruit fly.

In yet another embodiment, certain of the subject CDK inhibitors can be selected on the basis of inhibitory specificity for plant CDK's relative to the mammalian enzyme. For example, a plant CDK can be disposed in a differential screen with one or more of the human enzymes to select those compounds of greatest selectivity for inhibiting the plant enzyme. Thus, the present invention specifically contemplates formulations of the subject CDK inhibitors for agricultural applications, such as in the form of a defoliant or the like.

For agricultural and horticultural purposes the compounds of the invention may be used in the form of a composition formulated as appropriate to the particular use and intended purpose. Thus the compounds may be applied in the form of dusting powders, or granules, seed dressings, aqueous solutions, dispersions or emulsions, dips, sprays, aerosols or smokes. Compositions may also be supplied in the form of dispersible powders, granules or grains, or concentrates for dilution prior to use. Such compositions may contain such conventional carriers, diluents or adjuvants as are known and acceptable in agriculture and horticulture and they can be manufactured in accordance with conventional procedures. The compositions may also incorporate other active ingredients, for example, compounds having herbicidal or insecticidal activity or a further fungicide. The compounds and compositions can be applied in a number of ways, for example they can be applied directly to the plant foliage, stems, branches, seeds or roots or to the soil or other growing medium, and they may be used not only to eradicate disease, but also prophylactically to protect the plants or seeds from attack. By way of example, the compositions may contain from 0.01 to 1 wt. % of the active ingredient. For field

use, likely application rates of the active ingredient may be from 50 to 5000 g/hectare.

The invention also contemplates the use of the compounds of the formula (I) and sub-groups thereof as defined herein in the control of wood decaying fungi and in the treatment of soil where plants grow, paddy fields for seedlings, or water for perfusion. Also contemplated by the invention is the use of the compounds of the formula (I) and sub-groups thereof as defined herein to protect stored grain and other non-plant loci from fungal infestation.

EXAMPLES

The invention will now be illustrated, but not limited, by reference to the specific embodiments described in the following examples.

In the examples, the following abbreviations are used.

AcOH	acetic acid
BOC	*tert*-butyloxycarbonyl
CDI	1,1-carbonyldiimidazole
DMAW90	Solvent mixture: DCM: MeOH, AcOH, H_2O (90:18:3:2)
DMAW120	Solvent mixture: DCM: MeOH, AcOH, H_2O (120:18:3:2)
DMAW240	Solvent mixture: DCM: MeOH, AcOH, H_2O (240:20:3:2)
DCM	dichloromethane
DMF	dimethylformamide
DMSO	dimethyl sulphoxide
EDC	1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide
Et_3N	triethylamine
EtOAc	ethyl acetate
Et_2O	diethyl ether
HOAt	1-hydroxyazabenzotriazole
HOBt	1-hydroxybenzotriazole
MeCN	acetonitrile
MeOH	methanol

P.E.	petroleum ether
SiO_2	silica
TBTU	N,N,N',N'-tetramethyl-O-(benzotriazol-1-yl)uronium tetrafluoroborate
THF	tetrahydrofuran

Analytical LC-MS System and Method Description

In the examples, the compounds prepared were characterised by liquid chromatography and mass spectroscopy using the systems and operating conditions set out below. Where atoms with different isotopes are present, and a single mass quoted, the mass quoted for the compound is the monoisotopic mass (i.e. ^{35}Cl; ^{79}Br etc.). Several systems were used, as described below, and these were equipped with, and were set up to run under, closely similar operating conditions. The operating conditions used are also described below.

Waters Platform LC-MS system:

HPLC System:	Waters 2795
Mass Spec Detector:	Micromass Platform LC
PDA Detector:	Waters 2996 PDA

Analytical Acidic conditions:

Eluent A:	H_2O (0.1% Formic Acid)
Eluent B:	CH_3CN (0.1% Formic Acid)
Gradient:	5-95% eluent B over 3.5 minutes
Flow:	0.8 ml/min
Column:	Phenomenex Synergi 4μ MAX-RP 80A, 2.0 x 50 mm

Analytical Long Acidic conditions:

Eluent A:	H_2O (0.1% Formic Acid)
Eluent B:	CH_3CN (0.1% Formic Acid)
Gradient:	05-95% eluent B over 15 minutes
Flow:	0.4 ml/min

Column: Phenomenex Synergi 4μ MAX-RP 80A, 2.0 x 150 mm

Platform MS conditions:

Capillary voltage:	3.6 kV (3.40 kV on ES negative)
Cone voltage:	25 V
Source Temperature:	120 °C
Scan Range:	100-800 amu
Ionisation Mode:	ElectroSpray Positive or
	ElectroSpray Negative or
	ElectroSpray Positive & Negative

Waters Fractionlynx LC-MS system:

HPLC System:	2767 autosampler – 2525 binary gradient pump
Mass Spec Detector:	Waters ZQ
PDA Detector:	Waters 2996 PDA

Analytical Acidic conditions:

Eluent A:	H_2O (0.1% Formic Acid)
Eluent B:	CH_3CN (0.1% Formic Acid)
Gradient:	5-95% eluent B over 4 minutes
Flow:	2.0 ml/min
Column:	Phenomenex Synergi 4μ MAX-RP 80A, 4.6 x 50 mm

Fractionlynx MS conditions:

Capillary voltage:	3.5 kV (3.2 kV on ES negative)
Cone voltage:	25 V (30 V on ES negative)
Source Temperature:	120 °C
Scan Range:	100-800 amu
Ionisation Mode:	ElectroSpray Positive or
	ElectroSpray Negative or
	ElectroSpray Positive & Negative

Mass Directed Purification LC-MS System

Preparative LC-MS is a standard and effective method used for the purification of small organic molecules such as the compounds described herein. The methods for the liquid chromatography (LC) and mass spectrometry (MS) can be varied to provide better separation of the crude materials and improved detection of the samples by MS. Optimisation of the preparative gradient LC method will involve varying columns, volatile eluents and modifiers, and gradients. Methods are well known in the art for optimising preparative LC-MS methods and then using them to purify compounds. Such methods are described in Rosentreter U, Huber U.; Optimal fraction collecting in preparative LC/MS; *J Comb Chem.*; 2004; 6(2), 159-64 and Leister W, Strauss K, Wisnoski D, Zhao Z, Lindsley C., Development of a custom high-throughput preparative liquid chromatography/mass spectrometer platform for the preparative purification and analytical analysis of compound libraries; *J Comb Chem.*; 2003; 5(3); 322-9.

One such system for purifying compounds via preparative LC-MS is described below although a person skilled in the art will appreciate that alternative systems and methods to those described could be used. In particular, normal phase preparative LC based methods might be used in place of the reverse phase methods described here. Most preparative LC-MS systems utilise reverse phase LC and volatile acidic modifiers, since the approach is very effective for the purification of small molecules and because the eluents are compatible with positive ion electrospray mass spectrometry. Employing other chromatographic solutions e.g. normal phase LC, alternatively buffered mobile phase, basic modifiers etc as outlined in the analytical methods described above could alternatively be used to purify the compounds.

Preparative LC-MS Systems:

Waters Fractionlynx System:

- **Hardware:**

2767 Dual Loop Autosampler/Fraction Collector

2525 preparative pump

CFO (column fluidic organiser) for column selection

RMA (Waters reagent manager) as make up pump

Waters ZQ Mass Spectrometer

Waters 2996 Photo Diode Array detector

Waters ZQ Mass Spectrometer

- **Software:**

Masslynx 4.0

- **Waters MS running conditions:**

Capillary voltage:	3.5 kV (3.2 kV on ES Negative)
Cone voltage:	25 V
Source Temperature:	120 °C
Multiplier:	500 V
Scan Range:	125-800 amu
Ionisation Mode:	ElectroSpray Positive or ElectroSpray Negative

Agilent 1100 LC-MS preparative system:

- **Hardware:**

Autosampler: 1100 series "prepALS"

Pump: 1100 series "PrepPump" for preparative flow gradient and 1100 series "QuatPump" for pumping modifier in prep flow

UV detector: 1100 series "MWD" Multi Wavelength Detector

MS detector: 1100 series "LC-MSD VL"

Fraction Collector: 2 x "Prep-FC"

Make Up pump: "Waters RMA"

Agilent Active Splitter

- **Software:**

Chemstation: Chem32

- **Agilent MS running conditions:**

Capillary voltage:	4000 V (3500 V on ES Negative)
Fragmentor/Gain:	150/1
Drying gas flow:	13.0 L/min
Gas Temperature:	350 °C
Nebuliser Pressure:	50 psig
Scan Range:	125-800 amu
Ionisation Mode:	ElectroSpray Positive or ElectroSpray Negative

Chromatographic Conditions :

- **Columns:**

1. Low pH chromatography:

Phenomenex Synergy MAX-RP, 10µ, 100 x 21.2mm

(alternatively used Thermo Hypersil-Keystone HyPurity Aquastar, 5µ, 100 x 21.2mm for more polar compounds)

2. High pH chromatography:

Phenomenex Luna C18 (2), 10µ, 100 x 21.2mm

(alternatively used Phenomenex Gemini, 5µ, 100 x 21.2mm)

- **Eluents:**

1. Low pH chromatography:

Solvent A: H_20 + 0.1% Formic Acid, pH~1.5

Solvent B: CH_3CN + 0.1% Formic Acid

2. High pH chromatography:

Solvent A: H_20 + 10 mM NH_4HCO_3 + NH4OH, pH=9.2

Solvent B: CH_3CN

3. Make up solvent:

MeOH + 0.2% Formic Acid (for both chromatography type)

- **Methods:**

According to the analytical trace the most appropriate preparative chromatography type was chosen. A typical routine was to run an analytical LC-MS using the type of chromatography (low or high pH) most suited for compound structure. Once the analytical trace showed good chromatography a suitable preparative method of the same type was chosen. Typical running condition for both low and high pH chromatography methods were:

Flow rate: 24 ml/min

Gradient: Generally all gradients had an initial 0.4 min step with 95% A + 5% B. Then according to analytical trace a 3.6 min gradient was chosen in order to achieve good separation (e.g. from 5% to 50% B for early retaining compounds; from 35% to 80% B for middle retaining compounds and so on)

Wash: 1.2 minute wash step was performed at the end of the gradient

Re-equilibration: 2.1 minutes re-equilibration step was ran to prepare the system for the next run

Make Up flow rate: 1 ml/min

- **Solvent:**

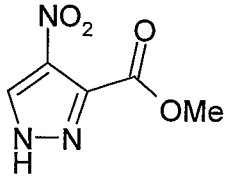
All compounds were usually dissolved in 100% MeOH or 100% DMSO

From the information provided someone skilled in the art could purify the compounds described herein by preparative LC-MS.

The starting materials for each of the Examples are commercially available unless otherwise specified.

EXAMPLE 1

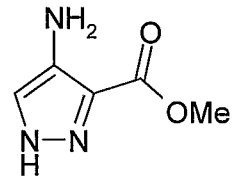
1A. 4-Nitro-1H-pyrazole-3-carboxylic acid methyl ester



Thionyl chloride (2.90 ml, 39.8 mmol) was slowly added to a mixture of 4-nitro-3-pyrazolecarboxylic acid (5.68 g, 36.2 mmol) in MeOH (100 ml) at ambient temperature and the mixture stirred for 48 hours. The mixture was reduced *in vacuo* and dried through azeotrope with toluene to afford 4-nitro-1H-pyrazole-3-carboxylic acid methyl ester as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 14.4 (s, 1H), 8.9 (s, 1H), 3.9 (s, 3H)

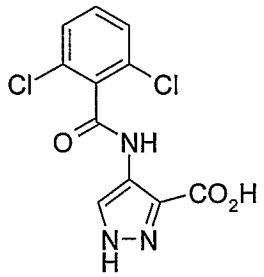
1B. 4-Amino-1H-pyrazole-3-carboxylic acid methyl ester



A mixture of 4-nitro-1H-pyrazole-3-carboxylic acid methyl ester and 10% Pd/C in EtOH was stirred under an atmosphere of hydrogen for 20 hours. The mixture was filtered through a plug of Celite, reduced *in vacuo* and dried through azeotrope with toluene to afford 4-amino-1H-pyrazole-3-carboxylic acid methyl ester.

^{1}H NMR (400 MHz, MeOD) δ 7.2 (s, 1H), 3.9 (s, 3H)

1C. 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid



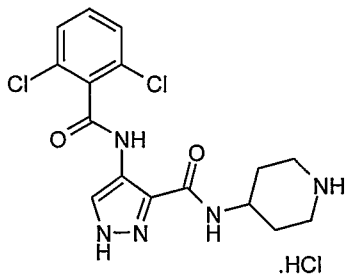
2,6-dichlorobenzoyl chloride (8.2 g; 39.05 mmol) was added cautiously to a solution of 4-amino-1H-pyrazole-3-carboxylic acid methyl ester (5 g; 35.5 mmol) and triethylamine (5.95 ml; 42.6 mmol) in dioxane (50 ml) then stirred at room temperature for 5 hours. The reaction mixture was filtered and the filtrate treated with methanol (50 ml) and 2M sodium hydroxide solution (100 ml), heated at 50 °C for 4 hours, and then evaporated. 100 ml of water was added to the residue then

acidified with concentrated hydrochloric acid. The solid was collected by filtration, washed with water (100 ml) and sucked dry to give 10.05 g of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid as a pale violet solid. (LC/MS: R_t 2.26, $[M+H]^+$ 300 / 302).

1D. 4-{[4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carbonyl]-amino}-piperidine-1-carboxylic acid *tert*-butyl ester

A mixture of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid (6.5 g, 21.6 mmol), 4-amino-1-BOC-piperidine (4.76 g, 23.8 mmol), EDC (5.0 g, 25.9 mmol) and HOBt (3.5 g, 25.9 mmol) in DMF (75 ml) was stirred at room temperature for 20 hours. The reaction mixture was reduced *in vacuo* and the residue partitioned between ethyl acetate (100 ml) and saturated aqueous sodium bicarbonate solution (100 ml). The organic layer was washed with brine, dried ($MgSO_4$) and reduced *in vacuo*. The residue was taken up in 5 % MeOH-DCM (~30 ml). The insoluble material was collected by filtration and, washed with DCM and dried in vacuo to give 4-{[4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carbonyl]-amino}-piperidine-1-carboxylic acid *tert*-butyl ester (5.38 g) as a white solid. The filtrate was reduced *in vacuo* and the residue purified by column chromatography using gradient elution 1:2 EtOAc / hexane to EtOAc to give further 4-{[4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carbonyl]-amino}-piperidine-1-carboxylic acid *tert*-butyl ester (2.54 g) as a white solid.

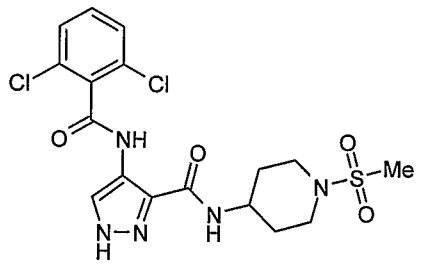
1E. 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide hydrochloride



A solution of 4-{[4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carbonyl]-amino}-piperidine-1-carboxylic acid tert-butyl ester (7.9 g) in MeOH (50 mL) and EtOAc

(50ml) was treated with sat. HCl-EtOAc (40 mL) then stirred at r.t. overnight. The product did not crystallise due to the presence of methanol, and therefore the reaction mixture was evaporated and the residue triturated with EtOAc. The resulting off white solid was collected by filtration, washed with EtOAc and sucked dry on the sinter to give 6.3g of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide as the hydrochloride salt. (LC/MS: R_t 5.89, $[M+H]^+$ 382 / 384).

1F. 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid (1-methanesulphonyl-piperidin-4-yl)-amide

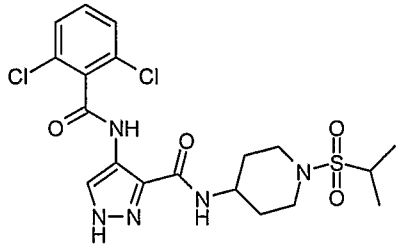


To a mixture of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide hydrochloride (1 mmol) in acetonitrile (10 ml) was added diisopropylethylamine (2.2 mmol) followed by the methanesulphonyl chloride (1 mmol). The mixture was stirred at ambient temperature for 16 hours then reduced *in vacuo*. The residue was partitioned between ethyl acetate and water, the layers separated and the organic portion washed with brine, dried ($MgSO_4$) and reduced *in vacuo* to give the title compound. $[M+H]^+$ 460 R_t 2.67. LC/MS. r.t. 2.67 min; m/z 460.11

^1H NMR: (400 MHz, DMSO-d_6) δ 13.51 (s, 1H), 10.20 (s, 1H), 8.50 (d, J = 8.0 Hz, 1H), 8.41 (s, 1H), 7.66 – 7.56 (m, 3H), 3.95 – 3.89 (m, 1H), 3.61 (d, J = 12.0 Hz, 2H), 2.92 (s, 3H), 2.84 (t, J = 12.0 Hz, 2H), 1.89 – 1.86 (m, 2H), 1.79 – 1.70 (m, 2H)

EXAMPLE 2

4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid (1-isopropyl-sulphonyl-piperidin-4-yl)-amide

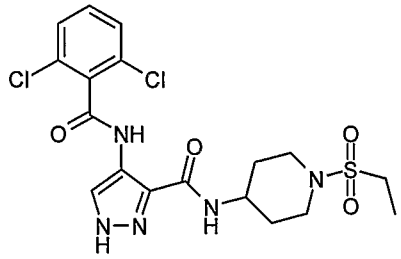


The title compound was prepared by the methods described in Example 1 but using isopropylsulphonyl chloride instead of methanesulphonyl chloride and was purified by preparative LC/MS. r.t. 2.83 min; m/z 488.22

^{1}H NMR: (400 MHz, DMSO-d_6) δ 13.42 (s, 1H), 10.16 (s, 1H), 8.46 (d, J = 8.0 Hz, 1H), 8.35 (s, 1H), 7.60 – 7.51 (m, 3H), 3.92 – 3.87 (m, 1H), 3.65 (d, J = 12.0 Hz, 2H), 3.35 – 3.27 (m, 1H), 2.95 (t, J = 12.0 Hz, 2H), 1.80 – 1.76 (m, 2H), 1.66 – 1.59 (m, 2H), 1.22 (d, J = 8.0 Hz, 6H)

EXAMPLE 3

4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid (1-ethyl-sulphonyl-piperidin-4-yl)-amide

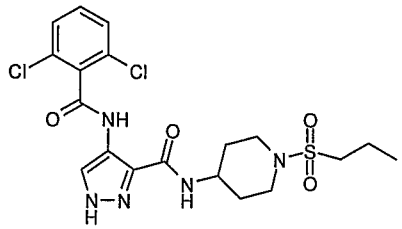


The title compound was prepared by the methods described in Example 1, but using ethylsulphonyl chloride instead of methanesulphonyl chloride, and was purified by column chromatography, eluting with P.E.-EtOAc (1:1 – 0:1). LC/MS. r.t. 2.74 min; m/z 474.17

^{1}H NMR (400 MHz, DMSO-d_6) δ 13.45 (s, 1H), 10.17 (s, 1H), 8.51 (d, J = 8.0 Hz, 1H), 8.37 (s, 1H), 7.60 – 7.51 (m, 3H), 3.91 – 3.85 (m, 1H), 3.61 (d, J = 12.0 Hz, 2H), 3.04 (q, J = 8.0 Hz, 2H), 2.86 (t, J = 12.0 Hz, 2H), 1.80 – 1.78 (m, 2H), 1.69 – 1.60 (m, 2H), 1.21 (t, J = 8.0 Hz, 3H)

EXAMPLE 4

4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid (1-propyl-sulphonyl-piperidin-4-yl)-amide



The title compound was prepared by the methods described in Example 1, but using propanesulphonyl chloride instead of methanesulphonyl chloride, and was purified by preparative LC/MS r.t. 3.11 min; m/z 488.18

^{1}H NMR: (400 MHz, DMSO-d_6) δ 13.42 (s, 1H), 10.15 (s, 1H), 8.46 (d, J = 8.0 Hz, 1H), 8.36 (s, 1H), 7.60 – 7.51 (m, 3H), 3.91 – 3.84 (m, 1H), 3.60 (d, J = 12.0 Hz, 2H), 3.00 (t, J = 8.0 Hz, 2H), 2.85 (t, J = 12.0 Hz, 2H), 1.82 – 1.78 (m, 2H), 1.72 – 1.62 (m, 4H), 0.99 (t, J = 8.0 Hz, 3H)

BIOLOGICAL ACTIVITY

EXAMPLE 5

Measurement of Activated CDK2/CyclinA Kinase Inhibitory Activity Assay (IC_{50})

Compounds of the invention were tested for kinase inhibitory activity using the following protocol.

Activated CDK2/CyclinA (Brown et al, Nat. Cell Biol., 1, pp438-443, 1999; Lowe, E.D., et al Biochemistry, 41, pp15625-15634, 2002) is diluted to 125pM in 2.5X strength assay buffer (50mM MOPS pH 7.2, 62.5 mM β-glycerophosphate, 12.5mM EDTA, 37.5mM $MgCl_2$, 112.5 mM ATP, 2.5 mM DTT, 2.5 mM sodium

orthovanadate, 0.25 mg/ml bovine serum albumin), and 10 µl mixed with 10 µl of histone substrate mix (60 µl bovine histone H1 (Upstate Biotechnology, 5 mg/ml), 940 µl H_2O, 35 µCi γ^{33}P-ATP) and added to 96 well plates along with 5 µl of various dilutions of the test compound in DMSO (up to 2.5%). The reaction is allowed to proceed for 2 to 4 hours before being stopped with an excess of ortho-phosphoric acid (5 µl at 2%). γ^{33}P-ATP which remains unincorporated into the histone H1 is separated from phosphorylated histone H1 on a Millipore MAPH filter plate. The wells of the MAPH plate are wetted with 0.5% orthophosphoric acid, and then the results of the reaction are filtered with a Millipore vacuum filtration unit through the wells. Following filtration, the residue is washed twice with 200 µl of 0.5% orthophosphoric acid. Once the filters have dried, 20 µl of Microscint 20 scintillant is added, and then counted on a Packard Topcount for 30 seconds.

The % inhibition of the CDK2 activity is calculated and plotted in order to determine the concentration of test compound required to inhibit 50% of the CDK2 activity (IC_{50}).

EXAMPLE 6

Measurement of Activated CDK1/CyclinB Kinase Inhibitory Activity Assay (IC_{50})

CDK1/CyclinB assay.is identical to the CDK2/CyclinA above except that CDK1/CyclinB (Upstate Discovery) is used and the enzyme is diluted to 6.25 nM.

Compounds of invention have IC_{50} values less than 20 µM or provide at least 50% inhibition of the CDK2 activity at a concentration of 10 µM. Preferred compounds of invention have IC_{50} values of less than 1 µM in the CDK2 or CDK1 assay.

EXAMPLE 7

GSK3-B Kinase Inhibitory Activity Assay

GSK3-β (Upstate Discovery) are diluted to 7.5nM in 25mM MOPS, pH 7.00, 25mg/ml BSA, 0.0025% Brij-35, 1.25% glycerol, 0.5mM EDTA, 25mM $MgCl_2$, 0.025% β-mercaptoethanol, 37.5mM ATP and and 10 µl mixed with 10 µl of

substrate mix. The substrate mix for GSK3-β is 12.5 μM phospho-glycogen synthase peptide-2 (Upstate Discovery) in 1ml of water with 35 μCi γ^{33}P-ATP. Enzyme and substrate are added to 96 well plates along with 5 μl of various dilutions of the test compound in DMSO (up to 2.5%). The reaction is allowed to proceed for 3 hours (GSK3-β) before being stopped with an excess of ortho-phosphoric acid (5 μl at 2%). The filtration procedure is as for Activated CDK2/CyclinA assay above.

EXAMPLE 8

Anti-proliferative Activity

The anti-proliferative activities of compounds of the invention can be determined by measuring the ability of the compounds to inhibition of cell growth in a number of cell lines. Inhibition of cell growth is measured using the Alamar Blue assay (Nociari, M. M, Shalev, A., Benias, P., Russo, C. *Journal of Immunological Methods* 1998, 213, 157-167). The method is based on the ability of viable cells to reduce resazurin to its fluorescent product resorufin. For each proliferation assay cells are plated onto 96 well plates and allowed to recover for 16 hours prior to the addition of inhibitor compounds for a further 72 hours. At the end of the incubation period 10% (v/v) Alamar Blue is added and incubated for a further 6 hours prior to determination of fluorescent product at 535nM ex / 590nM em. In the case of the non-proliferating cell assay cells are maintained at confluence for 96 hour prior to the addition of inhibitor compounds for a further 72 hours. The number of viable cells is determined by Alamar Blue assay as before. Cell lines can be obtained from the ECACC (European Collection of cell Cultures).

In particular, compounds of the invention were tested against the HCT-116 cell line (ECACC Reference: 91091005) derived from human colon carcinoma.

Many compounds of the invention were found to have IC_{50} values of less than 20 μM in this assay and preferred compounds have IC_{50} values of less than 1 μM.

EXAMPLE 9

Determination of Oral Bioavailability

The oral bioavailability of the compounds of formula (I) may be determined as follows.

The test compound is administered as a solution both I.V. and orally to balb/c mice at the following dose level and dose formulations;

- 1mg/kg IV formulated in 10%DMSO/90% (2-hydroxypropyl)-β-cyclodextrin (25% w/v); and
- 5mg/kg PO formulated in 10% DMSO/20%water/70%PEG200.

At various time points after dosing, blood samples are taken in heparinised tubes and the plasma fraction is collected for analysis. The analysis is undertaken by LC-MS/MS after protein precipitation and the samples are quantified by comparison with a standard calibration line constructed for the test compound. The area under the curve (AUC) is calculated from the plasma level vs time profile by standard methods. The oral bioavailability as a percentage is calculated from the following equation:

$$\frac{AUCpo}{AUCiv} \times \frac{doseIV}{dosePO} \times 100$$

By following this protocol, the compound 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid (1-methanesulphonyl-piperidin-4-yl)-amide, was found to have 40-50% bioavailability when administered to mice by the oral route.

EXAMPLE 10

Xenograph Studies

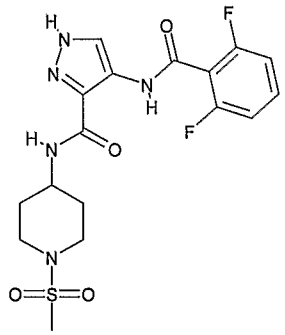
The compound of Example 1 has an anti-tumour action in nude mice engrafted with human tumour derived cell lines. Treatment with the compound of Example 1 causes inhibition of tumour growth in such xenografts implanted sub-cutaneously when dosed orally at doses which cause inhibition of the tumour biomarkers. These biomarkers include suppression of phosphorylation of substrates of the cyclin

dependent kinases e.g. retinoblastoma protien. The compound of Example 1 is effective when given in a range of different schedules including chronic dosing for several weeks.

EXAMPLE 11

COMPARATIVE EXAMPLE

The biological activities of the compound of Example 1, which contains a 2,6-dichlorophenyl group, were compared with the biological activities of its 2,6-difluorophenyl analogue. The 2,6-difluorophenyl analogue, which is described in Example 131 in our earlier application PCT/GB2004/003179 (publication number WO 2005/012256), has the following structure



More particularly, the compounds were compared with regard to their activities against CDK2 kinase and GSK3β kinase and their ability to inhibit the proliferation of HCT-116 human colon cancer cells. The kinase inhibitory activities and the HCT-116 inhibitory activity were determined using the assay methods set out above and the results are shown in the table below.

	Prior Art Compound (Example 131 of PCT/GB2004/003179)	**Compound of Example 1**
CDK2 IC_{50}	0.0022uM	43% @ 0.0003 μM
GSK3β IC_{50}	0.014uM	0.22 μM

HCT-116 cell proliferation IC_{50}	0.74uM	0.11 μM

The compound of Example 1 of the present application has advantages over the compound of its difluoro-analogue for the following reasons:

- The compound of Example 1 has a 6-7-fold more potent anti-proliferative effect on human colon cancer HCT-116 cell line, when compared to its difluoro-analogue.
- The compound of Example 1 has greater in vitro kinase (CDK2) inhibitory activity compared to its difluoro-analogue.
- The compound of Example 1 has lower activity versus GSK3β (0.22 μM) than its difluoro-analogue (0.014 μM).
- The compound of Example 1 has greater selectivity for CDK inhibition over GSK3β (>200-fold) compared to its difluoro-analogue (~6-fold).

PHARMACEUTICAL FORMULATIONS

EXAMPLE 12

(i) Tablet Formulation

A tablet composition containing a compound of the formula (I) is prepared by mixing 50 mg of the compound with 197 mg of lactose (BP) as diluent, and 3 mg magnesium stearate as a lubricant and compressing to form a tablet in known manner.

(ii) Capsule Formulation

A capsule formulation is prepared by mixing 100 mg of a compound of the formula (I) with 100 mg lactose and filling the resulting mixture into standard opaque hard gelatin capsules.

(iii) Injectable Formulation I

A parenteral composition for administration by injection can be prepared by dissolving a compound of the formula (I) (e.g. in a salt form) in water containing 10% propylene glycol to give a concentration of active compound of 1.5 % by weight. The solution is then sterilised by filtration, filled into an ampoule and sealed.

(iv) Injectable Formulation II

A parenteral composition for injection is prepared by dissolving in water a compound of the formula (I) (e.g. in salt form) (2 mg/ml) and mannitol (50 mg/ml), sterile filtering the solution and filling into sealable 1 ml vials or ampoules.

v) Injectable formulation III

A formulation for i.v. delivery by injection or infusion can be prepared by dissolving the compound of formula (I) (e.g. in a salt form) in water at 20 mg/ml. The vial is then sealed and sterilised by autoclaving.

vi) Injectable formulation IV

A formulation for i.v. delivery by injection or infusion can be prepared by dissolving the compound of formula (I) (e.g. in a salt form) in water containing a buffer (e.g. 0.2 M acetate pH 4.6) at 20mg/ml. The vial is then sealed and sterilised by autoclaving.

(vii) Subcutaneous Injection Formulation

A composition for sub-cutaneous administration is prepared by mixing a compound of the formula (I) with pharmaceutical grade corn oil to give a concentration of 5 mg/ml. The composition is sterilised and filled into a suitable container.

viii) Lyophilised formulation

Aliquots of formulated compound of formula (I) are put into 50 mL vials and lyophilized. During lyophilisation, the compositions are frozen using a one-step freezing protocol at (–45 °C). The temperature is raised to –10 °C for annealing, then lowered to freezing at –45 °C, followed by primary drying at +25 °C for

approximately 3400 minutes, followed by a secondary drying with increased steps if temperature to 50 °C. The pressure during primary and secondary drying is set at 80 millitor.

(ix) Solid Solution Formulation

The compound of Example 1 is dissolved in dichloromethane/ethanol (1:1) at a concentration of 5 to 50 % (for example 16 or 20 %) and the solution is spray dried using conditions corresponding to those set out in the table below. The data given in the table include the concentration of the compound of Example 1, the inlet and outlet temperatures of the spray drier, the total yield of spray dried solid, the concentration of the compound of Example 1 in the spray dried solid (assay), and the particle size distribution (P.S.D.) of the particles making up the spray dried solid.

Batch	conc sol. w/vol	temp. inlet	temp. outlet	% yield	assay (mg/g)	PSD (range) (µm)
BR1A	16 %	140 °C	80 °C	87.00	246.41	4.46 - 52.76
BR1B	16 %	180 °C	80 °C	97.00	246.65	14,83 - 91.70
BR2A	20 %	160 °C	80 °C	99.40	239.60	15,86 - 85.01
BR3A	20 %	180 °C	100 °C	79.50	246.64	15,09 - 91.84

The solid solution of the compound of Example 1 and PVP can either be filled directly into hard gelatin or HPMC (hydroxypropylmethyl cellulose) capsules, or be mixed with pharmaceutically acceptable excipients such as bulking agents, glidants or dispersants. The capsules could contain the compound of Example 1 in amounts of between 2 mg and 200 mg, for example 10, 20 and 80 mg.

EXAMPLE 13

Determination of Antifungal Activity

The antifungal activity of the compounds of the formula (I) can be determined using the following protocol.

The compounds are tested against a panel of fungi including Candida parpsilosis, Candida tropicalis, Candida albicans-ATCC 36082 and Cryptococcus neoformans. The test organisms are maintained on Sabourahd Dextrose Agar slants at 4 °C. Singlet suspensions of each organism are prepared by growing the yeast overnight at 27 °C on a rotating drum in yeast-nitrogen base broth (YNB) with amino acids (Difco, Detroit, Mich.), pH 7.0 with 0.05 M morpholine propanesulphonic acid (MOPS). The suspension is then centrifuged and washed twice with 0.85% NaCl before sonicating the washed cell suspension for 4 seconds (Branson Sonifier, model 350, Danbury, Conn.). The singlet blastospores are counted in a haemocytometer and adjusted to the desired concentration in 0.85% NaCl.

The activity of the test compounds is determined using a modification of a broth microdilution technique. Test compounds are diluted in DMSO to a 1.0 mg/ml ratio then diluted to 64 μg/ml in YNB broth, pH 7.0 with MOPS (Fluconazole is used as the control) to provide a working solution of each compound. Using a 96-well plate, wells 1 and 3 through 12 are prepared with YNB broth, ten fold dilutions of the compound solution are made in wells 2 to 11 (concentration ranges are 64 to 0.125 μg/ml). Well 1 serves as a sterility control and blank for the spectrophotometric assays. Well 12 serves as a growth control. The microtitre plates are inoculated with 10 μl in each of well 2 to 11 (final inoculum size is 10^4 organisms/ml). Inoculated plates are incubated for 48 hours at 35 °C. The IC50 values are determined spectrophotometrically by measuring the absorbance at 420 nm (Automatic Microplate Reader, DuPont Instruments, Wilmington, Del.) after agitation of the plates for 2 minutes with a vortex-mixer (Vorte-Genie 2 Mixer, Scientific Industries, Inc., Bolemia, N.Y.). The IC50 endpoint is defined as the lowest drug concentration exhibiting approximately 50% (or more) reduction of the growth compared with the control well. With the turbidity assay this is defined as the lowest drug concentration at which turbidity in the well is <50% of the control (IC50). Minimal Cytolytic Concentrations (MCC) are determined by sub-culturing all wells from the 96-well plate onto a Sabourahd Dextrose Agar (SDA) plate, incubating for 1 to 2 days at 35 °C and then checking viability.

EXAMPLE 14

Protocol for the Biological Evaluation of Control of in vivo Whole Plant Fungal Infection

Compounds of the formula (I) are dissolved in acetone, with subsequent serial dilutions in acetone to obtain a range of desired concentrations. Final treatment volumes are obtained by adding 9 volumes of 0.05% aqueous Tween-20 ™ or 0.01% Triton X-100™, depending upon the pathogen.

The compositions are then used to test the activity of the compounds of the invention against tomato blight (Phytophthora infestans) using the following protocol. Tomatoes (cultivar Rutgers) are grown from seed in a soil-less peat-based potting mixture until the seedlings are 10-20 cm tall. The plants are then sprayed to run-off with the test compound at a rate of 100 ppm. After 24 hours the test plants are inoculated by spraying with an aqueous sporangia suspension of Phytophthora infestans, and kept in a dew chamber overnight. The plants are then transferred to the greenhouse until disease develops on the untreated control plants.

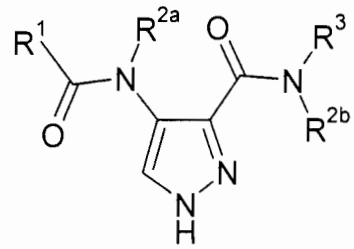
Similar protocols are also used to test the activity of the compounds of the invention in combatting Brown Rust of Wheat (Puccinia), Powdery Mildew of Wheat (Ervsiphe vraminis), Wheat (cultivar Monon), Leaf Blotch of Wheat (Septoria tritici), and Glume Blotch of Wheat (Leptosphaeria nodorum).

Equivalents

The foregoing examples are presented for the purpose of illustrating the invention and should not be construed as imposing any limitation on the scope of the invention. It will readily be apparent that numerous modifications and alterations may be made to the specific embodiments of the invention described above and illustrated in the examples without departing from the principles underlying the invention. All such modifications and alterations are intended to be embraced by this application.

CLAIMS

1. A compound of the formula (I):



(I)

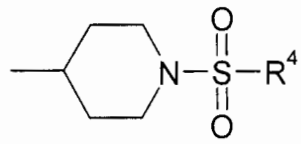
or salts, tautomers, solvates and N-oxides thereof;

wherein:

R^1 is 2,6-dichlorophenyl;

R^{2a} and R^{2b} are both hydrogen;

and R^3 is a group:



where R^4 is C_{1-4} alkyl.

2. A compound according to claim 1 wherein R^4 is C_{1-3} alkyl.

3. A compound according to claim 2 wherein R^4 is methyl.

4. A compound according to claim 2 wherein R^4 is ethyl.

5. A compound according to claim 2 wherein R^4 is n-propyl.

6. A compound according to claim 2 wherein R^4 is isopropyl.

7. A compound according to any one of the preceding claims which is not in the form of a salt or N-oxide.

8. A compound according to any one of claims 1 to 6 in the form of a salt, solvate or N-oxide.

9. A compound according to any one of claims 1 to 8 for use in the prophylaxis or treatment of a disease state or condition mediated by a cyclin dependent kinase or glycogen synthase kinase-3.

10. A compound according to any one of claims 1 to 8 for use in the prophylaxis or treatment of a disease state as described herein.

11. The use of a compound according to any one of claims 1 to 8 for the manufacture of a medicament, wherein the medicament is for any one or more of the uses defined herein.

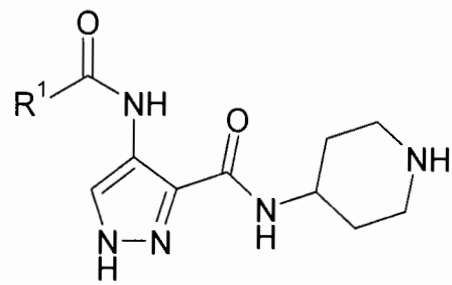
12. A pharmaceutical composition comprising a compound according to any one of claims 1 to 8 and a pharmaceutically acceptable carrier.

13. A pharmaceutical composition comprising a compound according to any one of claims 1 to 8 and a pharmaceutically acceptable carrier in a form suitable for oral administration.

14. A compound according to any one of claims 1 to 8 for use in medicine.

15. A compound according to any one of claims 1 to 8 for any of the uses and methods set forth above, and as described elsewhere herein.

16. A process for the preparation of a compound as defined in any one of claims 1 to 8, which process comprises the reaction of a compound of the formula (XVII):



(XVII)

a sulphonylating agent (for example a sulphonyl chloride such as methanesulphonyl chloride) suitable for introducing the group SO_2R^4.

17. The use of a compound according to any one of claims 1 to 8 for the manufacture of a medicament for the treatment or prophylaxis of a disease state or condition in a patient who has been screened and has been determined as suffering from, or being at risk of suffering from, a disease or condition which would be susceptible to treatment with a compound having activity against cyclin dependent kinase.

18. A compound according to any one of claims 1 to 8 for use in inhibiting tumour growth in a mammal.

19. A compound according to any one of claims 1 to 8 for use in inhibiting the growth of tumour cells (e.g. in a mammal).