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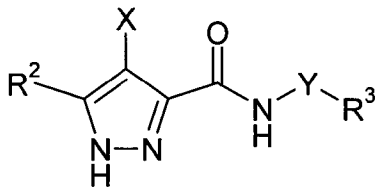

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(57) **Abstract:** The invention provides a combination comprising an ancillary compound and a compound having the formula (0): or salts or tautomers or N-oxides or solvates thereof; wherein X is a group R^1-A-NR^4- or a 5- or 6-membered carbocyclic or heterocyclic ring; A is a bond, SO_2, C=O, NR^9(C=O) or 0(C=O) wherein R^9 is hydrogen or C_{1-4} hydrocarbyl optionally substituted by hydroxy or C_{1-4} alkoxy; Y is a bond or an alkylene chain of 1, 2 or 3 carbon atoms in length; R^1 is hydrogen; a carbocyclic or heterocyclic group having from 3 to 12 ring members; or a C_{1-8} hydrocarbyl group optionally substituted by one or more substituents selected from halogen, hydroxy, C_{1-4} hydrocarbyloxy, amino, mono- or di-C_{1-4} hydrocarbylamino, and carbocyclic or heterocyclic groups having from 3 to 12 ring members, and wherein 1 or 2 of the carbon atoms of the hydrocarbyl group may optionally be replaced by an atom or group selected from O, S, NH, SO, SO_2; R^2 is hydrogen; halogen; C_{1-4} alkoxy; or a C_{1-4} hydrocarbyl group optionally substituted by halogen, hydroxyl or C_{1-4} alkoxy; R^3 is selected from hydrogen and carbocyclic and heterocyclic groups having from 3 to 12 ring members; and R4 is hydrogen or a C_{1-4} hydrocarbyl group optionally substituted by halogen, hydroxyl or C_{1-4} alkoxy.

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PHARMACEUTICAL COMBINATIONS

This invention relates to combinations of pyrazole compounds that inhibit or modulate the activity of cyclin dependent kinase (CDK) and/or glycogen synthase kinase (GSK, e.g. GSK-3) with one or more ancillary compounds, and to the therapeutic uses of such combinations. Also provided are pharmaceutical compositions containing the combinations.

Background of the Invention

The compounds of Formula (I) and subgroups thereof and the compound 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide and the hydrochloric acid addition salt thereof are disclosed in our earlier International patent application number PCT/GB2004/003179 (Publication No. WO 2005/012256) as being inhibitors of Cyclin Dependent Kinases (CDK kinases) and Glycogen Synthase Kinase-3 (GSK3).

The methanesulphonic acid and acetic acid addition salts of compound 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide and crystals thereof and method of making them are disclosed in our earlier International patent application WO 2006/077426.

The combinations of the invention comprise pyrazole compounds that inhibit or modulate the activity of Cyclin Dependent Kinases (CDK) and/or Glycogen Synthase Kinases (GSK, e.g. GSK-3) and one or more ancillary compounds. The ancillary compounds may themselves exhibit protein kinase modulatory or inhibitory activity and such activity may be quite distinct from that of the pyrazole component of the combinations (as described *infra*). Thus, depending on the identity of the ancillary compound(s) present, the combination as a whole may inhibit or modulate the activity of one or more of a range of different protein kinases, including those described below.

Protein kinases

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, diseases and conditions of the immune system, diseases 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 appears 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. Inhibition 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).

Aurora Kinases

Relatively recently, a new family of serine/threonine kinases known as the Aurora kinases has been discovered that are involved in the G2 and M phases of the cell cycle, and which are important regulators of mitosis.

The precise role of Aurora kinases has yet to be elucidated but that they play a part in mitotic checkpoint control, chromosome dynamics and cytokinesis (Adams *et al.*, T*rends Cell Biol.*, 11: 49-54 (2001). Aurora kinases are located at the centrosomes of interphase cells, at the poles of the bipolar spindle and in the mid-body of the mitotic apparatus.

Three members of the Aurora kinase family have been found in mammals so far (E. A. Nigg, *Nat. Rev. Mol. Cell Biol.* 2: 21-32, (2001)). These are:

- Aurora A (also referred to in the literature as Aurora 2);
- Aurora B (also referred to in the literature as Aurora 1); and
- Aurora C (also referred to in the literature as Aurora 3).

The Aurora kinases have highly homologous catalytic domains but differ considerably in their N-terminal portions (Katayama H, Brinkley WR, Sen S.; The Aurora kinases: role in cell transformation and tumorigenesis; Cancer Metastasis Rev. 2003 Dec;22(4):451-64).

The substrates of the Aurora kinases A and B have been identified as including a kinesin-like motor protein, spindle apparatus proteins, histone H3 protein, kinetochore protein and the tumour suppressor protein p53.

Aurora A kinases are believed to be involved in spindle formation and become localised on the centrosome during the early G2 phase where they phosphorylate spindle-associated proteins (Prigent *et al.*, *Cell,* 114: 531-535 (2003). Hirota *et al, Cell*, 114:585-598, (2003) found that cells depleted of Aurora A protein kinase were unable to enter mitosis. Furthermore, it has been found (Adams, 2001) that mutation or disruption of the Aurora A gene in various species leads to mitotic abnormalities, including centrosome separation and maturation defects, spindle aberrations and chromosome segregation defects.

The Aurora kinases are generally expressed at a low level in the majority of normal tissues, the exceptions being tissues with a high proportion of dividing cells such as the thymus and testis. However, elevated levels of Aurora kinases have been found in many human cancers (Giet *et al.*, *J. Cell. Sci.*112: 3591-361, (1999) and Katayama (2003). Furthermore, Aurora A kinase maps to the chromosome 20q13 region that has frequently been found to be amplified in many human cancers.

Thus, for example, significant Aurora A over-expression has been detected in human breast, ovarian and pancreatic cancers (see Zhou *et al.*, *Nat. Genet.* 20: 189-193, (1998), Tanaka *et al.*, *Cancer Res.*, 59: 2041-2044, (1999) and Han *et al.*, *cancer Res.*, 62: 2890-2896, (2002).

Moreover, Isola, *American Journal of Pathology* 147,905-911 (1995) has reported that amplification of the Aurora A locus (20ql3) correlates with poor prognosis for patients with node-negative breast cancer.

Amplification and/or over-expression of Aurora-A is observed in human bladder cancers and amplification of Aurora-A is associated with aneuploidy and aggressive clinical behaviour, see Sen *et al.*, *J. Natl.Cancer Inst*, 94: 1320-1329 (2002).

Elevated expression of Aurora-A has been detected in over 50% of colorectal cancers, (see Bischoff *et al.*, *EMBO J.*, 17: 3052-3065, (1998) and Takahashi et al., *Jpn. J. Cancer Res.* , 91: 1007-1014 (2000)) ovarian cancers (see Gritsko *et al. Clin. Cancer Res.*, 9: 1420-1426 (2003), and gastric tumours Sakakura *et al.*, *British Journal of Cancer*, 84: 824-831 (2001).

Tanaka *et al. Cancer Research*, 59: 2041-2044 (1999) found evidence of over-expression of Aurora A in 94% of invasive duct adenocarcinomas of the breast.

High levels of Aurora A kinase have also been found in renal, cervical, neuroblastoma, melanoma, lymphoma, pancreatic and prostate tumour cell lines Bischoff *et al.* (1998), EMBO J., 17: 3052-3065 (1998) ; Kimura et al. J. Biol. Chem., 274: 7334-7340 (1999) ; Zhou et al., Nature Genetics, 20: 189-193 (1998); Li et al., Clin Cancer Res. 9 (3): 991-7 (2003)].

Aurora-B is highly expressed in multiple human tumour cell lines, including leukemic cells [Katayama et al., Gene 244: 1-7)]. Levels of this enzyme increase as a function of Duke's stage in primary colorectal cancers [Katayama et al., J. Natl Cancer Inst., 91: 1160-1162 (1999)].

High levels of Aurora-3 (Aurora-C) have been detected in several tumour cell lines, even though this kinase tends to be restricted to germ cells in normal tissues (see Kimura *et al. Journal of Biological Chemistry*, 274: 7334-7340 (1999)). Over-expression of Aurora-3 in approximately 50% of colorectal cancers has also been reported in the article by Takahashi *et al., Jpn J. Cancer Res.* 91: 1007-1014 (2001)].

Other reports of the role of Aurora kinases in proliferative disorders may be found in Bischoff *et al., Trends in Cell Biology* 9: 454-459 (1999); Giet *et al. Journal of Cell Science*, 112: 3591-3601 (1999) and Dutertre, *et al. Oncogene*, 21: 6175-6183 (2002).

Royce *et al* report that the expression of the Aurora 2 gene (known as STK15 or BTAK) has been noted in approximately one-fourth of primary breast tumours. (Royce ME, Xia W, Sahin AA, Katayama H, Johnston DA, Hortobagyi G, Sen S, Hung MC; STK15/Aurora-A expression in primary breast tumours is correlated with nuclear grade but not with prognosis; *Cancer.* 2004 Jan 1;100(1):12-9).

Endometrial carcinoma (EC) comprises at least two types of cancer: endometrioid carcinomas (EECs) are estrogen-related tumours, which are frequently euploid and have a good prognosis. Nonendometrioid carcinomas (NEECs; serous and clear cell forms) are not estrogen related, are frequently aneuploid, and are clinically aggressive. It has also been found that Aurora was amplified in 55.5% of NEECs but not in any EECs (P <or= 0.001) (Moreno-Bueno G, Sanchez-Estevez C, Cassia R, Rodriguez-Perales S, Diaz-

Uriarte R, Dominguez O, Hardisson D, Andujar M, Prat J, Matias-Guiu X, Cigudosa JC, Palacios *J. Cancer Res.* 2003 Sep 15;63(18):5697-702).

Reichardt *et al* (*Oncol Rep.* 2003 Sep-Oct;10(5):1275-9) have reported that quantitative DNA analysis by PCR to search for Aurora amplification in gliomas revealed that five out of 16 tumours (31%) of different WHO grade (1x grade II, 1x grade III, 3x grade IV) showed DNA amplification of the Aurora 2 gene. It was hypothesized that amplification of the Aurora 2 gene may be a non-random genetic alteration in human gliomas playing a role in the genetic pathways of tumourigenesis.

Results by Hamada *et al* (*Br. J. Haematol.* 2003 May;121(3):439-47) also suggest that Aurora 2 is an effective candidate to indicate not only disease activity but also tumourigenesis of non-Hodgkin's lymphoma. Retardation of tumour cell growth resulting from the restriction of this gene's functions could be a therapeutic approach for non-Hodgkin's lymphoma.

In a study by Gritsko *et al* (*Clin Cancer Res.* 2003 Apr; 9(4):1420-6)), the kinase activity and protein levels of Aurora A were examined in 92 patients with primary ovarian tumours. *In vitro* kinase analyses revealed elevated Aurora A kinase activity in 44 cases (48%). Increased Aurora A protein levels were detected in 52 (57%) specimens. High protein levels of Aurora A correlated well with elevated kinase activity.

Results obtained by Li *et al* (*Clin. Cancer Res.* 2003 Mar; 9(3):991-7) showed that the Aurora A gene is overexpressed in pancreatic tumours and carcinoma cell lines and suggest that overexpression of Aurora A may play a role in pancreatic carcinogenesis.

Similarly, it has been shown that Aurora A gene amplification and associated increased expression of the mitotic kinase it encodes are associated with aneuploidy and aggressive clinical behaviour in human bladder cancer. (*J. Natl. Cancer Inst.* 2002 Sep 4; 94(17):1320-9).

Investigation by several groups (Dutertre S, Prigent C.,Aurora-A overexpression leads to override of the microtubule-kinetochore attachment checkpoint; *Mol. Interv.* 2003 May; 3(3):127-30 and Anand S, Penrhyn-Lowe S, Venkitaraman AR., Aurora-A amplification overrides the mitotic spindle assembly checkpoint, inducing resistance to Taxol, *Cancer Cell.* 2003 Jan;3(1):51-62) suggests that overexpression of Aurora kinase activity is associated with resistance to some current cancer therapies. For example overexpression

of Aurora A in mouse embryo fibroblasts can reduce the sensitivity of these cells to the cytotoxic effects of taxane derivatives. Therefore Aurora kinase inhibitors may find particular use in patients who have developed reistance to existing therapies.

On the basis of work carried out to date, it is apparent that inhibition of Aurora kinases, particularly Aurora kinase A and Aurora kinase B, will prove an effective means of arresting tumour development.

Harrington et al (*Nat Med.* 2004 Mar; 10(3):262-7) have demonstrated that an inhibitor of the Aurora kinases suppresses tumour growth and induces tumour regression *in vivo*. In the study, the Aurora kinase inhibitor blocked cancer cell proliferation, and also triggered cell death in a range of cancer cell lines including leukaemic, colorectal and breast cell lines. In addition, it has shown potential for the treatment of leukemia by inducing apoptosis in leukemia cells. VX-680 potently killed treatment-refractory primary Acute Myelogenous Leukemia (AML) cells from patients (Andrews, *Oncogene*, 2005, 24, 5005-5015).

Recent reports indicate that Aurora kinases A and B are overexpressed in human leukaemia cells and that a small molecule Aurora kinase inhibitor is active against the growth of primary acute myeloid cells in vitro (Harrington *et al*, 2004). Moreover it has recently been reported that the product of the PML gene that is disrupted in acute promyelocytic leukaemia by a t(15:17) translocation (PML3), interacts with Aurora A and suppresses its kinase activity. Further evidence is emerging that PML is a tumor suppressor and that its disruption is not limited to leukaemias but may also be common in lymphomas and some solid tumors (Xu *et al*, *Molecular Cell* 17: 721–732, 2005).

Cancers which may be particularly amenable to Aurora inhibitors include breast, bladder, colorectal, pancreatic, ovarian, non-Hodgkin's lymphoma, gliomas and nonendometrioid endometrial carcinomas. Leukemias particularly amenable to Aurora inhibitors include Acute Myelogenous Leukemia (AML), chronic myelogenous leukaemia (CML), B-cell lymphoma (Mantle cell), and Acute Lymphoblastic Leukemia (ALL). Further leukemias include acute promyelocytic leukaemia.

C-Abl

A chromosomal translocation event which fuses a BCR encoded sequence to a truncated c-abl gene greatly increases c-abl's tyrosine kinase activity and is the transforming agent in

95% of all Chronic Myeloid Leukaemia (CML) patients. This translocation occurs between chromosomes 9 and 22 resulting in an altered chromosome 22, the Philadelphia (Ph+) chromosome, which can be distinguished by cytogenetic methods. The fusion of BCR and Abl gene sequences results in the oligomerization of the Bcr-Abl gene product, increased trans-autophosphorylation and activation. An auto-inhibitory domain of the c-abl protein is also deleted as a result of the gene fusion. The sub-cellular localization of c-abl is also affected as a result of the gene fusion. The oncogenic effects of Bcr-Abl are complicated, but are believed to involve induction of G1 to S phase transition through activation of Ras, Erk and Jun pathways. Bcr-Abl also affects cell survival through the PI3K/Akt pathway. The oncogenic effects of Bcr-Abl have been demonstrated in animal models which indicate that the Bcr-Abl protein is able to establish CML symptoms in mice.

CML is a fatal disease, which progresses through three stages: chronic phase, accelerated phase, and blast crisis. CML is characterized in early stages by the proliferation of terminally differentiated neutrophils. As the disease progresses an excessive number of myeloid or lymphoid progenitor cells are produced. This chronic phase of the disease may last for years before advancing to an acute blast stage, characterized by multiple additional genetic mutations. CML primarily affects adults who have a mean survival of 5 years after the disease is manifested. CML has been successfully treated in early phases by an ATP competitive inhibitor of c-abl, imatinib (Gleevec). A 95% remission rate was demonstrated for this drug in a phase I clinical trial. Durable responses to imatinib have been observed for CML patients in the chronic phase, however remissions in blast phase only last 2-6 months. Unfortunately the development of acquired resistance to imatinib in CML patients is estimated to be as high as 15% /year.

Kinase domain mutations in BCR-ABL represent the most common mechanism of acquired resistance to imatinib, occurring in 50%–90% of cases. The most common cause of imatinib resistance is through the development of point mutations in the c-abl kinase domain, which directly or indirectly affect imatinib binding. More than 25 distinct Abl kinase domain mutations have been identified in imatinib treated CML patients and are associated with clinical resistance to imatinib (Hematology Shah 2005 (1): 183). These mutations have varying degrees of sensitivity to imatinib. Imatinib has been shown to bind to the ABL kinase domain in the inactive, or closed, conformation and to induce a variety of conformational changes to the protein upon binding. While some resistance-associated mutations occur at amino acid positions implicated in directly contacting imatinib, the majority are felt to prevent the kinase domain from adopting the specific conformation to

which imatinib binds. Studies have shown that some mutations confer only a moderate degree of resistance, and as a result, dose escalation is predicted to recapture responses in some cases. Co-administration of second generation BCR-ABL inhibitors (e.g. BMS354825, AMN-107) have been shown to effectively inhibit many imatinib resistant c-abl mutants. However there are no drugs in the clinic which have been shown to be efficacious against the most imatinib resistant c-abl mutation, T315I.

FMS-Like Tyrosine-kinase 3 (FLT3)

FLT3 (short for fms-like tyrosine-kinase 3) is a class III receptor tyrosine kinase (RTK) structurally related to the receptors for platelet derived growth factor (PDGF), colony stimulating factor 1 (CSF1), and KIT ligand (KL). FLT3 contains an intracellular tyrosine kinase domain split in two by a specific hydrophilic insertion termed a kinase insert.

FLT3 and its specific ligand FLT3-ligand (FL) plays a role in regulation of haematopoietic progenitor cells and is expressed on haematopoietic cells including CD34-positive bone marrow cells, corresponding to multipotential, myeloid and B-lymphoid progenitor cells, and on monocytic cells.

Activating mutations of FLT3 are one of the most frequent mutations observed in acute myeloid leukaemia. The most frequent mutations are referred to as length mutations (LM) or internal tandem duplications (ITD) and consist of a duplicated sequence or insert belonging to exon 11 and sometimes involving intron 11 and exon 12.

Internal tandem duplications and/or insertions and, rarely, deletions in the FLT3-gene are implicated in 20-25% of all acute myeloid leukemias (AML) and 5-10 % myelodysplastic syndromes (MDS) and some cases with acute lymphoblastic leukemia (ALL).

The mutation of the FLT3 protein causes constitutive activation of the tyrosine kinase activity due to disruption of a negative regulatory domain. This activation results in stimulation of several growth factor dependent pathways including the raf-MEK-ERK pathway and contributes to the growth and survival of the leukaemic cells. Thus inhibition of the kinase activity of FLT3 would be an effective treatment for diseases such as those described above which are dependent upon the FLT3 activity.

3-Phosphoinositide-Dependent protein Kinase-1 (PDK1)

The 3-phosphoinositide-dependent protein kinase-1 (PDK1) plays a key role in regulating the activity of a number of kinases belonging to the AGC subfamily of protein kinases (Alessi, D. et al., Biochem. Soc. Trans, 29, p1-14, 2001). These include protein kinase B (PKB/AKT), p70 ribosomal S6 kinase (S6K) (Avruch, J. et al., Prog. Mol. Subcell. Biol., 2001, p115-154, 2001) and p90 ribosomal S6 kinase (Frodin, M. et al., EMBO J., 19, p2924-2934, 2000). Kinase activity of serum and glucocordicoid regulated kinase (SGK) can also be phosphorylated and activated by PDK-1. Other potential substrates include protein kinase C, cAMP-dependent protein kinase (PKA), PRK1 and Protein kinase G.

PDK1 mediated signalling is activated in response to insulin and growth factors and as a consequence of attachment of the cell to the extracellular matrix (integrin signalling). Once activated these enzymes mediate many diverse cellular events by phosphorylating key regulatory proteins that play important roles controlling processes such as cell survival, growth, proliferation and glucose regulation (Lawlor, M.A. et al., J. Cell Sci., 114, p2903-2910, 2001), (Lawlor, M.A. et al., EMBO J., 21, p3728- 3738, 2002). PDK-1 inhibitors therefore may provide novel therapeutic treatment for diseases such as diabetes and cancer.

PDK1 is a 556 amino acid protein, with an N-terminal catalytic domain and a C-terminal pleckstrin homology (PH) domain, which activates its substrates by phosphorylating these kinases at their activation loop (Belham, C. et al., Curr. Biol., 9, pR93-96, 1999). Many human cancers including prostate and NSCL have elevated PDK1 signalling pathway function resulting from a number of distinct genetic events such as PTEN mutations or over-expression of certain key regulatory proteins [(Graff, J.R., Expert Opin. Ther. Targets, 6, p103-13, 2002), (Brognard, J., et al., Cancer Res., 61 p3986-97, 2001)]. Inhibition of PDK1 as a potential mechanism to treat cancer was demonstrated by transfection of a PTEN negative human cancer cell line (IJ87MG) with antisense oligonucleotides directed against PDK1. The resulting decrease in PDK1 protein levels led to a reduction in cellular proliferation and survival (Flynn, P., et al., Curr. Biol., 10, p1439-42, 2000). Therefore inhibition of PDK-1 could offer an attractive target for cancer therapy.

PDK-1-mediated phosphorylation of PKB/AKT, which is largely present in an inactive form in unstimulated cells, converts the enzyme to a catalytically active form. This occurs through the phosphorylation of the activation loop domain of AKT at threonine-309 in AKT2 and theonine-308 in AKT1. Although AKT displays low, basal levels of activation in normal, unstimulated cells, AKT often becomes constitutively activated in tumor cells. This occurs

through the up-regulation of a variety of different signalling molecules or the presence of oncogenenic mutations commonly found in cancer cells that can promote the activation of AKT, such as PI- 3 kinase, growth factor receptors (e.g., EGFR family members), Ras, Src, and BCR- ABL activation. Loss of the tumor suppressor PTEN is another means of greatly increasing AKT activity in cancer cells (Besson, A. et al., Eur. J. Biochem. (1999), Vol. 263, No. 3, pp. 605-611). PTEN mutation or down regulation of PTEN protein is found in a large number of tumors and cancer cell lines. PTEN is a phosphatase that removes the D-3 phosphate from the products of PI-3 kinase such as phosphatidylinositol 3,4,5-trisphosphate and phosphatidylinosito13,4-bisphosphate (Myers, M. P. et al., Proc. Natl. Acad. Sci. USA (1998), Vol.95, No. 23, pp.13513-13518; Stambolic, V. etal., Cell (1998), Vol. 95 p29-39). Loss of PTEN, therefore has the effect of increasing products of PI-3 kinase and promoting constitutive activation of AKT. Cancers with highly upregulated levels of AKT may be especially sensitive to the effects of PDK-1/AKT pathway inhibitors.

Therefore PDK1 is a critical mediator of the PI3K signalling pathway, which regulates a multitude of cellular function including growth, proliferation and survival. Consequently, inhibition of this pathway affects many defining requirements for cancer progression, so that a PDK1 inhibitor has an effect on the growth of a very wide range of human cancers.

Vascular Endothelial Growth Factor (VEGFR)

Chronic proliferative diseases are often accompanied by profound angiogenesis, which can contribute to or maintain an inflammatory and/or proliferative state, or which leads to tissue destruction through the invasive proliferation of blood vessels. (Folkman, *EXS*, 79, 1-81 (1997); Folkman, *Nature Medicine*, 1, 27-31 (1995); Folkman and Shing, *J. Biol. Chem.*, 267, 10931 (1992)).

Angiogenesis is generally used to describe the development of new or replacement blood vessels, or neovascularisation. It is a necessary and physiological normal process by which vasculature is established in the embryo. Angiogenesis does not occur, in general, in most normal adult tissues, exceptions being sites of ovulation, menses and wound healing. Many diseases, however, are characterized by persistent and unregulated angiogenesis. For instance, in arthritis, new capillary blood vessels invade the joint and destroy cartilage (Colville-Nash and Scott, *Ann. Rhum. Dis.*, 51, 919 (1992)). In diabetes (and in many different eye diseases), new vessels invade the macula or retina or other ocular structures, and may cause blindness (Brooks, *et al.*, *Cell*, 79, 1157 (1994)). The process of

atherosclerosis has been linked to angiogenesis (Kahlon, *et al.*, *Can. J. Cardiol.*, 8, 60 (1992)). Tumor growth and metastasis have been found to be angiogenesis-dependent (Folkman, *Cancer Biol*, 3, 65 (1992); Denekamp, *Br. J. Rad.*, 66,181 (1993); Fidler and Ellis, *Cell*, 79,185 (1994)).

The recognition of the involvement of angiogenesis in major diseases has been accompanied by research to identify and develop inhibitors of angiogenesis. These inhibitors are generally classified in response to discrete targets in the angiogenesis cascade, such as activation of endothelial cells by an angiogenic signal; synthesis and release of degradative enzymes; endothelial cell migration; proliferation of endothelial cells; and formation of capillary tubules. Therefore, angiogenesis occurs in many stages and attempts are underway to discover and develop compounds that work to block angiogenesis at these various stages.

There are publications that teach that inhibitors of angiogenesis, working by diverse mechanisms, are beneficial in diseases such as cancer and metastasis (O'Reilly, *et al.*, *Cell*, 79, 315 (1994); Ingber, *et al.*, *Nature*, 348, 555 (1990)), ocular diseases (Friedlander, *et al.*, *Science*, 270,1500 (1995)), arthritis (Peacock, *et al.*, *J. Exp. Med.*, 175, 1135 (1992); Peacock *et al.*, *Cell. Immun.*, 160,178 (1995)) and hemangioma (Taraboletti, *et al.*, *J. Natl. Cancer Inst.*, 87, 293 (1995)).

Receptor tyrosine kinases (RTKs) are important in the transmission of biochemical signals across the plasma membrane of cells. These transmembrane molecules characteristically consist of an extracellular ligand-binding domain connected through a segment in the plasma membrane to an intracellular tyrosine kinase domain. Binding of ligand to the receptor results in stimulation of the receptor-associated tyrosine kinase activity that leads to phosphorylation of tyrosine residues on both the receptor and other intracellular proteins, leading to a variety of cellular responses. To date, at least nineteen distinct RTK subfamilies, defined by amino acid sequence homology, have been identified.

Vascular endothelial growth factor (VEGF), a polypeptide, is mitogenic for endothelial cells in vitro and stimulates angiogenic responses in vivo. VEGF has also been linked to inappropriate angiogenesis (Pinedo, H.M., *et al.*, *The Oncologist*, 5(90001), 1-2 (2000)). VEGFR(s) are protein tyrosine kinases (PTKs). PTKs catalyze the phosphorylation of specific tyrosine residues in proteins involved in cell function thus regulating cell growth,

survival and differentiation. (Wilks, A.F., *Progress in Growth Factor Research*, 2, 97-111 (1990); Courtneidge, S.A., *Dev. Supp.I*,57-64 (1993); Cooper, J.A., *Semin. Cell Biol.*, 5(6), 377-387 (1994); Paulson, R.F., *Semin. Immunol.*, 7(4), 267-277 (1995); Chan, A.C., *Curr. Opin.Immunol.*, 8(3), 394-401 (1996)).

Three PTK receptors for VEGF have been identified: VEGFR-1 (Flt-1) ; VEGFR-2 (Flk-1 or KDR) and VEGFR-3 (Flt-4). These receptors are involved in angiogenesis and participate in signal transduction (Mustonen, T., *et al.*, *J. Cell Biol.*, 129, 895-898 (1995)).

Of particular interest is VEGFR-2, which is a transmembrane receptor PTK expressed primarily in endothelial cells. Activation of VEGFR-2 by VEGF is a critical step in the signal transduction pathway that initiates tumour angiogenesis. VEGF expression may be constitutive to tumour cells and can also be upregulated in response to certain stimuli. One such stimuli is hypoxia, where VEGF expression is upregulated in both tumour and associated host tissues. The VEGF ligand activates VEGFR-2 by binding with its extracellular VEGF binding site. This leads to receptor dimerization of VEGFRs and autophosphorylation of tyrosine residues at the intracellular kinase domain of VEGFR- 2. The kinase domain operates to transfer a phosphate from ATP to the tyrosine residues, thus providing binding sites for signalling proteins downstream of VEGFR-2 leading ultimately to initiation of angiogenesis (McMahon, G. ,*The Oncologist*, 5(90001), 3-10 (2000)).

Inhibition at the kinase domain binding site of VEGFR-2 would block phosphorylation of tyrosine residues and serve to disrupt initiation of angiogenesis.

FGFR

The fibroblast growth factor (FGF) family of protein tyrosine kinase (PTK) receptors regulates a diverse array of physiologic functions including mitogenesis, wound healing, cell differentiation and angiogenesis, and development. Both normal and malignant cell growth as well as proliferation are affected by changes in local concentration of these extracellular signaling molecules, which act as autocrine as well as paracrine factors. Autocrine FGF signaling may be particularly important in the progression of steroid hormone-dependent cancers and to a hormone independent state (Powers, et al., Endocr. Relat. Cancer, 7, 165-197 (2000)).

FGFs and their receptors are expressed at increased levels in several tissues and cell lines and overexpression is believed to contribute to the malignant phenotype. Furthermore, a number of oncogenes are homologues of genes encoding growth factor receptors, and there is a potential for aberrant activation of FGF-dependent signaling in human pancreatic cancer (Ozawa, et al., Teratog. Carcinog. Mutagen., 21, 27-44 (2001)).

The two prototypic members are acidic fibroblast growth factor (aFGF or FGF1) and basic fibroblast growth factor (bFGF or FGF2), and to date, at least twenty distinct FGF family members have been identified. The cellular response to FGFs is transmitted via four types of high affinity transmembrane protein tyrosine-kinase fibroblast growth factor receptors numbered 1 to 4 (FGFR1 to FGFR4). Upon ligand binding, the receptors dimerize and auto- or trans-phosphorylate specific cytoplasmic tyrosine residues to transmit an intracellular signal that ultimately regulates nuclear transcription factor effectors.

Disruption of the FGFR1 pathway should affect tumor cell proliferation since this kinase is activated in many tumor types in addition to proliferating endothelial cells. The over-expression and activation of FGFR1 in tumor- associated vasculature has suggested a role for these molecules in tumor angiogenesis.

Fibroblast growth factor receptor 2 has high affinity for the acidic and/or basic fibroblast growth factors, as well as the keratinocyte growth factor ligands. Fibroblast growth factor receptor 2 also propagates the potent osteogenic effects of FGFs during osteoblast growth and differentiation. Mutations in fibroblast growth factor receptor 2, leading to complex functional alterations, were shown to induce abnormal ossification of cranial sutures(craniosynostosis), implying a major role of FGFR signaling in intramembranous bone formation. For example, in Apert (AP) syndrome, characterized by premature cranial suture ossification, most cases are associated with point mutations engendering gain-of-function in fibroblast growth factor receptor 2 (Lemonnier, et al., J. Bone Miner. Res., 16, 832-845 (2001)).

Several severe abnormalities in human skeletal development, including Apert, Crouzon, Jackson-Weiss, Beare-Stevenson cutis gyrata, and Pfeiffer syndromes are associated with the occurrence of mutations in fibroblast growth factor receptor 2. Most, if not all, cases of Pfeiffer Syndrome (PS) are also caused by de novo mutation of the fibroblast growth factor receptor 2 gene (Meyers, et al., Am. J. Hum. Genet., 58, 491-498 (1996); Plomp, et al., Am. J. Med. Genet., 75, 245-251 (1998)), and it was recently shown that mutations in fibroblast growth factor receptor 2 break one of the cardinal rules governing ligand

specificity. Namely, two mutant splice forms of fibroblast growth factor receptor, FGFR2c and FGFR2b, have acquired the ability to bind to and be activated by atypical FGF ligands. This loss of ligand specificity leads to aberrant signaling and suggests that the severe phenotypes of these disease syndromes result from ectopic ligand-dependent activation of fibroblast growth factor receptor 2 (Yu, et al., Proc. Natl. Acad. Sci. U.S.A., 97, 14536-14541 (2000)).

Genetic aberrations of the FGFR3 receptor tyrosine kinase such as chromosomal translocations or point mutations result in ectopically expressed or deregulated, constitutively active, FGFR3 receptors. Such abnormalities are linked to a subset of multiple myelomas and in bladder, hepatocellular, oral squamous cell carcinoma and cervical carcinomas (Powers, C.J., et al., Endocr. Rel. Cancer, 7, 165 (2000), Qiu, W., et. al., World Journal Gastroenterol, 11(34) 2005). Accordingly, FGFR3 inhibitors would be useful in the treatment of multiple myeloma, bladder and cervical carcinomas.

As such, the compounds are useful in providing a means of preventing the growth or inducing apoptosis of neoplasias and in tumours, particularly by inhibiting angiogenesis. The compounds are useful in treating or preventing proliferative disorders such as cancers. In particular tumours with activating mutants of receptor tyrosine kinases or upregulation of receptor tyrosine kinases may be particularly sensitive to the inhibitors. Patients with activating mutants of any of the isoforms of the specific RTKs discussed herein may also find treatment with RTK inhibitors particularly beneficial.

Over expression of FGFR4 has been linked to poor prognosis in both prostate and thyroid carcinomas (Ezzat, S., et al. The Journal of Clinical Investigation, 109, 1 (2002), Wang et al. Clinical Cancer Research, 10 (2004)). In addition a germline polymorphism (Gly388Arg) is associated with increased incidence of lung, breast, colon and prostate cancers (Wang et al. Clinical Cancer Research, 10 (2004)).

RET

The Ret proto-oncogene encodes a receptor tyrosine kinase that is expressed during development in a variety of tissues, including the peripheral and central nervous systems and the kidney. The abnormalities present in ret null mice suggest that Ret is critical for the migration and innervation of enteric neurons to the hindgut, and for proliferation and branching of the ureteric bud epithelium during kidney development (Nature 367, 380-383, 1994).

Mutations in the RET receptor tyrosine kinase provides a classic example of phenotypic heterogeneity in a variety of diseases. Gain-of-function mutations of RET are associated with human cancer and in particular cause inherited and non-inherited thyroid cancer. Gene rearrangements juxtaposing the tyrosine kinase domain of RET to heterologous gene partners have been found in sporadic papillary carcinomas of the thyroid (PTC). These rearrangements generate chimeric RET/PTC oncogenes. In germline cancers, point mutations of RET are responsible for multiple endocrine neoplasia type 2 (MEN 2A and 2B) and familial medullary thyroid carcinoma (FMTC). Both MEN 2 mutations and PTC gene rearrangements potentiate the intrinsic tyrosine kinase activity of RET and, ultimately, activate targets downstream of RET.

Thus somatic gene rearrangements of RET have been found in papillary thyroid carcinoma (PTC) and germline point mutations in multiple endocrine neoplasia (MEN) types 2A and 2B and familial medullary thyroid carcinoma (FMTC). Conversely, loss-of-function mutations are responsible for the development of Hirschsprung's disease, a congenital malformation of the enteric nervous system. (Naoya Asai et al, Pathology International, Volume 56 Page 164, April 2006)

SRC

The Src family kinases (SFK) comprises nine members of which three (Src, Fyn Yes) are ubiquitously expressed. Src itself is implicated in the pathogenesis of human malignancies. Activated mutants of c-Src can transform human cells in culture and Src protein expression and/or activity is increased in epithelial cancers. In colon cancer there is frequent elevation of Src activity compared to adjacent normal mucosa. Furthermore the Src activation is often elevated in metastases compared to the primary tumour implying a possible role for the protein in invasion and metastasis. Moreover Src expression is strongly correlated with disease progression. Similarly Src expression and activation are also elevated in breast, pancreatic, oesophageal, ovarian, lung, head and neck and gastric cancers compared to normal tissues.

EGFR and PDGFR

A malignant tumour is the product of uncontrolled cell proliferation. Cell growth is controlled by a delicate balance between growth-promoting and growth-inhibiting factors. In normal tissue the production and activity of these factors results in differentiated cells growing in a controlled and regulated manner that maintains the normal integrity and functioning of the

organ. The malignant cell has evaded this control; the natural balance is disturbed (via a variety of mechanisms) and unregulated, aberrant cell growth occurs. One driver for growth is the epidermal growth factor (EGF), and the receptor for EGF (EGFR) has been implicated in the development and progression of a number of human solid tumours including those of the lung, breast, prostate, colon, ovary, head and neck. EGFR is a member of a family of four receptors, namely EGFR (HER1 or ErbB1), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4). These receptors are large proteins that reside in the cell membrane, each having a specific external ligand binding domain, a transmembrane domain and an internal domain which has tyrosine kinase enzyme activity. When EGF attaches to EGFR, it activates the tyrosine kinase, triggering reactions that cause the cells to grow and multiply. EGFR is found at abnormally high levels on the surface of many types of cancer cells, which may divide excessively in the presence of EGF. Inhibition of EGFR activity has therefore been a target for chemotherapeutic research in the treatment of cancer. Such inhibition can be effected by direct interference with the target EGFR on the cell surface, for example by the use of antibodies, or by inhibiting the subsequent tyrosine kinase activity.

Examples of agents which target EGFR tyrosine kinase activity include the tyrosine kinase inhibitors gefitinib and erlotinib. Gefitinib which has the chemical name 4-(3-chloro-4-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy)quinazoline, is used for the treatment of non-small-cell lung cancer, and is also under development for other solid tumours that over-express EGF receptors such as breast and colorectal cancer. Erlotinib, which has the chemical name N-(3-ethynyl-phenyl)-6,7-bis(2-methoxyethoxy)-4-quinazoline, has also been used for the treatment of non-small-cell lung cancer, and is being developed for the treatment of various other solid tumours such as pancreatic cancer.

Another growth factor of importance in tumour development is the platelet-derived growth factor (PDGF) that comprises a family of peptide growth factors that signal through cell surface tyrosine kinase receptors (PDGFR) and stimulate various cellular functions including growth, proliferation, and differentiation. PDGF expression has been demonstrated in a number of different solid tumours including glioblastomas and prostate carcinomas. The tyrosine kinase inhibitor imatinib mesylate, which has the chemical name 4-[(4-methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)- 2-ylpyridinyl]amino]-phenyl]benzamide methanesulfonate, blocks activity of the Bcr-Abl oncoprotein and the cell surface tyrosine kinase receptor c-Kit, and as such is approved for the treatment of chronic

myeloid leukemia and gastrointestinal stromal tumours. Imatinib mesylate is also a potent inhibitor of PDGFR kinase and is currently being evaluated for the treatment of chronic myelomonocytic leukemia and glioblastoma multiforme, based upon evidence in these diseases of activating mutations in PDGFR. In addition, sorafenib (BAY 43-9006) which has the chemical name 4-(4-(3-(4-chloro-3 (trifluoromethyl)phenyl)ureido)phenoxy)-N2-methylpyridine-2-carboxamide, targets both the Raf signalling pathway to inhibit cell proliferation and the VEGFR/PDGFR signalling cascades to inhibit tumour angiogenesis. Sorafenib is being investigated for the treatment of a number of cancers including liver and kidney cancer.

Ancillary compounds

A wide variety of ancillary compounds find application in the combinations of the invention, as described in detail below. The ancillary compounds may be anti-cancer agents.

It is an object of the invention to provide therapeutic combinations comprising (or consisting essentially of) one or more ancillary compounds and a pyrazole compound that inhibits or modulates (in particular inhibits) the activity of cyclin dependent kinases (CDK) and/or glycogen synthase kinase (e.g. GSK-3). Such combinations may have an advantageous efficacious effect against tumour cell growth, in comparison with the respective effects shown by the individual components of the combination.

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.

WO 2005/012256 (Astex Technology Limited) discloses various compounds of formula (0) having activity as inhibitors of various kinases for use in the treatment of disease states and conditions such as cancer.

WO 2006/077424 (Astex Therapeutics Limited) discloses a combination of a cytotoxic compound or signalling inhibitor and a compound having the formula (0).

WO 2006/077426 (Astex Therapeutics Limited) discloses various compounds and salts of formula (0) having activity as inhibitors of cyclin dependent kinases, and glycogen synthase kinase-3.

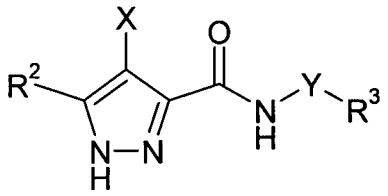
Summary of the Invention

The invention provides combinations of one or more ancillary compounds with pyrazole compounds that have cyclin dependent kinase inhibiting or modulating activity, wherein the

combinations have efficacy against abnormal cell growth. The invention further provides combinations as described above which are further combined with other classes of therapeutic agents or treatments that may be administered together (whether concurrently or at different time intervals) as described in more detail hereinafter.

Thus, for example, the combinations of the invention will be useful in alleviating or reducing the incidence of cancer.

Accordingly, in one aspect, the invention provides a combination comprising (or consisting essentially of) an ancillary compound and a compound having the formula (0):



(0)

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

wherein

X is a group R^1-A-NR^4- or a 5- or 6-membered carbocyclic or heterocyclic ring;

A is a bond, SO_2, C=O, NR^g(C=O) or O(C=O) wherein R^g is hydrogen or C_{1-4} hydrocarbyl optionally substituted by hydroxy or C_{1-4} alkoxy;

Y is a bond or an alkylene chain of 1, 2 or 3 carbon atoms in length;

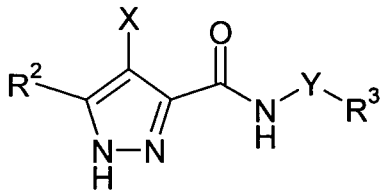
R^1 is hydrogen; a carbocyclic or heterocyclic group having from 3 to 12 ring members; or a C_{1-8} hydrocarbyl group optionally substituted by one or more substituents selected from halogen (e.g. fluorine), hydroxy, C_{1-4} hydrocarbyloxy, amino, mono- or di-C_{1-4} hydrocarbylamino, and carbocyclic or heterocyclic groups having from 3 to 12 ring members, and wherein 1 or 2 of the carbon atoms of the hydrocarbyl group may optionally be replaced by an atom or group selected from O, S, NH, SO, SO_2;

R^2 is hydrogen; halogen; C_{1-4} alkoxy (e.g. methoxy); or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy);

R^3 is selected from hydrogen and carbocyclic and heterocyclic groups having from 3 to 12 ring members; and

R^4 is hydrogen or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy).

In one embodiment, the invention provides a combination comprising (or consisting essentially of) an ancillary compound and a compound having the formula (I^0):



(I⁰)

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

wherein

X is a group R^1-A-NR^4- or a 5- or 6-membered carbocyclic or heterocyclic ring;

A is a bond, C=O, NR^9(C=O) or O(C=O) wherein R^9 is hydrogen or C_{1-4} hydrocarbyl optionally substituted by hydroxy or C_{1-4} alkoxy;

Y is a bond or an alkylene chain of 1, 2 or 3 carbon atoms in length;

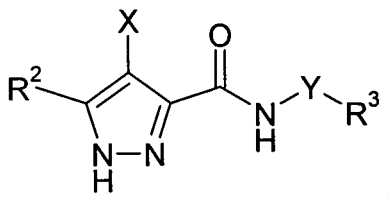
R^1 is hydrogen; a carbocyclic or heterocyclic group having from 3 to 12 ring members; or a C_{1-8} hydrocarbyl group optionally substituted by one or more substituents selected from halogen (e.g. fluorine), hydroxy, C_{1-4} hydrocarbyloxy, amino, mono- or di-C_{1-4} hydrocarbylamino, and carbocyclic or heterocyclic groups having from 3 to 12 ring members, and wherein 1 or 2 of the carbon atoms of the hydrocarbyl group may optionally be replaced by an atom or group selected from O, S, NH, SO, SO_2;

R^2 is hydrogen; halogen; C_{1-4} alkoxy (e.g. methoxy); or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy);

R^3 is selected from hydrogen and carbocyclic and heterocyclic groups having from 3 to 12 ring members; and

R^4 is hydrogen or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy).

In a further embodiment, the invention provides a combination comprising (or consisting essentially of) an ancillary compound and a compound having the formula (I):



(I)

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

wherein

X is a group R^1-A-NR^4-;

A is a bond, C=O, $NR^g(C=O)$ or O(C=O) wherein R^g is hydrogen or C_{1-4} hydrocarbyl optionally substituted by hydroxy or C_{1-4} alkoxy;

Y is a bond or an alkylene chain of 1, 2 or 3 carbon atoms in length;

R^1 is hydrogen; a carbocyclic or heterocyclic group having from 3 to 12 ring members; or a C_{1-8} hydrocarbyl group optionally substituted by one or more substituents selected from halogen (e.g. fluorine), hydroxy, C_{1-4} hydrocarbyloxy, amino, mono- or di-C_{1-4} hydrocarbylamino, and carbocyclic or heterocyclic groups having from 3 to 12 ring members, and wherein 1 or 2 of the carbon atoms of the hydrocarbyl group may optionally be replaced by an atom or group selected from O, S, NH, SO, SO_2;

R^2 is hydrogen; halogen; C_{1-4} alkoxy (e.g. methoxy); or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy);

R^3 is selected from hydrogen and carbocyclic and heterocyclic groups having from 3 to 12 ring members; and

R^4 is hydrogen or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy).

Any one or more of the following optional provisos, in any combination, may apply to the compounds of formulae (0), (I^0), (I) and sub-groups thereof:

(a-i) When A is a bond and Y-R^3 is an alkyl, cycloalkyl, optionally substituted phenyl or optionally substituted phenylalkyl, then R^1 is other than a substituted or unsubstituted dihydronaphthalene, dihydrochroman, dihydrothiochroman, tetrahydroquinoline or tetrahydrobenzfuranyl group.

(a-ii) X and R^3 are each other than a moiety containing a maleimide group wherein the maleimide group has nitrogen atoms attached to the 3-and 4-positions thereof.

(a-iii) R^1 is other than a moiety containing a purine nucleoside group.

(a-iv) X and R^3 are each other than a moiety containing a cyclobutene-1,2-dione group wherein the cyclobutene-1,2-dione group has nitrogen atoms attached to the 3-and 4-positions thereof.

(a-v) R^3 is other than a moiety containing a 4-monosubsituted or 4,5-disubstituted 2-pyridyl or 2-pyrimidinyl group or a 5-monosubstituted or 5,6-disubstituted 1,2,4-triazin-3-yl or 3-pyridazinyl group.

(a-vi) X and R^3 are each other than a moiety containing a substituted or unsubstituted pyrazol-3-ylamine group linked to a substituted or unsubstituted pyridine, diazine or triazine group.

(a-vii) When A is C=O and Y-R^3 is an alkyl, cycloalkyl, optionally substituted phenyl or optionally substituted phenylalkyl group, then R^1 is other than a substituted or unsubstituted tetrahydronaphthalene, tetrahydroquinolinyl, tetrahydrochromanyl or tetrahydrothiochromanyl group.

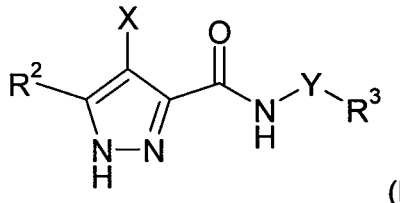
(a-viii) When R^3 is H and A is a bond, R^1 is other than a moiety containing a bis-aryl, bis-heteroaryl or aryl heteroaryl group.

(a-ix) R^3 is other than a moiety containing a 1,2,8,8a-tetrahydro-7-methyl-cyclopropa[c]pyrrolo[3,2,e]indole-4-(5H)-one group.

(a-x) When Y is a bond, R^3 is hydrogen, A is CO and R^1 is a substituted phenyl group, each substituent on the phenyl group is other than a group CH_2-P(O)R^xR^y where R^x and R^y are each selected from alkoxy and phenyl groups.

(a-xi) X is other than 4-(*tert*-butyloxycarbonylamino)-3-methylimidazol-2-ylcarbonylamino.

In another embodiment, the invention provides a combination comprising (or consisting essentially of) an ancillary compound and a compound having the formula (Ia):



(Ia)

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

wherein

X is a group R^1-A-NR^4-;

A is a bond, C=O, NR^g(C=O) or O(C=O) wherein R^g is hydrogen or C_{1-4} hydrocarbyl optionally substituted by hydroxy or C_{1-4} alkoxy;

Y is a bond or an alkylene chain of 1, 2 or 3 carbon atoms in length;

R^1 is a carbocyclic or heterocyclic group having from 3 to 12 ring members; or a C_{1-8} hydrocarbyl group optionally substituted by one or more substituents selected from fluorine, hydroxy, C_{1-4} hydrocarbyloxy, amino, mono- or di-C_{1-4} hydrocarbylamino, and

carbocyclic or heterocyclic groups having from 3 to 12 ring members, and wherein 1 or 2 of the carbon atoms of the hydrocarbyl group may optionally be replaced by an atom or group selected from O, S, NH, SO, SO_2;

R^2 is hydrogen; halogen; C_{1-4} alkoxy (e.g. methoxy); or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy);

R^3 is selected from hydrogen and carbocyclic and heterocyclic groups having from 3 to 12 ring members; and

R^4 is hydrogen or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy).

Any one or more of the following optional provisos, in any combination, may apply to the compounds of formula (Ia) and sub-groups thereof:

Provisos (a-i) to (a-xi) above.

(b-i) R^3 is other than a bridged azabicyclo group.

(b-ii) When A is a bond, then R^3 is other than a moiety containing an unsubstituted or substituted phenyl group having attached to an *ortho* position thereof, a substituted or unsubstituted carbamoyl or thiocarbamoyl group.

(b-iii) When A is a bond, then R^3 is other than a moiety containing an isoquinoline or quinoxaline group each having attached thereto a substituted or unsubstituted piperidine or piperazine ring.

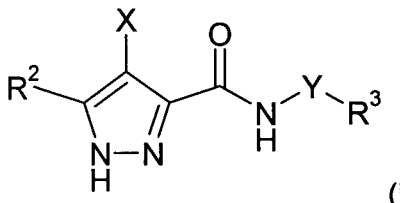
(b-iv) When A is a bond and R^1 is an alkyl group, then R^3 is other than a moiety containing a thiatriazine group.

(b-v) When R^1 or R^3 contain a moiety in which a heterocyclic ring having an $S(=O)_2$ ring member is fused to a carbocyclic ring, the said carbocyclic ring is other than a substituted or unsubstituted benzene ring

(b-vi) When A is a bond, R^1 is other than an arylalkyl, heteroarylalkyl or piperidinylalkyl group each having attached thereto a substituent selected from cyano, and substituted or unsubstituted amino, aminoalkyl, amidine, guanidine, and carbamoyl groups.

(b-vii) When X is a group R^1-A-NR^4-, A is a bond and R^1 is a non-aromatic group, then R^3 is other than a six membered monocyclic aryl or heteroaryl group linked directly to a 5,6-fused bicyclic heteroaryl group.

In another embodiment, the invention provides a combination comprising (or consisting essentially of) an ancillary compound and a compound of the formula (Ib):



(Ib)

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

wherein

X is a group R^1-A-NR^4-;

A is a bond, C=O, NR^9(C=O) or O(C=O) wherein R^9 is hydrogen or C_{1-4} hydrocarbyl optionally substituted by hydroxy or C_{1-4} alkoxy;

Y is a bond or an alkylene chain of 1, 2 or 3 carbon atoms in length;

R^1 is a carbocyclic or heterocyclic group having from 3 to 12 ring members; or a C_{1-8} hydrocarbyl group optionally substituted by one or more substituents selected from fluorine, hydroxy, C_{1-4} hydrocarbyloxy, amino, mono- or di-C_{1-4} hydrocarbylamino, and carbocyclic or heterocyclic groups having from 3 to 12 ring members, and wherein 1 or 2 of the carbon atoms of the hydrocarbyl group may optionally be replaced by an atom or group selected from O, S, NH, SO, SO_2;

R^2 is hydrogen; halogen; C_{1-4} alkoxy (e.g. methoxy); or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy);

R^3 is selected from carbocyclic and heterocyclic groups having from 3 to 12 ring members; and

R^4 is hydrogen or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy).

Any one or more of the following optional provisos, in any combination, may apply to the compounds of formula (Ib) and sub-groups thereof:

Provisos (a-i) to (a-vii), (a-ix) and (a-xi).

Provisos (b-i) to (b-vii).

(c-i) When A is a bond, R^1 is other than a substituted arylalkyl, heteroarylalkyl or piperidinylalkyl group.

(c-ii) When X is an amino or alkylamino group and Y is a bond, R^3 is other than a disubstituted thiazolyl group wherein one of the substituents is selected from cyano and fluoroalkyl.

The reference in proviso (a-iii) to a purine nucleoside group refers to substituted and unsubstituted purine groups having attached thereto a monosaccharide group (e.g. a pentose or hexose) or a derivative of a monosaccharide group, for example a deoxy monosaccharide group or a substituted monosaccharide group.

The reference in proviso (b-i) to a bridged azabicyclo group refers to bicycloalkane bridged ring systems in which one of the carbon atoms of the bicycloalkane has been replaced by a nitrogen atom. In bridged ring systems, two rings share more than two atoms, see for example *Advanced Organic Chemistry*, by Jerry March, 4th Edition, Wiley Interscience, pages 131-133, 1992.

The provisos (a-i) to (a-x), (b-i) to (b-vii), (c-i) and (c-ii) in formulae (I), (Ia) and (Ib) above refer to the disclosures in the following prior art documents.

(a-i)	US 2003/0166932, US 6,127,382, US 6,093,838
(a-ii)	WO 03/031440
(a-iii)	WO 03/014137
(a-iv)	WO 02/083624
(a-v)	WO 02/064586
(a-vi)	WO 02/22608, WO 02/22605, WO 02/22603 & WO 02/22601
(a-vii)	WO 97/48672, WO 97/19052
(a-viii)	WO 00/06169
(a-ix)	US 5,502,068
(a-x)	JP 07188269
(b-i)	WO 03/040147
(b-ii)	WO 01/70671
(b-iii)	WO 01/32626
(b-iv)	WO 98/08845
(b-v)	WO 00/59902
(b-vi)	US 6,020,357, WO 99/32454 & WO 98/28269

(b-vii) WO 2004/012736

(c-i) US 6,020,357, WO 99/32454 & WO 98/28269

(c-ii) US 2004/0082629

Any one or more of the foregoing optional provisos, (a-i) to (a-xi), (b-i) to (b-vii), (c-i) and (c-ii) in any combination, may also apply to the compounds of formulae (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof or salts or tautomers or N-oxides or solvates thereof as defined herein.

In the following aspects and embodiments of the invention, references to "a combination according to the invention" refer to the combination comprising (or consisting essentially of) an ancillary compound and a compound of formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII). In this section, as in all other sections of this application, unless the context indicates otherwise, references to a compound of formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) includes all other subgroups as defined herein. The term 'subgroups' includes all preferences, examples and particular compounds defined herein.

Moreover, a reference to a compound of formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof includes ionic, salt, solvate, isomers, tautomers, N-oxides, ester, prodrugs, isotopes and protected forms thereof, as discussed below. Preferably, the salts or tautomers or isomers or N-oxides or solvates thereof. More preferably, the salts or tautomers or N-oxides or solvates thereof.

The invention also provides:

- A combination according to the invention for use in alleviating or reducing the incidence of a disease or condition comprising or arising from abnormal cell growth in a mammal.

- A combination of the invention 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 combination of the invention.

- 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 combination of the invention.

- 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 combination according to the invention 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, which method comprises administering to the mammal a combination according to the invention in an amount effective in inhibiting abnormal cell growth.

- A combination according to the invention for use in inhibiting tumour growth in a mammal.

- A method of inhibiting tumour growth in a mammal, which method comprises administering to the mammal an effective tumour growth-inhibiting amount of a combination according to the invention.

- A combination according to the invention for use in inhibiting the growth of tumour cells.

- A method of inhibiting the growth of tumour cells, which method comprises contacting the tumour cells with administering to the mammal an effective tumour cell growth-inhibiting amount of a combination according to the invention.

- A pharmaceutical composition comprising a combination according to the invention and a pharmaceutically acceptable carrier.

- A combination according to the invention for use in medicine.

- The use of a combination according to the invention, for the manufacture of a medicament for the prophylaxis or treatment of any one of the disease states or conditions disclosed herein.

- A method for the treatment or prophylaxis of any one of the disease states or conditions disclosed herein, which method comprises administering to a patient (e.g. a patient in need thereof) a combination according to the invention.

- A method for alleviating or reducing the incidence of a disease state or condition disclosed herein, which method comprises administering to a patient (e,g, a patient in need thereof) a combination according to the invention.

- A method for the diagnosis and treatment of a cancer in a mammalian patient, which method comprises (i) screening a patient to determine whether a cancer 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 an ancillary compound; and (ii) where it is indicated that the disease or condition from which the patient is thus susceptible, thereafter administering to the patient a combination according to the invention.

- The use of a combination according to the invention for the manufacture of a medicament for the treatment or prophylaxis of a cancer in a patient who has been screened and has been determined as suffering from, or being at risk of suffering from, a cancer which would be susceptible to treatment with a combination comprising (or consisting essentially of) an ancillary compound and a compound having activity against cyclin dependent kinase.

- A method for treating a cancer in a patient comprising administration of a combination according to the invention to said patient in an amount and in a schedule of administration that is therapeutically efficaceous in the treatment of said cancer.

- A method for preventing, treating or managing cancer in a patient in need thereof, said method comprising administering to said patient a prophylactically or therapeutically effective amount of a combination according to the invention.

- The use of a combination according to the invention for the manufacture of a medicament for use in the production of an anti-cancer effect in a warm-blooded animal such as a human.

- A kit comprising a combination according to the invention.

- A method for the treatment of a cancer in a warm-blooded animal such as a human, which comprises administering to said animal an effective amount of an ancillary compound sequentially e.g. before or after, or simultaneously with an effective amount of a compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein.

- A pharmaceutical kit for anticancer therapy comprising an ancillary compound in dosage form and a compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein, also in dosage form (e.g. wherein the dosage forms are packaged together in common outer packaging).

- A method of combination cancer therapy in a mammal comprising administering a therapeutically effective amount of an ancillary compound and a therapeutically effective amount of a compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein.

- A compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein for use in combination therapy with an ancillary compound to alleviate or reduce the incidence of a disease or condition comprising or arising from abnormal cell growth in a mammal.

- A compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein for use in combination therapy with an ancillary compound to inhibit tumour growth in a mammal.

- A compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein for use in combination therapy with an ancillary compound to prevent, treat or manage cancer in a patient in need thereof.

- A compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein for use in

enhancing or potentiating the response rate in a patient suffering from a cancer where the patient is being treated with an ancillary compound.

- A method of enhancing or potentiating the response rate in a patient suffering from a cancer where the patient is being treated with an ancillary compound, which method comprises administering to the patient, in combination with the ancillary compound, a compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein.

- The use of a combination according to the invention for the manufacture of a medicament for any of the therapeutic uses as defined herein.

In each of the foregoing uses, methods and other aspects of the invention, as well as any aspects and embodiments of the invention as set out below, references to compounds of the formulae (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein include within their scope the salts or solvates or tautomers or N-oxides of the compounds.

The invention also provides the further combinations, uses, methods, compounds and processes as set out in the claims below.

General Preferences and Definitions

In this specification, unless the context indicates otherwise, references to formula (0) include formulae (I), (I^0), (Ia), (Ib), (II'), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) as described herein and in WO 2005/012256, and sub-groups, examples or embodiments of formulae (0), (I^0), (Ia), (Ib), (II'), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) as described herein and in WO 2005/012256.

As used herein, the term "modulation", as applied to the activity of cyclin dependent kinase (CDK), Aurora kinases and glycogen synthase kinase (GSK, e.g. GSK-3) and/or any other kinase as described herein, is intended to define a change in the level of biological activity of the kinase(s). Thus, modulation encompasses physiological changes which effect an increase or decrease in the relevant kinase activity. In the latter case, the modulation may be described as "inhibition". The modulation may arise directly or indirectly, and may be mediated by any mechanism and at any physiological level, including for example at the level of gene expression (including for example transcription, translation and/or post-

translational modification), at the level of expression of genes encoding regulatory elements which act directly or indirectly on the levels of the kinase actitvty e.g. Aurora kinase, cyclin dependent kinase (CDK) and/or glycogen synthase kinase-3 (GSK-3) activity and/or activity of any other kinase described herein, or at the level of enzyme (e.g. cyclin dependent kinase (CDK) and/or glycogen synthase kinase-3 (GSK-3)) activity and/or any other kinase described herein (for example by allosteric mechanisms, competitive inhibition, active-site inactivation, perturbation of feedback inhibitory pathways etc.). Thus, modulation may imply elevated/suppressed expression or over- or under-expression of the kinase (e.g. cyclin dependent kinase (CDK) and/or glycogen synthase kinase-3 (GSK-3) or any other kinase described herein), including gene amplification (i.e. multiple gene copies) and/or increased or decreased expression by a transcriptional effect, as well as hyper- (or hypo-)activity and (de)activation of the protein kinase(s) (e.g. cyclin dependent kinase (CDK) and/or glycogen synthase kinase-3 (GSK-3) and/or any other protein kinase described herein) (including (de)activation) by mutation(s). The terms "modulated", "modulating" and "modulate" are to be interpreted accordingly.

The term "upregulation of Aurora kinase" as used herein is defined as including elevated expression or over-expression of Aurora kinase, including gene amplification (i.e. multiple gene copies) and increased expression by a transcriptional effect, and hyperactivity and activation of Aurora kinase, including activation by mutations.

As used herein, the term "mediated", as used e.g. in conjunction with a kinase as described herein (e.g. cyclin dependent kinases (CDK) and/or glycogen synthase kinase-3 (GSK-3) as described herein) (and applied for example to various physiological processes, diseases, states, conditions, therapies, treatments or interventions) is intended to operate limitatively so that the various processes, diseases, states, conditions, treatments and interventions to which the term is applied are those in which the kinase (e.g. cyclin dependent kinase (CDK) and/or glycogen synthase kinase-3 (GSK-3)) plays a biological role. In cases where the term is applied to a disease, state or condition, the biological role played by the kinase (e.g. cyclin dependent kinase (CDK) and/or glycogen synthase kinase-3 (GSK-3)) may be direct or indirect and may be necessary and/or sufficient for the manifestation of the symptoms of the disease, state or condition (or its aetiology or progression). Thus, kinase acitivty (e.g. cyclin dependent kinase (CDK) and/or glycogen synthase kinase-3 (GSK-3) activity) (and in particular aberrant levels of cyclin dependent kinase (CDK) and/or glycogen synthase kinase-3 (GSK-3)activity, e.g. cyclin dependent kinases (CDK) and/or glycogen synthase kinase-3 (GSK-3) over-expression) need not

necessarily be the proximal cause of the disease, state or condition: rather, it is contemplated that the kinase mediated diseases, states or conditions (e.g. CDK- and/or GSK- (e.g. GSK-3-) mediated diseases, states or conditions) include those having multifactorial aetiologies and complex progressions in which the kinase (e.g. CDK and/or GSK-3) is only partially involved. In cases where the term is applied to treatment, prophylaxis or intervention (e.g. in the "CDK-mediated treatments" and "GSK-3-mediated prophylaxis" of the invention), the role played by the kinase (e.g. CDK and/or GSK-3) may be direct or indirect and may be necessary and/or sufficient for the operation of the treatment, prophylaxis or outcome of the intervention. Thus, a disease state or condition mediated by the kinase (e.g. cyclin dependent kinases (CDK) and/or glycogen synthase kinase-3 (GSK-3) and/or Aurora kinase and/or any other kinase as described herein) includes a disease state or condition which has arisen as a consequence of the development of resistance to any particular cancer drug or treatment (including in particular resistance to one or more of the ancillary compounds described herein).

The term "intervention" is a term of art used herein to define any agency which effects a physiological change at any level. Thus, the intervention may comprise the induction or repression of any physiological process, event, biochemical pathway or cellular/biochemical event. The interventions of the invention typically effect (or contribute to) the therapy, treatment or prophylaxis of a disease or condition.

The combinations of the invention may produce a therapeutically efficacious effect relative to the therapeutic effect of the individual compounds when administered separately.

The term 'efficacious' includes advantageous effects such as additivity, synergism, reduced side effects, reduced toxicity, increased time to disease progression, increased time of survival, sensitization or resensitization of one agent to another, or improved response rate. Advantageously, an efficacious effect may allow for lower doses of each or either component to be administered to a patient, thereby decreasing the toxicity of chemotherapy, whilst producing and/or maintaining the same therapeutic effect.

A "synergistic" effect in the present context refers to a therapeutic effect produced by the combination which is larger than the sum of the therapeutic effects of the components of the combination when presented individually.

An "additive" effect in the present context refers to a therapeutic effect produced by the combination which is larger than the therapeutic effect of any of the components of the combination when presented individually.

The term "response rate" as used herein refers, in the case of a solid tumour, to the extent of reduction in the size of the tumour at a given time point, for example 12 weeks. Thus, for example, a 50% response rate means a reduction in tumour size of 50%. References herein to a "clinical response" refer to response rates of 50% or greater. A "partial response" is defined herein as being a response rate of less than 50%.

As used herein, the term "combination", as applied to two or more compounds and/or agents (also referred to herein as the *components*), is intended tomay define material in which the two or more compounds/agents are associated. The terms "combined" and "combining" in this context are to be interpreted accordingly.

The association of the two or more compounds/agents in a combination may be physical or non-physical. Examples of physically associated combined compounds/agents include:

- compositions (e.g. unitary formulations) comprising the two or more compounds/agents in admixture (for example within the same unit dose);
- compositions comprising material in which the two or more compounds/agents are chemically/physicochemically linked (for example by crosslinking, molecular agglomeration or binding to a common vehicle moiety);
- compositions comprising material in which the two or more compounds/agents are chemically/physicochemically co-packaged (for example, disposed on or within lipid vesicles, particles (e.g. micro- or nanoparticles) or emulsion droplets);
- pharmaceutical kits, pharmaceutical packs or patient packs in which the two or more compounds/agents are co-packaged or co-presented (e.g. as part of an array of unit doses);

Examples of non-physically associated combined compounds/agents include:

- material (e.g. a non-unitary formulation) comprising at least one of the two or more compounds/agents together with instructions for the extemporaneous association of the at least one compound to form a physical association of the two or more compounds/agents;

- material (e.g. a non-unitary formulation) comprising at least one of the two or more compounds/agents together with instructions for combination therapy with the two or more compounds/agents;
- material comprising at least one of the two or more compounds/agents together with instructions for administration to a patient population in which the other(s) of the two or more compounds/agents have been (or are being) administered;
- material comprising at least one of the two or more compounds/agents in an amount or in a form which is specifically adapted for use in combination with the other(s) of the two or more compounds/agents.

As used herein, the term "combination therapy" is intended to define therapies which comprise the use of a combination of two or more compounds/agents (as defined above). Thus, references to "combination therapy", "combinations" and the use of compounds/agents "in combination" in this application may refer to compounds/agents that are administered as part of the same overall treatment regimen. As such, the posology of each of the two or more compounds/agents may differ: each may be administered at the same time or at different times. It will therefore be appreciated that the compounds/agents of the combination may be administered sequentially (e.g. before or after) or simultaneously, either in the same pharmaceutical formulation (i.e. together), or in different pharmaceutical formulations (i.e. separately). Simultaneously in the same formulation is as a unitary formulation whereas simultaneously in different pharmaceutical formulations is non-unitary. The posologies of each of the two or more compounds/agents in a combination therapy may also differ with respect to the route of administration.

As used herein, the term "pharmaceutical kit" defines an array of one or more unit doses of a pharmaceutical composition together with dosing means (e.g. measuring device) and/or delivery means (e.g. inhaler or syringe), optionally all contained within common outer packaging. In pharmaceutical kits comprising a combination of two or more compounds/agents, the individual compounds/agents may unitary or non-unitary formulations. The unit dose(s) may be contained within a blister pack. The pharmaceutical kit may optionally further comprise instructions for use.

As used herein, the term "pharmaceutical pack" defines an array of one or more unit doses of a pharmaceutical composition, optionally contained within common outer packaging. In pharmaceutical packs comprising a combination of two or more compounds/agents, the

individual compounds/agents may unitary or non-unitary formulations. The unit dose(s) may be contained within a blister pack. The pharmaceutical pack may optionally further comprise instructions for use.

As used herein, the term "patient pack" defines a package, prescribed to a patient, which contains pharmaceutical compositions for the whole course of treatment. Patient packs usually contain one or more blister pack(s). 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.

The combinations of the invention may produce a therapeutically efficacious effect relative to the therapeutic effect of the individual compounds/agents when administered separately.

The term "ancillary compound" as used herein may define a compound which yields an efficacious combination (as herein defined) when combined with the compound of the formulae (0) as defined herein. The ancillary compound may therefore act as an adjunct to the compound of the formulae (0) as defined herein, or may otherwise contribute to the efficacy of the combination (for example, by producing a synergistic or additive effect or improving the response rate, as herein defined).

The term "checkpoint targeting agent" is used herein to define a functional class of agents which act to initiate activation of a cell cycle checkpoint or agents that interfere with or modulate the normal action of the cell cycle checkpoint in replicating tumour cells. The term therefore covers various agents (including, for example, platinum compounds, nucleoside analogues, CDK inhibitors, taxanes, epothilones, vinca alkaloids, polo-like kinase inhibitors, CHK kinase inhibitors, inhibitors of the BUB kinase family and kinesin inhibitors) that target the cell cycle checkpoint.

The targeting of the checkpoint may be mediated by any mechanism, including for example *via* stabilisation of spindle microtubules (so preventing spindle contraction, as mediated e.g. by various taxanes) or by prevention of spindle formation (as mediated e.g. by various vinca alkaloids) or by agents which cause damage to cellular components (e.g. DNA as caused by the platinum compounds or nucleoside analogues) thus causing activation of the checkpoint during cell proliferation. Thus, the checkpoint targeting agents typically cause

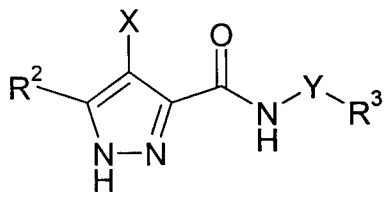
chromosome mis-alignment or premature cytokinesis leading to death of the tumour cell. Checkpoint targeting agents may be identified by various techniques known to those skilled in the art for assessing cell cycle dynamics (e.g. for detecting multinucleation events), including for example flow cytometry, DNA staining, Western blot analysis for cell cycle markers (e.g. cyclins) and direct visualization by various microscopic techniques (e.g. focal microscopy).

General Preferences and Definitions for compounds of formula (0)

A wide variety of compounds of the formula (0) find application in the combinations of the invention, as described in detail below. The compounds of formula (0) for use in the combinations of the invention correspond to those of formula (0) in WO 2005/012256 (the contents of which are incorporated herein by reference) and various possible substituents, sub-groups, embodiments and examples thereof as therein defined. The content of WO 2005/012256 describing the various possible substituents, subgroups, embodiments and examples of compounds of formula (0) is hereby incorporated herein by reference.

The formula (0) of WO 2005/012256 (PCT/GB2004/003179) is herein also referred to as formula (0) and references to formula (0) herein are to be interpreted accordingly.

Thus, the compound of formula (0) for use in the combinations of the invention has the formula:



(0)

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

wherein

X is a group R^1-A-NR^4- or a 5- or 6-membered carbocyclic or heterocyclic ring;

A is a bond, SO_2, C=O, NR^g(C=O) or O(C=O) wherein R^g is hydrogen or C_{1-4} hydrocarbyl optionally substituted by hydroxy or C_{1-4} alkoxy;

Y is a bond or an alkylene chain of 1, 2 or 3 carbon atoms in length;

R^1 is hydrogen; a carbocyclic or heterocyclic group having from 3 to 12 ring members; or a C_{1-8} hydrocarbyl group optionally substituted by one or more substituents selected from halogen (e.g. fluorine), hydroxy, C_{1-4} hydrocarbyloxy, amino, mono- or di-C_{1-4} hydrocarbylamino, and carbocyclic or heterocyclic groups having from 3 to 12 ring members, and wherein 1 or 2 of the carbon atoms of the hydrocarbyl group may optionally be replaced by an atom or group selected from O, S, NH, SO, SO_2;

R^2 is hydrogen; halogen; C_{1-4} alkoxy (e.g. methoxy); or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy);

R^3 is selected from hydrogen and carbocyclic and heterocyclic groups having from 3 to 12 ring members; and

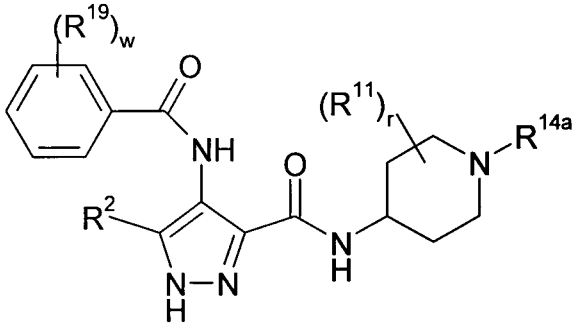
R^4 is hydrogen or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy),

so corresponding to formula (0) in WO 2005/012256 (PCT/GB2004/003179) and including the various possible substituents, subgroups, embodiments and examples thereof as defined in WO 2005/012256 (PCT/GB2004/003179), so that the general preferences and definitions defined in WO 2005/012256 (PCT/GB2004/003179) shall apply to each of the moieties X, Y, R^g, R^1 to R^4 and any substituent, moieties, sub-definition, sub-group or embodiment thereof, unless the context indicates otherwise.

In particular the carbocyclic and heterocyclic groups forming part of X, R^1 and R^3 may be optionally substituted as defined in WO 2005/012256.

Particular compounds of the formula (0) are those defined in, for example, the compounds of formulae (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII), and any sub-groups thereof in PCT/GB2004/003179 (WO 2005/012256), the compounds listed in PCT/GB2004/003179 (WO 2005/012256) and the compounds exemplified in the Examples section of PCT/GB2004/003179 (WO 2005/012256), the aforementioned sections of PCT/GB2004/003179 (WO 2005/012256) being hereby incorporated by reference.

A preferred sub-group of CDK inhibitor compounds within WO 2005/012256 is represented by the formula (Va):



(Va)

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

wherein R^{14a} is selected from hydrogen, C_{1-4} alkyl optionally substituted by fluoro (e.g. methyl, ethyl, n-propyl, i-propyl, butyl and 2,2,2-trifluoroethyl), cyclopropylmethyl, phenyl-C_{1-2} alkyl (e.g. benzyl), C_{1-4} alkoxycarbonyl (e.g. ethoxycarbonyl and t-butyloxycarbonyl), phenyl-C_{1-2} alkoxycarbonyl (e.g. benzyloxycarbonyl), C_{1-2}-alkoxy-C_{1-2} alkyl (e.g. methoxymethyl and methoxyethyl), and C_{1-4} alkylsulphonyl (e.g.methanesulphonyl), wherein the phenyl moieties when present are optionally substituted by one to three substituents selected from fluorine, chlorine, C_{1-4} alkoxy optionally substituted by fluoro or C_{1-2}-alkoxy, and C_{1-4} alkyl optionally substituted by fluoro or C_{1-2}-alkoxy;

w is 0, 1, 2 or 3;

R^2 is hydrogen or methyl, most preferably hydrogen;

r is 0, 1 or 2;

R^{11} is selected from hydrogen and C_{1-3} alkyl (and more preferably is selected from hydrogen and methyl and most preferably is hydrogen); and

R^{19} is selected from fluorine; chlorine; C_{1-4} alkoxy optionally substituted by fluoro or C_{1-2}-alkoxy; and C_{1-4} alkyl optionally substituted by fluoro or C_{1-2}-alkoxy.

Particular compounds within formula (VIb) of WO 2005/012256 include:

4-(2,6-difluoro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide;

4-(2,6-difluoro-benzoylamino)-1H-pyrazole-3-carboxylic acid (1-methyl-piperidin-4-yl)-amide;

4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide; and

4-(2-fluoro-6-methoxy-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide;

or salts or tautomers or N-oxides or solvates thereof.

The compound 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide may be present in the form of an acid addition salt which may be a salt formed with

hydrochloric acid or a salt as described in WO 2006/077426, the contents of which are incorporated herein by reference.

For example, the acid addition salt may be selected from salts formed with an acid selected from the group consisting of acetic, adipic, alginic, ascorbic (e.g. L-ascorbic), aspartic (e.g. L-aspartic), benzenesulphonic, benzoic, camphoric (e.g. (+) camphoric), capric, caprylic, carbonic, citric, cyclamic, dodecanoate, dodecylsulphuric, ethane-1,2-disulphonic, ethanesulphonic, fumaric, galactaric, gentisic, glucoheptonic, D-gluconic, glucuronic (e.g. D-glucuronic), glutamic (e.g. L-glutamic), α-oxoglutaric, glycolic, hippuric, isethionic, isobutyric, lactic (e.g. (+)-L-lactic and (±)-DL-lactic), lactobionic, laurylsulphonic, maleic, malic, (-)-L-malic, malonic, methanesulphonic, mucic, naphthalenesulphonic (e.g. naphthalene-2-sulphonic), naphthalene-1,5-disulphonic, nicotinic, oleic, orotic, oxalic, palmitic, pamoic, phosphoric, propionic, sebacic, stearic, succinic, sulphuric, tartaric (e.g. (+)-L-tartaric), thiocyanic, toluenesulphonic (e.g. *p*-toluenesulphonic), valeric and xinafoic acids.

One sub-group of acid addition salts includes salts formed with an acid selected from the group consisting of acetic, adipic, ascorbic (e.g. L-ascorbic), aspartic (e.g. L-aspartic), caproic, carbonic, citric, dodecanoic, fumaric, galactaric, glucoheptonic, gluconic (e.g. D-gluconic), glucuronic (e.g. D-glucuronic), glutamic (e.g. L-glutamic), glycolic, hippuric, lactic (e.g. (+)-L-lactic and (±)-DL-lactic), maleic, palmitic, phosphoric, sebacic, stearic, succinic, sulphuric, tartaric (e.g. (+)-L-tartaric) and thiocyanic acids.

More particularly the salts are acid addition salts formed with an acid selected from methanesulphonic acid and acetic acid, and mixtures thereof.

In one embodiment, the salt is an acid addition salt formed with methanesulphonic acid.

In another embodiment, the salt is an acid addition salt formed with acetic acid.

The salts may be prepared from 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide by the methods described in WO 2006/077426.

The salts may be substantially crystalline; i.e. they are from 50% to 100% crystalline, and more particularly they may be at least 50% crystalline, or at least 60% crystalline, or at least 70% crystalline, or at least 80% crystalline, or at least 90% crystalline, or at least 95% crystalline, or at least 98% crystalline, or at least 99% crystalline, or at least 99.5% crystalline, or at least 99.9% crystalline, for example 100% crystalline.

More preferably the salts may be those (or may be selected from the group consisting of those) that are 95% to 100 % crystalline, for example at least 98% crystalline, or at least 99% crystalline, or at least 99.5% crystalline, or at least 99.6% crystalline or at least 99.7% crystalline or at least 99.8% crystalline or at least 99.9% crystalline, for example 100% crystalline.

One example of a substantially crystalline salt is a crystalline salt formed with methanesulphonic acid. Another example of a substantially crystalline salt is a crystalline salt formed with acetic acid.

The salts of the invention, in the solid state, can be solvated (e.g. hydrated) or non-solvated (e.g. anhydrous).

In one embodiment, the salts are non-solvated (e.g. anhydrous). An example of a non-solvated salt is the crystalline salt formed with methanesulphonic acid.

In one embodiment, the salt is a methanesulphonic acid salt of 4-(2,6-dichlorobenzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide mesylate salt which is crystalline and is characterised by any one or more (in any combination) or all of the following parameters, namely that the salt:

(a) has a crystal structure as set out in Figures 1 and 2 of WO 2006/077426; and/or

(b) has a crystal structure as defined by the coordinates in Example 2 of WO 2006/077426; and/or

(c) has crystal lattice parameters at 93 K *a*=8.90(10), *b*=12.44(10), *c*=38.49(4) Å, $\alpha = \beta = \gamma = 90°$; and/or

(d) has a crystal structure that belongs belong to an orthorhombic space group such as P*bca* (# 61); and/or

(e) has an X-ray powder diffraction pattern characterised by the presence of major peaks at the diffraction angles (2θ) and interplanar spacings (d) set forth in Table A of WO 2006/077426, and optionally Table B of WO 2006/077426; for example wherein the X-ray powder diffraction pattern is characterised by the presence of major peaks at the diffraction angles (2θ), interplanar spacings (d) and intensities set forth in Table C of WO 2006/077426; and/or

(f) exhibits peaks at the same diffraction angles as those of the X-ray powder diffraction pattern shown in Figure 3 of WO 2006/077426 and optionally wherein the peaks have the same relative intensity as the peaks in Figure 3 of WO 2006/077426; and/or

(g) has an X-ray powder diffraction pattern substantially as shown in Figure 3 of WO 2006/077426; and/or

(h) is anhydrous and exhibits an endothermic peak at 379-380 °C e.g. 379.8 °C when subjected to DSC; and/or

(i) exhibits an infra-red spectrum, when analysed using the KBr disc method , that contains characteristic peaks at 3233, 3002, 2829, 1679, 1632, 1560, 1430, 1198, 1037, 909 and 784 cm^{-1} .

Particular pharmaceutical compositions comprising an aqueous solution containing an acid addition salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide (such as the mesylate and acetate and mixtures thereof, and preferably the mesylate) are also described in WO 2006/077426.

Methods of Treatment using this compound are described in WO 2005/012256 and WO 2006/077426. Methods of Diagnosis of 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 CDK.

A preferred compound of the formula (0) is 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide.

Accordingly, a preferred combination comprises (or consists essentially of) 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide.

A further combination of the invention comprises (or consists essentially of) 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide in the form of a salt selected from the acid addition salts formed with hydrochloric acid, methanesulphonic acid and/or acetic acid.

In addition combination of the invention comprises (or consists essentially of) the methane sulphonic acid salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide.

In addition combination of the invention comprises (or consists essentially of) methane sulphonic acid salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide is in crystalline form.

Methods for the Preparation of Compounds of the Formula (0) are as described in WO 2005/012256, WO 2006/077416 and WO 2006/077426, the contents of which are incorporated herein by reference. In particular, the contents of WO 2005/012556 which relate to the relevant processes at pages 91 to 101 are hereby incorporated herein by reference. In particular, the contents of WO 2006/074416 which relate to the relevant processes at pages 33 to 39 are hereby incorporated herein by reference. In particular, the contents of WO 2006/077426 which relate to the relevant processes at pages 30 to 36 are hereby incorporated herein by reference.

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

A reference to a particular ancillary compound or compound of the formulae (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) 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.

Such salts, solvates, tautomers, isomers, N-oxides, esters, prodrugs and isotopes are described in WO 2006/077416 at pages 18 to 25 in relation to compounds of "formula (I)" therein. This disclosure in WO 2006/077416 is hereby incorporated herein by reference, and the teachings relating to salts, solvates, tautomers, isomers, N-oxides, esters, prodrugs and isotopes of compounds of formula (I) are to be extended to the various compounds (including *inter alia* any of the compounds of formula (0) and the ancillary compounds described herein).

Methanesulphonic acid and acetic acid addition salts of compound 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide

The combinations of the invention may comprise any of the compounds, salts, solvates, tautomers and isotopes thereof and, where the context admits, N-oxides, other ionic forms and prodrugs, as described below.

References to the compound 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide and its acid addition salts include within their scope all solvates, tautomers and isotopes thereof and, where the context admits, N-oxides, other ionic forms and prodrugs.

The acid addition salt may be selected from salts formed with an acid selected from the group consisting of acetic, adipic, alginic, ascorbic (e.g. L-ascorbic), aspartic (e.g. L-aspartic), benzenesulphonic, benzoic, camphoric (e.g. (+) camphoric), capric, caprylic, carbonic, citric, cyclamic, dodecanoate, dodecylsulphuric, ethane-1,2-disulphonic, ethanesulphonic, fumaric, galactaric, gentisic, glucoheptonic, D-gluconic, glucuronic (e.g. D-glucuronic), glutamic (e.g. L-glutamic), α-oxoglutaric, glycolic, hippuric, isethionic, isobutyric, lactic (e.g. (+)-L-lactic and (±)-DL-lactic), lactobionic, laurylsulphonic, maleic, malic, (-)-L-malic, malonic, methanesulphonic, mucic, naphthalenesulphonic (e.g. naphthalene-2-sulphonic), naphthalene-1,5-disulphonic, nicotinic, oleic, orotic, oxalic, palmitic, pamoic, phosphoric, propionic, sebacic, stearic, succinic, sulphuric, tartaric (e.g. (+)-L-tartaric), thiocyanic, toluenesulphonic (e.g. *p*-toluenesulphonic), valeric and xinafoic acids.

One sub-group of acid addition salts includes salts formed with an acid selected from the group consisting of acetic, adipic, ascorbic (e.g. L-ascorbic), aspartic (e.g. L-aspartic), caproic, carbonic, citric, dodecanoic, fumaric, galactaric, glucoheptonic, gluconic (e.g. D-gluconic), glucuronic (e.g. D-glucuronic), glutamic (e.g. L-glutamic), glycolic, hippuric, lactic (e.g. (+)-L-lactic and (±)-DL-lactic), maleic, palmitic, phosphoric, sebacic, stearic, succinic, sulphuric, tartaric (e.g. (+)-L-tartaric) and thiocyanic acids.

More particularly the salts are acid addition salts formed with an acid selected from methanesulphonic acid and acetic acid, and mixtures thereof.

In one embodiment, the salt is an acid addition salt formed with methanesulphonic acid.

In another embodiment, the salt is an acid addition salt formed with acetic acid.

For convenience the salts formed from methanesulphonic acid and acetic acid may be referred to herein as the methanesulphonate or mesylate salts and acetate salts respectively.

In the solid state, the salts can be crystalline or amorphous or a mixture thereof.

In one embodiment, the salts are amorphous.

In an amorphous solid, the three dimensional structure that normally exists in a crystalline form does not exist and the positions of the molecules relative to one another in the

amorphous form are essentially random, see for example Hancock *et al. J. Pharm. Sci.* (1997), 86, 1).

In another embodiment, the salts are substantially crystalline; i.e. they are from 50% to 100% crystalline, and more particularly they may be at least 50% crystalline, or at least 60% crystalline, or at least 70% crystalline, or at least 80% crystalline, or at least 90% crystalline, or at least 95% crystalline, or at least 98% crystalline, or at least 99% crystalline, or at least 99.5% crystalline, or at least 99.9% crystalline, for example 100% crystalline.

In a further embodiment, the salts are selected from the group consisting of salts that are from 50% to 100% crystalline, salts that are at least 50% crystalline, salts that are at least 60% crystalline, salts that are at least 70% crystalline, salts that are at least 80% crystalline, salts that are at least 90% crystalline, salts that are at least 95% crystalline, salts that are at least 98% crystalline, salts that are at least 99% crystalline, salts that are at least 99.5% crystalline, and salts that are at least 99.9% crystalline, for example 100% crystalline.

More preferably the salts may be those (or may be selected from the group consisting of those) that are 95% to 100 % crystalline, for example at least 98% crystalline, or at least 99% crystalline, or at least 99.5% crystalline, or at least 99.6% crystalline or at least 99.7% crystalline or at least 99.8% crystalline or at least 99.9% crystalline, for example 100% crystalline.

One example of a substantially crystalline salt is a crystalline salt formed with methanesulphonic acid.

Another example of a substantially crystalline salt is a crystalline salt formed with acetic acid.

The salts, in the solid state, can be solvated (e.g. hydrated) or non-solvated (e.g. anhydrous).

In one embodiment, the salts are non-solvated (e.g. anhydrous). An example of a non-solvated salt is the crystalline salt formed with methanesulphonic acid as defined herein.

The term "anhydrous" as used herein does not exclude the possibility of the presence of some water on or in the salt (e.g a crystal of the salt). For example, there may be some

water present on the surface of the salt (e.g. salt crystal), or minor amounts within the body of the salt (e.g. crystal). Typically, an anhydrous form contains fewer than 0.4 molecules of water per molecule of compound, and more preferably contains fewer than 0.1 molecules of water per molecule of compound, for example 0 molecules of water.

In another embodiment, the salts are solvated. Where the salts are hydrated, they can contain, for example, up to three molecules of water of crystallisation, more usually up to two molecules of water, e.g. one molecule of water or two molecules of water. Non-stoichiometric hydrates may also be formed in which the number of molecules of water present is less than one or is otherwise a non-integer. For example, where there is less than one molecule of water present, there may be for example 0.4, or 0.5, or 0.6, or 0.7, or 0.8, or 0.9 molecules of water present per molecule of compound.

Other solvates include alcoholates such as ethanolates and isopropanolates.

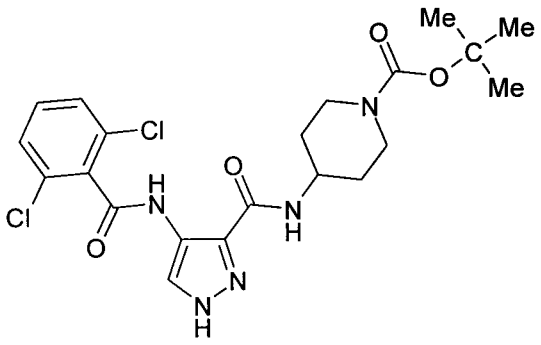
The salts can be synthesized from the parent compound 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide by conventional chemical methods such as methods described in *Pharmaceutical Salts: Properties, Selection, and Use*, P. Heinrich Stahl (Editor), Camille G. Wermuth (Editor), ISBN: 3-90639-026-8, Hardcover, 388 pages, August 2002. Generally, such salts can be prepared by reacting the parent compound 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide with the appropriate 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.

One method of preparing an acid addition salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide comprises forming a solution of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide free base in a solvent (typically an organic solvent) or mixture of solvents, and treating the solution with an acid to form a precipitate of the acid addition salt.

The acid may be added as a solution in a solvent which is miscible with the solvent in which the free base is dissolved. The solvent in which the free base is initially dissolved may be one in which the acid addition salt thereof is insoluble. Alternatively, the solvent in which the free base is initially dissolved may be one in which the acid addition salt is at least partially soluble, a different solvent in which the acid addition salt is less soluble subsequently being added such that the salt precipitates out of solution.

In an alternative method of forming an acid addition salt, 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide is dissolved in a solvent comprising a volatile acid and optionally a co-solvent, thereby to form a solution of the acid addition salt with the volatile acid, and the resulting solution is then concentrated or evaporated to isolate the salt. An example of an acid addition salt that can be made in this way is the acetate salt.

In another aspect, the combination of the invention includes an acid addition salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide as defined herein, obtained (or obtainable) by treating a compound of the formula (X):



(X)

with an organic or inorganic acid as defined herein, other than hydrochloric acid, in an organic solvent to remove the *tert*-butyloxycarbonyl group and form an acid addition salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide with the organic or inorganic acid, and optionally isolating the acid addition salt thus formed.

The salt is typically precipitated from the organic solvent as it is formed and hence can be isolated by separation of the solid from the solution, e.g. by filtration.

One salt form can be converted to the free base and optionally to another salt form by methods well known to the skilled person. For example, the free base can be formed by passing the salt solution through a column containing an amine stationary phase (e.g. a Strata-NH_2 column). Alternatively, a solution of the salt in water can be treated with sodium bicarbonate to decompose the salt and precipitate out the free base. The free base may then be combined with another acid by one of the methods described above or elsewhere herein.

The methanesulphonate salt form is particularly advantageous because of its good stability at elevated temperatures and in conditions of high relative humidity, its non-hygroscopicity (as defined herein), absence of polymorph and hydrate formation, and stability in aqueous

conditions. Moreover, it has excellent water solubility and has better physiochemical properties (such as a high melting point) relative to other salts.

The term 'stable' or 'stability' as used herein includes chemical stability and solid state (physical) stability. The term 'chemical stability' means that the compound can be stored in an isolated form, or in the form of a formulation in which it is provided in admixture with for example, pharmaceutically acceptable carriers, diluents or adjuvants as described herein, under normal storage conditions, with little or no chemical degradation or decomposition. 'Solid-state stability' means the compound can be stored in an isolated solid form, or the form of a solid formulation in which it is provided in admixture with, for example, pharmaceutically acceptable carriers, diluents or adjuvants as described herein, under normal storage conditions, with little or no solid-state transformation (e.g. hydration, dehydration, solvatisation, desolvatisation, crystallisation, recrystallisation or solid-state phase transition).

The terms "non-hygroscopic" and "non-hygroscopicity" and related terms as used herein refer to substances that absorb less than 5% by weight (relative to their own weight) of water when exposed to conditions of high relative humidity, for example 90% relative humidity, and/or do not undergo changes in crystalline form in conditions of high humidity and/or do not absorb water into the body of the crystal (internal water) in conditions of high relative humidity.

Preferred salts for use in the combinations of the invention are acid addition salts (such as the mesylate and acetate and mixtures thereof as defined herein) having a solubility in a given liquid carrier (e.g. water) of greater than 15 mg/ml of the liquid carrier (e.g. water), more typically greater than 20 mg/ml, preferably greater than 25 mg/ml, and more preferably greater than 30 mg/ml.

In another aspect, there is provided a combination comprising an aqueous solution containing an acid addition salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide (such as the mesylate and acetate and mixtures thereof as defined herein, and preferably the mesylate) in a concentration of greater than 15 mg/ml, typically greater than 20 mg/ml, preferably greater than 25 mg/ml, and more preferably greater than 30 mg/ml..

In a preferred embodiment, the combination comprises an aqueous solution containing an acid addition salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-

4-ylamide selected from an acetate or methanesulphonate salt or a mixture thereof in a concentration of greater than 15 mg/ml, typically greater than 20 mg/ml, preferably greater than 25 mg/ml, and more preferably greater than 30 mg/ml.

In another aspect, the combination of the invention includes an aqueous solution of an acid addition salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide (such as the mesylate and acetate and mixtures thereof as defined herein), wherein the aqueous solution has a pH of 2 to 12, for example 2 to 9, and more particularly 4 to 7.

In the aqueous solutions defined above, the acid addition salt may be any of the salts described herein but, in one preferred embodiment, is a mesylate or acetate salt as defined herein, and in particular the mesylate salt.

The combinations of the invention may include an aqueous solution of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide in protonated form together with one or more counter ions and optionally one or more further counter ions. In one embodiment one of the counter ions is selected from methanesulphonate and acetate. In another embodiment one of the counter ions is from the formulation buffer as described herein such as acetate. In a further embodiment there may be one or more further counter ions such as a chloride ion (e.g. from saline).

The combinations of the invention may include an aqueous solution of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide in protonated form together with one or more counter ions selected from methanesulphonate and acetate and optionally one or more further counter ions such as a chloride ion.

In the situation where there is more than one counter ion the aqueous solution of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide in protonated form will potentially contain a mixture of counter ions for example a mixture of methanesulphonate and acetate counter ions and optionally one or more further counter ions such as a chloride ion.

The combinations of the invention may include an aqueous solution of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide in protonated form together with one or more counter ions selected from methanesulphonate and acetate and optionally one or more further counter ions such as a chloride ion, and a mixture thereof.

The aqueous solutions can be formed *inter alia* by dissolving a mesylate salt in a solution of acetate ions (e.g an acetate buffer) or by dissolving an acetate salt in a solution of mesylate ions. The mesylate and acetate ions may be present in the solution in a mesylate:acetate ratio of from 10:1 or less, for example 10:1 to 1:10, more preferably less then 8:1, or less than 7:1, or less than 6:1, or less than 5:1 or less than 4:1 or less than 3:1 or less than 2:1 or less than 1:1, more particularly from 1:1 to 1:10. In one embodiment, the mesylate and acetate ions are present in the solution in a mesylate:acetate ratio of from 1:1 to 1:10, for example 1:1 to 1:8, or 1:1 to 1:7 or 1:1 to 1:6 or 1:1 to 1:5, e.g. approximately 1:4.8.

The aqueous solutions of the salts may be buffered or unbuffered but in one embodiment are buffered.

In the context of the acid addition salt formed with methanesulphonic acid, a preferred buffer is a buffer formed from acetic acid and sodium acetate, for example at a solution pH of approximately 4.6. At this pH and in the acetate buffer, the methanesulphonic acid salt has a solubility of about 35 mg/ml.

The salts for use in the combinations 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 salt forms therefore also form part of the invention.

Particular pharmaceutical compositions comprising an aqueous solution containing an acid addition salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide (such as the mesylate and acetate and mixtures thereof, and preferably the mesylate) are also described in WO 2006/077426.

Methods of Treatment using compounds of formula (0) are described in WO 2005/012256 pages 105 to 107, and WO 2006/077426 pages 58 to 61, and are further described herein. Methods of Diagnosis of 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 CDK are described in WO 2005/012256 pages 107 to 111, and WO 2006/077426 pages 62 to 65, and are further described herein.

Methods for the Preparation of Compounds of the Formula (0) are as described in WO 2005/012256, WO 2006/077416 and WO 2006/077426, the contents of which are incorporated herein by reference. In particular, the contents of WO 2005/012556 which relate to the relevant processes at pages 91 to 101 are hereby incorporated herein by reference. In particular, the contents of WO 2006/074416 which relate to the relevant processes at pages 33 to 39 are hereby incorporated herein by reference. In particular, the contents of WO 2006/077426 which relate to the relevant processes at pages 30 to 36 are hereby incorporated herein by reference.

Such salts, solvates, tautomers, isomers, N-oxides, esters, prodrugs and isotopes are described in WO 2005/012256 at pages 81 to 88 in relation to compounds of "formula (0)" therein (which correspond to the compounds of formula (0) herein, as explained above) and in WO 2006/077426. This disclosures in WO 2005/012256 and WO 2006/077426 are hereby incorporated herein by reference, and the teachings relating to salts, solvates, tautomers, isomers, N-oxides, esters, prodrugs and isotopes of compounds of formula (0) are to be extended to the various compounds (including *inter alia* any of the compounds of formula (0) or the auxiliary compounds described herein).

Biological Activity of the compounds of formula (0)

The biological activity of the compounds of formula (0) are described at pages 88 to 91 of WO 2005/012556 and at pages 39 to 49 of WO 2006/077426, which disclosure is hereby incorporated herein by reference.

The compounds of the formula (0) are inhibitors of cyclin dependent kinases. For example, the compounds of the formula (0) are inhibitors of 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.

The compounds of the formula (0) 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, the compounds of the formula (0) will be useful in providing a means of arresting, or recovering control of, the cell cycle in abnormally dividing cells. The compounds will therefore prove useful in treating or preventing proliferative disorders such as cancers. The compounds of the formula (0) will also 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 the compounds of the formula (0) 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.

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 formula (0) as inhibitors of cyclin dependent kinases and glycogen synthase kinase-3 can be measured using the assays set forth herein and the level of activity exhibited by a given compound can be defined in terms of the IC_{50} value.

Biological Activity of the ancillary compounds

Some of the ancillary agents for use in the combinations of the invention are inhibitors of VEGFR activity. In addition some are inhibitors of FGFR activity. As such, they are expected to be useful in providing a means of preventing the growth or inducing apoptosis of neoplasias, particularly by inhibiting angiogenesis. It is therefore anticipated that the combinations of the invention will prove useful in treating or preventing proliferative disorders such as cancers. In particular tumours with activating mutants of VEGFR or upregulation of VEGFR may be particularly sensitive to the inhibitors. Patients with activating mutants of any of the isoforms of the specific VEGFR as discussed herein may also find treatment with VEGFR inhibitors particularly beneficial. Also particular tumours with activating mutants or upregulation or overexpression of any of the isoforms of FGFR such as FGFR2 or FGFR3 may be particularly sensitive to the combinations of the invention and thus patients as discussed herein with such particular tumours may also find treatment with the combinations of the invention particularly beneficial. It may be preferred that the treatment is related to or directed at a mutated form of a receptor tyrosine kinase, such as discussed above.

The the ancillary agents for use in the combinations of the invention having Flt3, C-abl, and PDK1 inhibitory activity, will be particularly useful as constituents of combinations in the treatment or prevention of the following diseases and leukemias: Chronic Myeloid Leukaemia (CML); imatinib resistant CML; acute myeloid leukemias (AML); and acute lymphoblastic leukemia (ALL).

Therefore, in a further embodiment the combinations of the invention are used to treat Chronic Myeloid Leukaemia (CML); imatinib resistant CML; acute myeloid leukemias (AML); and acute lymphoblastic leukemia (ALL).

It may be preferred that the treatment is related to or directed at a mutated form of a kinase, such as discussed herein. Diagnosis of tumours with such mutations could be performed using techniques known to a person skilled in the art and as described herein such as RTPCR and FISH.

The the ancillary agents for use in the combinations of the invention having FGFR such as FGFR3, Ret, or cSrc inhibitory activity, will be particularly useful in the treatment or prevention of the following diseases: papillary thyroid carcinoma; multiple endocrine neoplasia (MEN) types 2A and 2B; familial medullary thyroid carcinoma (FMTC); Hirschsprung's disease; Apert (AP) syndrome; Crouzon syndrome; Jackson-Weiss syndrome; Beare-Stevenson cutis gyrata syndrome; Pfeiffer Syndrome (PS); and multiple myelomas.

Therefore, in a further embodiment the combinations of the invention are used to treat multiple myelomas, abnormalities in human skeletal development such as Apert (AP) syndrome, Crouzon syndrome, Jackson-Weiss syndrome, Beare-Stevenson cutis gyrata syndrome and Pfeiffer Syndrome (PS), thyroid cancers such as papillary thyroid carcinoma, familial medullary thyroid carcinoma (FMTC), multiple endocrine neoplasia (MEN) types 2A and 2B and Hirschsprung's disease.

Ancillary compounds for use according to the invention

Any of a wide variety of ancillary compounds may be used in the combinations of the invention. The ancillary compounds may be anti-cancer agents.

Preferably, the ancillary compounds for use in the combinations of the invention are selected from the following classes:

1. epothilones;
2. aurora inhibitors;
3. Hsp90 inhibitors;
4. tyrosine kinase inhibitors
5. EGF antibodies;
6. decitabine and azacytidine DNA methyl transferase inhibitors;
7. cytokines and cytokine activating agents;
8. retinoids and rexinoids;
9. Selective immunoresponse modulators;

10. Checkpoint targeting agents;
11. DNA repair inhibitors;
12. Inhibitors of G-protein coupled receptor inhibitors;
13. a combination of two or more of the foregoing classes.

In one embodiment of the invention the one or more ancillary agents of the invention are selected from the classes 1 to 6 above.

In embodiments where the combination of the invention comprises two or more ancillary compounds, then the two or more ancillary compounds are preferably independently selected from the classes 1 to 12 set out above.

A reference to a particular ancillary compound herein is intended to include ionic, salt, solvate, isomers, tautomers, N-oxides, ester, prodrugs, isotopes and protected forms thereof (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).

1. Epothilones

Definition: As used herein, the term "epothilone" is used to define a class of cytotoxic macrolides with a similar mechanism of action to paclitaxel but with the potential advantage of activity in taxane-resistant settings in preclinical models. The epothilones ixabepilone, patupilone, BMS-310705, KOS-862 and ZK-EPO are in early clinical trials for cancer treatment. Phase I studies have shown that dose-limiting toxicities of epothilones are generally neurotoxicity and neutropenia although initial studies with patupilone indicated that diarrhoea was dose limiting. Neuropathy induced by ixabepilone may be schedule dependent. Response rates in taxane-refractory metastatic breast cancer are relatively modest, but ixabepilone and patupilone have shown promising efficacy in hormone-refractory metastatic prostate cancer and in taxane-refractory ovarian cancer.

Technical Background; Epothilones A and B were originally isolated as anti-fungal fermentation products of the myxobacteria *Sorangium cellulosum*. Shortly thereafter these agents were demonstrated to stabilize microtubules and induce mitotic arrest. Though their cytotoxic activity relies on the same mechanism as that of the taxanes, the epothilones have a couple of key advantages. Firstly they are not substrates for the multi-drug

resistance pump P-gylycoprotein. Secondly they are easier both to produce (because of their bacterial origin) and to manipulate. Chemical syntheses, either total or partial, of these molecules and their analogs allows for modification to enhance their efficacy Mani *et al.* Anticancer Drugs 2004;15(6):553–8). Several epothilones or epothilone-derivatives have been shown effective against cell lines and tumor xenografts and are now in clinical trials (Goodin *et al.* J Clin Oncol 2004;22(10): 2015–25). An unexpected source for the identification of microtubule stabilizing agents has been marine organisms. Laulimalide and isolaulimalide are natural products of the marine sponge *Cacospongia mycofijiensis* with strong paclitaxel-like activity, even against P-gp expressing cell lines. Eluetherobin, similar in both respects, is a product of the *Eleutherobia* species of soft coral.

Biological Activity; Formation of microtubules involves polymerization of heterodimeric α/ß-tubulin subunits with multiple isoforms of both α- and ß-tubulin present in human cells. Intact microtubule function is required for formation and functioning of the mitotic spindle, and cells treated with agents that bind either tubulin subunits or polymerized microtubules exhibit alterations in spindle formation, as well as arrest at the G2/M phase of the cell cycle, which is associated with induction of apoptosis. Compounds that target microtubules are potent cytotoxic agents, exemplified by the convergent evolution of microtubule-targeting compounds by a variety of plant and marine species. Published studies of three epothilones in current clinical development, epothilone B, aza-epothilone B, and desoxyepothilone B, indicate that these compounds exhibit broad spectrum antitumor activity in cell culture models and in xenografts. Furthermore, epothilones are generally more cytotoxic than paclitaxel in cell culture studies, with IC_{50} values in the sub- or low nanomolar range in a variety of tumor cell lines (Bollag *et al.* Cancer Res 55:2325-2333, 1995; Lee *et al.* Clin Cancer Res 7:1429-1437, 2001; Chou *et al.* Proc Natl Acad Sci U S A 95:9642-9647, 1998; Newman *et al.* Cancer Chemother Pharmacol 48:319-326, 2001). Preclinical studies also demonstrated important differences with regard to drug resistance mechanisms between epothilones and taxanes. In particular, overexpression of P-glycoprotein minimally affects the cytotoxicity of epothilone B, aza-epothilone B, and desoxyepothilones in cell culture models. Comparison of the cytotoxic effects of epothilone B, aza-epothilone B, and desoxyepothilone B among P-glycoprotein-overexpressing cell lines suggests that desoxyepothilone B is least affected, whereas aza-epothilone B is most affected by P-glycoprotein expression. However, it should be noted that differences among the IC_{50}s of these compounds in P-glycoprotein–overexpressing cell lines are small compared with the differences between these values and IC_{50}s for paclitaxel in these cell

lines. Although the significance of P-glycoprotein expression in clinical resistance to taxanes remains uncertain, these results suggest that epothilones may be more active than taxanes in patients with malignancies characterized by high levels of P-glycoprotein expression. *In vivo* studies indicate that epothilones are active in paclitaxel-sensitive and -resistant tumor models using a variety of schedules. When administered intravenously to mice using intermittent daily or weekly schedules, aza-epothilone B is highly active in ovarian, colon, and breast xenografts and induces cures in an ovarian xenograft model (Pat-7) that is resistant to paclitaxel. Notably, unlike paclitaxel, aza-epothilone B is effective when administered orally in preclinical models. This phenomenon likely relates to the expression of P-glycoprotein in intestinal mucosa, resulting in poor absorption of paclitaxel but not epothilones.

Problems; Sensory neuropathy and myelosuppression has been documented with epothilones

Preferences; Existing structure-activity data provide some insight into the interaction between epothilones and microtubules. Results from several groups indicate that modifications at or near the C12–13 epoxide can affect microtubule-stabilizing activity (Wartmann and Altmann, Curr Med Chem Anti-Canc Agents 2:123-148, 2002). For example, addition of a methyl group to epothilone A at position C12 yields epothilone B, which is approximately twice as potent as epothilone A or paclitaxel in inducing tubulin polymerization *in vitro* (Kowalski *et al.* J Biol Chem 272: 2534-2541, 1997; Nicolaou *et al.* Nature 387:268-272, 1997, abstr 428). In addition, it is clear that an epoxide at C12–13 is not required for microtubule-binding, because desoxyepothilone B (also known as epothilone D or KOS-862) lacks the C12–13 epoxide and is a more potent microtubule stabilizer *in vitro* than epothilone A or B. Less data are available regarding the effects of modifying other regions of epothilone. Despite attempts to improve microtubule binding by altering the C9–C12 region (on the basis of molecular modeling), alterations in this area resulted in loss of cytotoxic activity. By contrast, replacement of the lactone oxygen of epothilone B with a lactam (aza-epothilone B, also known as BMS-247550) does not impair microtubule-polymerizing activity or cytotoxicity. Although a variety of other epothilone analogs have been synthesized, it should be noted that increasing microtubule-stabilizing activity does not always result in increased cytotoxicity, presumably because of the importance of other variables such as cellular accumulation and metabolic stability (Wartmann and Altmann, Curr Med Chem Anti-Canc Agents 2:123-148, 2002). Indeed,

replacement of the methyl group at C12 position of desoxyepothilone B with a propanol group results in a compound that is as effective as desoxyepothilone B against the leukemic cell line CCRF-CEM but is significantly less active against a P-glycoprotein-overexpressing subline (IC_{50} of 17 nmol/L for desoxyepothilone B *v* 167 nmol/L for the propanol derivative) (Chou *et al.* Proc Natl Acad Sci U S A 95:9642-9647, 1998). Additional modifications of naturally occurring epothilones have been made in an effort to improve solubility, such as BMS-310705, which is a C-21-substituted derivative of epothilone B (Lee *et al.* Proc Am Assoc Cancer Res 43:a3928, 2002).

Specific embodiments: In one embodiment, the epothilone compound is BMS-247550. In another embodiment, the epothilone compound is Desoxyeopthilone and in another embodiment the epothilone compound is BMS-310705

Posology: BMS-247550 is dosed either 40 mg/m^2 over 3 hours every 21 days or 6 mg/m^2 administered over 1 hour daily times 5 days every 3 weeks. Because of the frequency of mucositis and neutropenia in the first 18 patients on the single-dose every-3-week schedule, the dose was reduced to 32 mg/m^2. EPO906 is dosed either at 2.5 mg/m^2 weekly for 3 weeks followed by 1 week of rest in one trial, and 6 mg/m^2 once every 3 weeks. KOS-862 is scheduled at either a single dose every 3 weeks, a daily dose times 3 every 3 weeks, a fixed rate dose every 3 weeks, and a weekly dose for 3 weeks with 1 week rest.

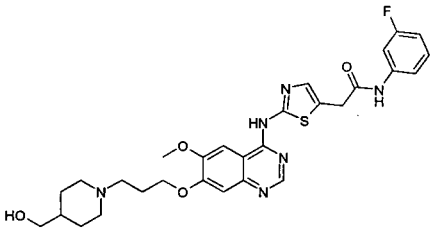
2. Aurora inhibitors

In one embodiment of the invention, the ancillary compound is an inhibitor of Aurora kinase(s).

Definition: The term "Aurora kinase inhibitor" (or simply "Aurora inhibitor") as used herein refers to compounds that inhibit or modulate the activity of any of the Aurora kinase isoforms A, B and/or C as described herein, including the ionic, salt, solvate, isomers, tautomers, N-oxides, ester, prodrugs, isotopes and protected forms thereof (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), as described above.

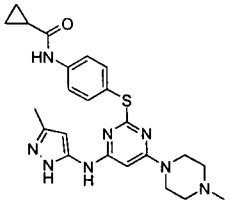
Technical Background: Aurora kinases play a role in regulating the cell cycle and in particular in the process of cellular mitosis (they have an important role in the mitotic phase of the cell cycle). Therefore, Aurora kinase inhibitors may find application in the treatment of diseases in which there is a disorder of proliferation, cell division, differentiation such as cancer. In particular tumours with mitotic and or spindle defects may be particularly sensitive to CDK inhibitors.

Examples of Aurora kinase inhibitors include AZD1152, MK0457 (VX680), PHA-739358, MLN-8054, MP-235 in particular MK0457 (VX680), PHA-739358, MLN-8054, MP-235. AZD1152 is undergoing clinical evaluation. AZD1152 is a pro-drug which is converted rapidly to the active moiety AZD1152-HQPA in the plasma (AZD-1152 hydroxy-QPA, structure shown below). In early studies in patients with advanced solid malignancies, AZD1152 given in a 2hr infusion weekly, induces p53 independent cellular multinucleation and polyploidy, resulting in apoptosis. These early studies indicate neutropenia is the dose-limiting toxicology (ASCO 2006).



AZD1152 and AZD1152-HQPA can be synthesized as described in WO 02/00649 or by processes analogous thereto.

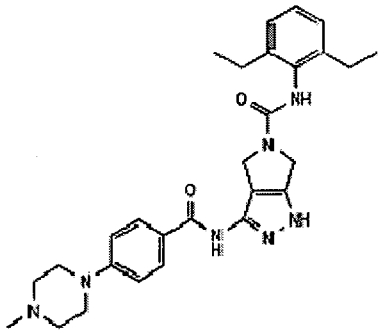
MK0457 (VX-680) is undergoing clinical evaluation. MK0457 has been given to patients with refractory malignancies in a continuous 5 day infusion every 28 days. These early studies indicate neutropenia is the dose-limiting toxicology (ASCO 2006).



MK0457 can be synthesised as described in Harrington et al, *Nat Med.* 2004 Mar; 10(3):262-7 and WO 02/057259, WO 02/059111, WO 02/059112, WO 02/062789, WO

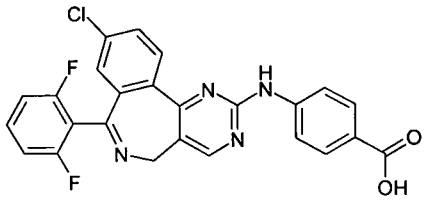
02/068415, WO 02/066461, WO 02/050065, WO 02/050066 and in particular WO 2004/000833, and by processes analogous thereto.

PHA-739358, the structure of which is shown below, is currently being evaluated by Nerviano Medical Sciences Srl in a multicenter phase 1 dose escalation clinical trials.



PHA-739358 can be synthesised as described in Fancelli et al, Journal of Medicinal Chemistry (2005), 48(8), 3080-3084 and WO02/12242 and by processes analogous thereto.

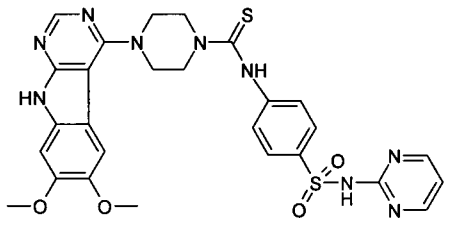
MLN-8054 the chemical name of which is 4-[9-Chloro-7-(2,6-difluoro-phenyl)-5H-benzo[c]pyrimido[4,5-e]azepin-2-ylamino]-benzoic acid (structure shown below) is currently being evaluated in multicenter phase 1 dose escalation clinical trials in patients with refractory solid tumours including lymphomas.



MLN-8054 can be synthesised as described in WO 2005/111039, and by processes analogous thereto.

SuperGen, following the acquisition of Montigen in April 2006, is investigating a series of small molecule Aurora-2 kinase inhibitors that induce apoptosis and inhibit cell division, including MP-235 (HPK-62) (4-(6,7-Dimethoxy-9H-1,3,9-triaza-fluoren-4-yl)-piperazine-1-carbothioic acid [4-(pyrimidin-2-ylsulfamoyl)-phenyl]-amide, structure shown), for the

potential treatment of various cancers, including pancreatic cancer. MP-235 can be synthesised as described in WO 2005/037825 and by processes analogous thereto



3. Hsp90 Inhibitors

In one embodiment of the invention, the ancillary agent is an inhibitor of HSP90.

Definition: The term Hsp90 inhibitor as used herein refers to compounds that inhibit or modulate the activity of Heat Shock Protein 90 as described herein.

Technical Background: In response to cellular stresses including heat, toxins, radiation, infection, inflammation, and oxidants, all cells produce a common set of heat shock proteins (Hsps) (Macario & de Macario 2000). Most heat shock proteins act as molecular chaperones. Chaperones bind and stabilize proteins at intermediate stages of folding and allow proteins to fold to their functional states. Hsp90 is the most abundant cytosolic Hsp under normal conditions. There are two human isoforms of Hsp90, a major form Hsp90α and minor form Hsp90β. Hsp90 binds proteins at a late stage of folding and is distinguished from other Hsps in that most of its protein substrates are involved in signal transduction. It has been shown that ATP bound at the N-terminal pocket of Hsp90 is hydrolysed. This ATPase activity results in a conformational change in Hsp90 that is required to enable conformational changes in the client protein.

Activation of Hsp90 is further regulated through interactions with a variety of other chaperone proteins and can be isolated in complex with other chaperones including Hsp70, Hip, Hop, p23, and p50cdc37. Many other co-chaperone proteins have also been demonstrated to bind Hsp90. There is some evidence that Hsp90 is found primarily within "activated" multichaperone complexes in the tumour cells as opposed to "latent" complexes in normal cells.

Increased genetic instability associated with the cancer phenotype leads to an increase in the production of non-native or mutant proteins. The ubiquitin pathway also serves to protect the cell from non-native or misfolded proteins, by targeting these proteins for proteasomal degradation. Mutant proteins are by their nature not native and therefore have the potential to show structural instability and an increased requirement for the chaperone system. (Giannini *et al.*, Mol Cell Biol. 2004; 24(13):5667-76).

The number of reported Hsp90 client proteins now exceeds 100. Since many of its client proteins are involved in cell signalling proliferation and survival, Hsp90 has received major interest as an oncology target. Hsp90 protein kinase client proteins implicated in cell proliferation and survival include the following; Cellular Src (c-Src), ErbB2 (Her2/neu), Polo-like kinases (Plks), Akt (PKB), c-Raf, B-RAF, Mek, epidermal growth factor receptor (EGFR), FMS-like tyrosine kinase 3 (FLT3), c-met, Cdk1, Cdk2, Cdk4, and Cdk6, Wee-1, Mutant p53, Hypoxia inducible factor-1a (HIF-1a)

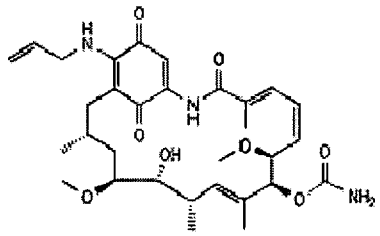
Examples of Hsp90 inhibitors include herbimycin, geldanamycin (GA), 17-AAG e.g. Kos-953 and CNF-1010, 17-DMAG (Kos-1022), CNF-2024 (an oral purine), and IPI-504, in particular 17-AAG e.g. Kos-953 and CNF-1010, 17-DMAG (Kos-1022), CNF-2024, and IPI-504. Preferred compounds are geldanamycin analogs such as 17-AAG e.g. Kos-953 and CNF-1010, 17-DMAG (Kos-1022), and IPI-504.

Ansamycin antibiotics herbimycin, geldanamycin (GA) and 17-allylamino-17-desmethoxygeldanamycin (17-AAG) are ATP binding site inhibitors that block the binding of ATP and prevent conversion to the mature complex (Grenert *et. al.*, 1997. J Biol Chem., 272:23834-23850). Despite Hsp90 being ubiquitously expressed, GA and its analogues have a higher binding affinity for Hsp90 derived from tumour vs. normal cell lines (Kamal *et. al.*, Nature 2003; 425: 407-410). GA also shows more potent cytotoxic activity in tumour cells and is sequestered at higher concentrations within tumours in xenograft mouse models (Brazidec *J. Med. Chem.* 2004, *47,* 3865-3873). Furthermore the ATP-ase activity of Hsp90 is elevated in cancer cells and is an indication of the increased level of stress in these cells. Hsp90 gene amplification has also been reported to occur in the later stages of cancer (Jolly and Morimoto JNCI Vol. 92, No. 19, 1564-1572, 2000).

17-AAG (NSC-330507, 17-allylaminogeldanamycin) is an injectable semisynthetic derivative of geldanamycin and a polyketide inhibitor of Hsp90 identified at the University of

Maryland under development by Kosan Biosciences, in collaboration with the National Cancer Institute (NCI) and the UK Institute of Cancer Research, for the potential treatment of cancer. Studies of 17-AAG have been initiated in melanoma, multiple myeloma, non-Hodgkin's lymphoma (NHL) and Hodgkin's lymphoma (HL) and as a combination therapy with imatinib (qv) for chronic myelogenous leukemia (CML).

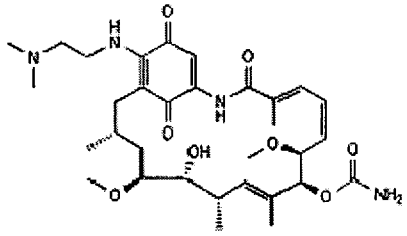
The structure of 17-AAG is outlined below. It can be prepared as described in WO 02/36574 and processes analogous those described therein.



KOS-953 is a 17-AAG formulation developed by Kosan that replaces the DMSO-egg lecithin vehicle used in the original formulation, with the aim of improving patient tolerability and providing greater stability. This can be prepared as described in WO 2005/110398 and processes analogous those described therein.

Conforma is developing CNF-1010, an organic solvent-free lipid-based formulation of 17-AAG (qv) for the potential iv treatment of cancer. This can be prepared as described in WO 03/026571, WO 02/069900 and WO 2006/050333 and processes analogous those described therein. An oral formulation of 17-AAG is described by Conforma in US 2006/0067953.

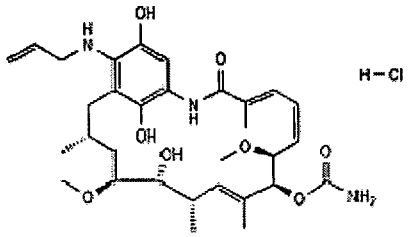
17-DMAG (17-dimethylaminoethylamino-17-demethoxygeldanamycin hydrochloride, NSC-707545; structure shown) is an analog of 17-AAG (qv). It is a water-soluble geldanamycin derivative and it is being investigated for advanced solid tumors. Kosan, under license from the National Cancer Institute (NCI), is developing an iv formulation of KOS-1022 (17-DMAG), for the potential treatment of solid tumors. Kosan is also developing an oral formulation of KOS-1022 (qv) for the same indication.



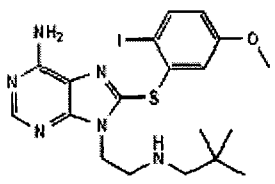
It can be prepared as described in WO 03/013430 and processes analogous to those described therein.

Infinity is developing the Hsp90 inhibitor IPI-504, a further analog of 17-AAG (qv) that is soluble in aqueous formulations for iv administration, for the potential treatment of cancer. Infinity started studies of IPI-504 in multiple myeloma (MM), and gastrointestinal stromal tumors (GIST), and the compound has potential for other haematological cancers and solid tumors.

The structure of IPI-504, a reduced form of 17-AAG called 18, 21-didehydro-17-demethoxy-18, 21-dideoxo-18, 21-dihydroxy-17-(2-propenylamino)-geldanamycin monohydrochloride, is shown below. It can be prepared as described in WO 2005/063714 and processes analogous those described therein.



Conforma Therapeutics is developing CNF-2024, a synthetic oral Hsp 90 inhibitor, for the potential treatment of cancer. CNF-2024 is an oral purine analogue.



It can be prepared as described in J Med Chem (2006) 49: 817-828.

4. Tyrosine kinase inhibitors

Definition: The term "tyrosine kinase inhibitor" as used herein refers to tyrosine kinase inhibitors or analogues of tyrosine kinase inhibitors as described herein, including the ionic, salt, solvate, isomers, tautomers, N-oxides, ester, prodrugs, isotopes and protected forms thereof (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), as described above.

Technical background: A malignant tumour is the product of uncontrolled cell proliferation. Cell growth is controlled by a delicate balance between growth-promoting and growth-inhibiting factors. In normal tissue the production and activity of these factors results in differentiated cells growing in a controlled and regulated manner that maintains the normal integrity and functioning of the organ. The malignant cell has evaded this control; the natural balance is disturbed (via a variety of mechanisms) and unregulated, aberrant cell growth occurs.

One driver for growth is the epidermal growth factor (EGF), and the receptor for EGF (EGFR) has been implicated in the development and progression of a number of human solid tumours including those of the lung, breast, prostate, colon, ovary, head and neck. EGFR is a member of a family of four receptors, namely EGFR (HER1 or ErbB1), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4). These receptors are large proteins that reside in the cell membrane, each having a specific external ligand binding domain, a transmembrane domain and an internal domain which has tyrosine kinase enzyme activity. When EGF attaches to EGFR, it activates the tyrosine kinase, triggering reactions that cause the cells to grow and multiply. EGFR is found at abnormally high levels on the surface of many types of cancer cells, which may divide excessively in the presence of EGF. Inhibition of EGFR activity has therefore been a target for chemotherapeutic research in the treatment of cancer. Such inhibition can be effected by direct interference with the target EGFR on the cell surface, for example by the use of antibodies, or by inhibiting the subsequent tyrosine kinase activity.

Biological activity: The tyrosine kinase inhibitors of the combinations of the invention are specific inhibitors of cell signalling proteins as described above and have activity against

various cancers. Combinations of compounds of formula I with tyrosine kinase inhibitors may be beneficial in the treatment and diagnosis of many types of cancer. Combination with a molecularly targeted agent such as a tyrosine kinase inhibitor (e.g.dasatinib, nilotinib, vatalinib or lapatinib) would find particular application in relation to cancers which express or have activated the relevant molecular target such as EGF receptor, VEGF receptor, ErbB2, BCRabl, c-kit, PDGF. Diagnosis of such tumours could be performed using techniques known to a person skilled in the art and as described herein such as RTPCR and FISH.

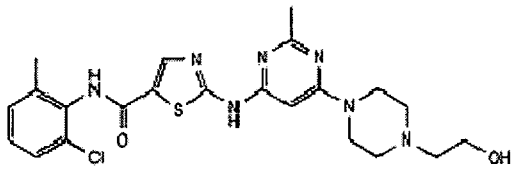
Problems: There is a need to increase the inhibitory efficacy of tyrosine kinase inhibitors against tumour growth and also to provide a means for the use of lower dosages of signaling inhibitors to reduce the potential for adverse toxic side effects to the patient.

Specific embodiments: In one embodiment, the tyrosine kinase inhibitor for use in the combinations of the invention is selected from agents which target the action of VEGF at alternate points in the signal transduction cascade intiated by this growth factor include sunitinib which is marketed under the trade name Sutent by Sugen/Pfizer and inhibits the kinase activity of the VEGF receptor. Sutent has demonstrated efficacy in Phase III trials in gastrointestinal stromal tumours.

Further combinations of the invention include the following tyrosine kinase inhibitors: dasatinib, lapatinib, nilotinib, vandetanib, vatalinib and CHIR-258, in particular dasatinib, lapatinib, nilotinib, vandetanib and vatalinib.

BMS is developing dasatinib (Sprycel or BMS-354825) an oral multitargeted kinase inhibitor, for the potential twice-daily treatment of chronic myelogenous leukemia (CML), Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) and solid tumors. The drug is also under investigation for multiple myeloma (MM) and other hematologic malignancies. Dasatanib has proved effective in Ph+ CML and AML in clinical trials given twice daily at 50 – 90mg and also in imatinib resistant patients. Thrombocytopenia and neutropenia were amongst the side effects observed during clinical evaluation of dasatinib.

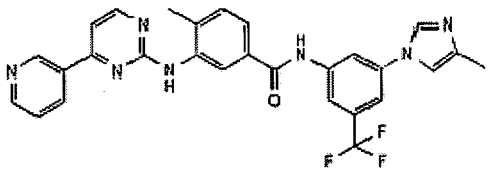
The structure of dasatinib, a Src/Abl kinase inhibitor is below:



Dasatinib can be prepared by processes described in or analogous to WO 00/062778, WO 2005/076990 and WO 2005/077945.

Novartis is developing nilotinib (AMN-107), an orally available signal transduction inhibitor that targets BCR-ABL, c-kit and PDGF, for the potential treatment of leukemias. The compound is being investigated for chronic myeloid leukemia (CML) and relapsed or refractory acute lymphoblastic leukemia (ALL), systemic mastocytosis or chronic eosinophilic leukemia (hypereosinophilic syndrome), refractory gastrointestinal stromal tumor (GIST). Adverse events included hematological adverse events, headache, fatigue, muscle spasms, and nausea and vomiting. In early clinical studies, doses of the order of 400mg given twice daily have proved effective in treating CML, AML and ALL.

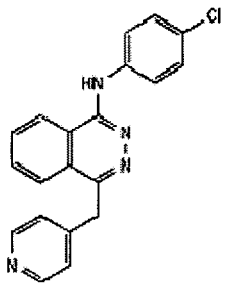
The structure of nilotinib is shown below. It can be prepared as described in or analogous to as described in WO 2004/005281 and WO 2005/049032.



Vatalanib (PTK787 / ZK222584) is a VEGF receptor tyrosine kinase angiogenesis inhibitor, under development by Novartis AG (formerly Ciba-Geigy) and Schering AG, for the potential treatment of colorectal cancer. The compound entered trials for colorectal cancer, the first- and second-line treatment of metastatic colorectal cancer (untreated and pretreated metastatic colorectal patients). Schering and Novartis are also investigating vatalanib in other solid tumors e.g. non-small cell lung cancer (NSCLC), as a second-line monotherapy in patients with stage IIIb/IV disease who had relapsed or were refractory to first-line therapy, renal cell cancer and glioblastoma, and potentially prostate, ovarian, breast, pancreas and small cell lung cancers. In addition vatalanib is also investigated for wet age-related macular degeneration (AMD). Vatalanib has been evaluated at doses up

to 1,250mg daily in clinical studies. Adverse events include nausea/vomiting, fatigue, ataxia, lethargy, hypertension, headache, dizziness, diarrhoea, hypertension as well as syncope and neurotoxicity.

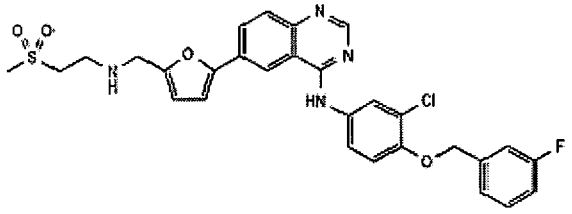
Vatalinib (structure shown below) can be prepared as described in or analogues to as described in WO 98/35958



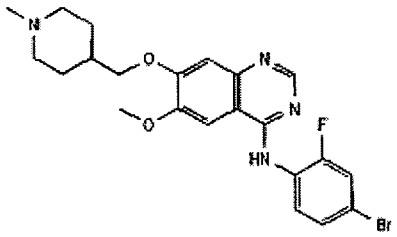
Lapatinib ditosylate (Tykerb or GW2016 / 572016), an ErbB2 and EGFR dual tyrosine kinase inhibitor, is being developed by GlaxoSmithKline plc (GSK) for the potential treatment of solid tumors.

It is under investigation for various tumors including breast, lung, stomach, bladder and head and neck cancers, in particular for the treatment of patients with refractory advanced or metastatic breast cancer whose tumours express HER-2 and who have failed previous therapies both as a single agent and in combination with other therapies including capecitabine and paclitaxel. The compound had also entered trials for renal cell cancer, advanced and metastatic non-small cell lung cancer (NSCLC) and in the treatment of brain metastases associated with breast cancer. In early clinical evaluation Lapatinib has been evaluated on a twice daily and once daily schedule at doses over the range 500 – 1500mg and at doses of 750 – 1250mg given twice daily. Side effects include gastrointestinal gaseous symptoms, rash, headache and abnormal liver function tests.

Quinazoline compounds, and ditosylate salts, anhydrate or hydrate forms such as of the structure shown below (lapatinib) can be synthesised using the process described in WO 00/202552 and WO 99/35146 or process analogues thereto.

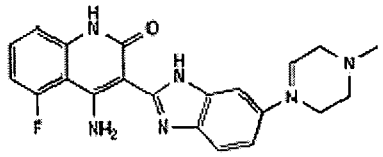


Vandetanib (ZD-6474; Zactima; formerly AZD-6474) is under development by AstraZeneca for the potential once-daily oral treatment of solid and haematological tumors including thyroid, lung, breast, head and neck, brain (i.e. glioma) and multiple myeloma. It is one of a series of inhibitors of vascular endothelial growth factor (VEGF) receptor tyrosine kinase) that also has activity against the EGF and RET receptor tyrosine kinases. Clinical studies have investigated doses of vandetanib in the region of 100 -300mg daily as monotherapy and in combinations. Common adverse effects observed were rash, fatigue, nausea, diarrhea, asymptomatic QTc prolongation



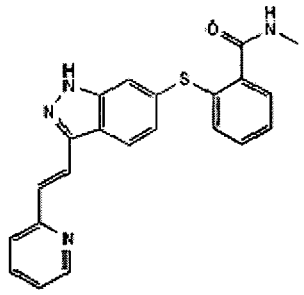
ZD-6474 can be prepared as described in WO 01/32651 and processes analogous therein.

CHIR-258 (GFKI-258; structure shown), is a potent VEGF, FGF and PDGF receptor kinase inhibitor, for the potential oral treatment of various types of cancer. Novartis (formerly Chiron), had initiated a study in acute myelogenous leukemia (AML) patients and multiple myeloma (MM).



CHIR-258 can be prepared as described in WO 02/22598 and WO 2005/046590 and processes analogous therein.

Another suitable tyrosine kinase inhibitor for use in the combinations of the invention is axitinib (AG-013736). Pfizer is developing axitinib (AG-13736, AG-013736), an oral inhibitor of the VEGF, PDGF and CSF-1 receptor tyrosine kinases which was discovered by Pfizer's wholly-owned subsidiary Agouron Pharmaceuticals, as an anti-angiogenic agent for the potential treatment of cancer. It is being studied for breast cancer, renal cell carcinoma (RCC), non-small cell lung cancer (NSCLC), melanoma, and carcinomas. The compound has also being investigated for the treatment of acute myeloid leukemia and myelodysplastic syndrome (MDS).



It can be prepared as described in WO 2004/087152, WO 2006/048746 and WO 2006/048745 and process analogous thereto. Axitinib may be dosed at 5mg PO BID.

Posology: With regard to tyrosine kinase inhibitors, these are generally administered in a daily oral dosage of 100 to 500 mg, for example gefitinib in a dosage of about 250 mg and erlotinib in a dosage of about 150 mg. These dosages may be administered for example once, twice or more per course of treatment, which may be repeated for example every 7, 14, 21 or 28 days.

5. EGF antibody and other inhibitors

Definition: The term "EGF antibody" as used herein refers to EGF antibodies or analogues of EGF antibody as described herein, including the ionic, salt, solvate, isomers, tautomers, N-oxides, ester, prodrugs, isotopes and protected forms thereof (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), as described above.

Technical background: A malignant tumour is the product of uncontrolled cell proliferation. Cell growth is controlled by a delicate balance between growth-promoting and growth-inhibiting factors. In normal tissue the production and activity of these factors results in differentiated cells growing in a controlled and regulated manner that maintains the normal integrity and functioning of the organ. The malignant cell has evaded this control; the natural balance is disturbed (via a variety of mechanisms) and unregulated, aberrant cell growth occurs.

One driver for growth is the epidermal growth factor (EGF), and the receptor for EGF (EGFR) has been implicated in the development and progression of a number of human solid tumours including those of the lung, breast, prostate, colon, ovary, head and neck. EGFR is a member of a family of four receptors, namely EGFR (HER1 or ErbB1), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4). These receptors are large proteins that reside in the cell membrane, each having a specific external ligand binding domain, a transmembrane domain and an internal domain which has tyrosine kinase enzyme activity. When EGF attaches to EGFR, it activates the tyrosine kinase, triggering reactions that cause the cells to grow and multiply. EGFR is found at abnormally high levels on the surface of many types of cancer cells, which may divide excessively in the presence of EGF. Inhibition of EGFR activity has therefore been a target for chemotherapeutic research in the treatment of cancer. Such inhibition can be effected by direct interference with the target EGFR on the cell surface, for example by the use of antibodies, or by inhibiting the subsequent tyrosine kinase activity.

Biological activity: The EGF antibodies of the combinations of the invention are specific inhibitors of cell signalling proteins as described above and have activity against various cancers. Combinations comprising these growth factor receptor antibodies may therefore be beneficial in the treatment and diagnosis of many types of cancer and in particular cancers which express or have activated the relevant molecular target (such as the EGF receptor). Diagnosis of such tumours could be performed using techniques known to a person skilled in the art and as described herein such as RTPCR and FISH.

Problems: There is a need to increase the inhibitory efficacy of EGF antibodies against tumour growth and also to provide a means for the use of lower dosages of growth factor receptor antibodies to reduce the potential for adverse toxic side effects to the patient.

Specific embodiments: In one embodiment the EGF for use in the combinations of the invention is the monoclonal antibody panitumumab. Amgen Inc (formerly Immunex and Abgenix Inc) is developing panitumumab (ABX-EGF), a fully human monoclonal antibody against the EGF receptor, for the potential treatment of cancer, such as monotherapy for renal cancer, non-small-cell lung cancer, and colorectal cancer (CRC) in combination with standard chemotherapy as first-line treatment, as third-line monotherapy in advanced CRC, in particular to treat metastatic colorectal cancer (MCC) and in patients who failed standard chemotherapy. Thus ABX-EGF can be administered as a monotherapy or in association with chemotherapy and radiotherapy in order to complement independent approaches for the treatment of cancer.

ABX-EGF is a fully humanized IgG2 monoclonal antibody against the human EGFR. Fully humanized monoclonal antibodies such as ABX-EGF have several advantages over chimeric antibodies, which contain significant amounts of mouse protein. They do not generate human anti-mouse antibodies (HAMA); the risk of inducing hypersensitivity reactions in patients is therefore reduced and the antibodies should demonstrate an increased in vivo lifetime. Such considerations may be important for long-term administration.

It can be prepared as described in WO98/50433 and process analogous thereto.

Panitumumab may be dosed ranging from 0.01 to 5.0 mg/kg once per week, 6.0 mg/kg once every two weeks or 9.0 mg/kg once every three weeks administered by intravenous infusion.

In a Phase 3 pivotal study examining panitumumab as third-line monotherapy in colorectal cancer patients, patients received panitumumab every two weeks.

Posology: With regard to the EGFR antibodies, these are generally administered in a dosage of 1 to 500 mg per square meter (mg/m^2) of body surface area.

6. Decitabine and azacytidine DNA methyl transferase inhibitors

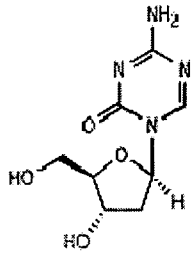
Definition: The term "DNA methylase inhibitor" or "DNA methyltransferase inhibitor" as used herein refers to a compound which directly or indirectly perturbs, disrupts, blocks, modulates or inhibits the methylation of DNA, including the ionic, salt, solvate, isomers, tautomers, N-oxides, ester, prodrugs, isotopes and protected forms thereof (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), as described above.

Biological activity: The DNA methylase inhibitors working via one or more pharmacological actions as described herein have been identified as suitable anti-cancer agents.

Technical background: One target for cancer chemotherapy is DNA synthesis, which may depend on appropriate methylation of tumour DNA. Compounds which directly or indirectly perturb, disrupt, block, modulate or inhibit the methylation of DNA may therefore be useful anticancer drugs.

Problems: The most common side effects associated with DNA methyltransferase inhibitor therapy are nausea, vomiting, headache, fatigue, and constipation. There is a need to increase the inhibitory efficacy of DNA\methylase inhibitors and to provide a means for the use of lower dosages of signaling inhibitors to reduce the potential for adverse toxic side effects to the patient.

Preferences and specific embodiments: In one embodiment, the DNA methylase inhibitor is decitabine (a.k.a. Dacogen) having the structure shown below:



SuperGen Inc and MGI Pharma Inc have developed decitabine (Dacogen), an inhibitor of DNA methyltransferase, preventing methylation of cytosine residues on DNA and leading to hypomethylation of gene promoters, thereby reactivating silenced genes. Decitabine/Dacogen is cytotoxic to a broad range of malignant cells in vitro. It shows

significant activity against acute myeloid leukemia (AML), chronic myeloid leukemia (CML) and myelodysplastic syndromes (MDS). Decitabine/Dacogen is indicated for the treatment of myelodysplastic syndromes (MDS) and secondary MDS (including chronic myelomonocytic leukemia, refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts and refractory anemia with excess blasts in transformation).

Decitabine/Dacogen is an analog of deoxycytidine (beta-D-anomer of 2'-deoxy-5-azacytidine). It differs from deoxycytidine by substitution at position 5 of the pyrimidine ring with nitrogen. Decitabine contains deoxyribose, in contrast to the related analog, Pharmion Corp's 5-azacytidine (Vidaza), which contains a ribose sugar. Decitabine is, therefore, a deoxynucleoside and is incorporated into DNA, but not RNA, in contrast to 5-azacytidine which is incorporated into RNA. Decitabine and 5-azacytidine differ from other pyrimidine analogs, such as cytosine arabinoside and gemcitabine, by modification at position 5 of the pyrimidine ring. This distinctive feature, which is not present in these latter drugs, is responsible for inhibition of DNA methyltransferase. Pseudoisocytidine and 5-fluoro-2'-deoxycytidine, further analogs with modifications of the 5 position of the pyrimidine ring, also inhibit demethylation.

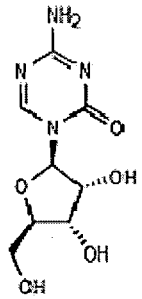
Decitabine/Dacogen is dosed at 15mg/m2 over a three hour period every 8 hours for 3 days every 6 weeks as a cycle of therapy or on a daily dosing schedule with a one hour infusion usually delivered at 20mg/m2 per day either for one week or two weeks every 6 weeks as a cycle

At toxic doses decitabine/Dacogen produces leukopenia, thrombocytopenia and weight loss. The major toxicity of decitabine is myelosuppression, which is proportional to dose and duration of therapy. The effects are pronounced at high doses (> 200 mg/m2/day), and myelosuppression is enhanced by concomitant administration of other cytotoxic drugs. Neutropenic infection and other complications of myelosuppression have proved fatal. Non-hematological side effects include nausea, vomiting, mucositis and alopecia.

Decitabine/Dacogen and other analogues thereof can be made as outlined in US-03432549 and further discussed on WO 006/017278 and WO 2006/037024 to SuperGen Inc.

A further DNA methyltransferase inhibitor for use in the combinations of the invention is azacytidine (a.k.a. 5-azacitidine, 5-azacytidine or Vidaza) is an sc administered hypomethylating agent and DNA methyltransferase inhibitor. It is indicated for the treatment of all five myelodysplastic syndrome (MDS) subtypes, including refractory anemia (RA) or RA with ringed sideroblasts, RA with excess blasts, RA with excess blasts in transformation and chronic myelomonocytic leukemia.

5-azacitidine (Vidaza) can be administered twice-daily subcutaneously or via the iv route administration for MDS treatment.



It can be prepared as described in DE 1922702, GB 1227691 and FR 2008048 from Ceskoslovenska Akademie Ved and WO 2004082618, WO 2004082619 and WO 2004082822 from Pharmion and process analogous thereto.

Posology: The DNA methylating agent can be administered in a dosage such as 0.5 to 2.5 mg per square meter (mg/m^2) of body surface area, particularly about 1.3 mg/m^2. These dosages may be administered for example once, twice or more per course of treatment, which may be repeated for example every 7, 14, 21 or 28 days.

7. Cytokines and cytokine-activating agents

Definition: The term "cytokine" is a term of art, and references to cytokines herein is intended to cover the cytokine *per se* together with the ionic, salt, solvate, isomers, tautomers, N-oxides, ester, prodrugs, isotopes and protected forms thereof (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), as described above. The term "cytokine-activating agent" is intended to cover any agent which (directly or indirectly) induces, potentiates, stimulates, activates or promotes endogenous cytokine production or

the activity thereof *in vivo*, together with the ionic, salt, solvate, isomers, tautomers, N-oxides, ester, prodrugs, isotopes and protected forms thereof (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), as described above.

Technical background: Cytokines are a class of proteins or polypeptides predominantly produced by cells of the immune system which have the capacity to control the function of a second cell. In relation to anticancer therapy cytokines are used to control the growth or kill the cancer cells directly and to modulate the immune system more effectively to control the growth of tumours.

Cytokines, such as interferon (IFN) alpha and Interleukin-2, induce growth arrest or tumour cell death. IFN-alpha is used the treatment of malignant melanoma, chronic myelogenous leukemia (CML), hairy cell leukemia, and Kaposi's sarcoma. Interleukin-2 is used in the treatment of malignant melanoma and renal cell cancer either alone or in combination with IFN-alpha.

Cytokines exhibit antitumour activity through a variety of different mechanisms including the stimulation of immune cells to fight tumors For example, the T cell growth factor, IL-2 promotes T-cell and natural killer (NK) cell activation. Other cytokines such as the interferons and granulocyte–macrophage colony-stimulating factor (GM-CSF) act on antigen presenting cells to facilitate the activation of the key immune effector B cells and T cells.

Preferences and specific embodiments: Any of the cytokines and cytokine-modulating agents described herein may find application in the invention, including in particular interferons (such as interferon-γ and interferon α) and interleukins (e.g. interleukin 2). Interferon α -2b (recombinant) is available commercially under the trade name of INTRON® A from Schering Plough.

Other preferred interferons include Interferon α -2a which is available under the trade name of ROFERON from Roche.

A particularly preferred interleukin is PROLEUKIN® IL-2 (aldesleukin) which is available from Chiron Corp.

Posology: The interferons are administered by injection in a schedule which is dependent on tha particular indication. For IntronA treatment of malignant melanoma preferably in a schedule that includes induction treatment on 5 consecutive days per week for 4 weeks as an intravenous (IV) infusion at a dose of 20 million IU/m2, followed by maintenance treatment three times per week for 48 weeks as a subcutaneous (SC) injection, at a dose of 10 million IU/m2. For Intron A treatment of non-Hodgkin's Lymphoma preferably in a schedule of 5 million IU subcutaneously three times per week for up to 18 months in conjunction with an anthracycline-containing chemotherapy regimen.

The recommended initial dose of Roferon-A for CML is 9 MIU daily administered as a subcutaneous or intramuscular injection. Based on clinical experience short-term tolerance may be improved by gradually increasing the dose of Roferon-A over the first week of administration from 3 MIU daily for 3 days to 6 MIU daily for 3 days to the target dose of 9 MIU daily for the duration of the treatment period. The induction dose of Roferon-A for Hairy cell leukaemia is 3 MIU daily for 16 to 24 weeks, administered as a subcutaneous or intramuscular injection. Subcutaneous administration is particularly suggested for, but not limited to, thrombocytopenic patients (platelet count <50,000) or for patients at risk for bleeding. The recommended maintenance dose is 3 MIU, three times a week (tiw).

For PROLEUKIN the following schedule has been used to treat adult patients with metastatic renal cell carcinoma (metastatic RCC) or metastatic melanoma (each course of treatment consists of two 5-day treatment cycles separated by a rest period): 600,000 IU/kg (0.037 mg/kg) dose administered every 8 hours by a 15-minute IV infusion for a maximum of 14 doses. Following 9 days of rest, the schedule is repeated for another 14 doses, for a maximum of 28 doses per course, as tolerated.

Cytokine-activating agents: Preferred cytokine-activating agents include: (a) Picibanil from Chugai Pharmaceuticals, an IFN-gamma-inducing molecule for carcinoma treatment; (b) Romurtide from Daiichi which activates the cytokine network by stimulation of colony stimulating factor release; (c) Sizofiran from Kaken Pharmaceutical, a beta1-3, beta1-6 D-glucan isolated from suehirotake mushroom, which stimulates production of IFN-gamma and IL-2 by mitogen-stimulated peripheral blood mononuclear cells, and is useful in uterine cervix tumour and lung tumour treatment; (d) Virulizin from Lorus Therapeutics Inc, a NK agonist and cytokine release modulator which stimulates IL-17 synthesis and IL-12 release

for the treatment of sarcoma, melanoma, pancreas tumours, breast tumours, lung tumours, and Kaposis sarcoma (e) Thymosin alpha 1, a synthetic 28-amino acid peptide with multiple biological activities primarily directed towards immune response enhancement for increased production of Th1 cytokines, which is useful in the treatment of non-small-cell lung cancer, hepatocellular carcinoma, melanoma, carcinoma, and lung brain and renal tumours.

8. Retinoids and rexinoids

Definition: The term "retinoid" is a term of art used herein in a broad sense to include not only the specific retinoids disclosed herein, but also the ionic, salt, solvate, isomers, tautomers, N-oxides, ester, prodrugs, isotopes and protected forms thereof (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), as described above. The term 'rexinoids' refers to synthetic agents that bind specifically to retinoid X receptors.

Technical background: Tretinoin is an endogenous metabolite of retinol. It induces terminal differentiation in several hemopoietic precursor cell lines, including human myeloid cell lines. Acute Promyelocytic Leukemia (APL) is associated with a specific translocation between chromosomes 15 and 17; the retinoic acid receptor - α is located on chromosome 17. The translocation appears to inhibit differentiation and lead to carcinogenesis; tretinoin may overcome this when used in high doses. Tretinoin induces remissions in 64-100% of APL patients, with time to remission usually between 8 and 119 days of therapy. Acquired resistance during therapy is common especially with prolonged dosing (4-6 months). Alitretinoin is a 9-cis-retinoic acid derivative which appears to be selective for the RXR subfamily of retinoid receptors. This selectivity may preserve therapeutic antineoplastic effects while reducing significant side effects of retinoid therapy including birth defects at fetal exposure, irritation of skin and mucosal surfaces or skeletal abnormalities. Topical alitretinoin is approved in the US for the treatment of Kaposi's Sarcoma. Oral and gel (topical) formulations of bexarotene (Targretin; LGD-1069), a retinoid X receptor (RXR)-selective antitumor retinoid, are available for the treatment of cutaneous T-cell lymphoma (CTCL).

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.

Preferences and specific embodiments: Preferred retinoids for use in accordance with the invention include any of the retinoids disclosed herein, including in particular tretinoin (all-trans retinoic acid), alitretinoin and bexarotene. Tretinoin (Retacnyl, Aknoten, Tretin M) is commercially available from Roche under the trade name Vesanoid and may be prepared for example as described in D. A. van Dorp, J. R. Arens, Rec. Trav. Chim. 65, 338 (1946); C. D. Robeson et al., J. Am. Chem. Soc. 77, 4111 (1955); R. Marbet, DE 2061507; US 3746730 (1971, 1973 both to Hoffmann-La Roche), or by processes analogous thereto. Alitretinoin (9-cis-Tretinoin, Panrexin) is commercially available from Ligand Pharmaceuticals under the trade name Panretin and may be prepared for example as described in C. D. Robeson et al., J. Am. Chem. Soc. 77, 4111 (1955); M. Matsui et al., J. Vitaminol. 4, 178 (1958); M. F. Boehm et al., J. Med. Chem. 37, 408 (1994), or by processes analogous thereto. Bexarotene (Targrexin, Targret) is commercially available from Eisai Inc under the trade name Targretin and may be prepared for example as described in M. F. Boehm et al., WO 9321146 (1993 to Ligand Pharm.); M. L. Dawson et al., US 5466861 (1995 to SRI Int.; La Jolla Cancer Res. Found.), or by processes analogous thereto.

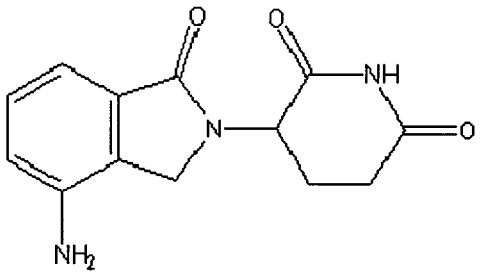
Posology: Tretinoin is advantageously administered in dosages of 25 mg/m^2/day to 45 mg/m^2/day by mouth in two divided doses for 30 days after complete remission or up to a maximum of 90 days. Alitretinoin gel 0.1% is advantageously administered initially by application two (2) times a day to cutaneous KS lesions. Bexarotene is advantageously administered initially as a single daily oral dose of 300 mg/ m^2/day. The dose may be adjusted to 200 mg/m^2/day then to 100 mg/m^2/day, or temporarily suspended, if necessitated by toxicity. If there is no tumor response after eight weeks of treatment and if the initial dose of 300 mg/m2/day is well tolerated, the dose may be escalated to 400 mg/m^2/day with careful monitoring. Bexarotene gel is advantageously applied initially once every other day for the first week. The application frequency may be increased at weekly intervals to once daily, then twice daily, then three times daily and finally four times daily according to individual lesion tolerance.

9. Selective immunoresponse modulators

Selective immunoresponse modulators include Lenalidomide and_Thalidomide.

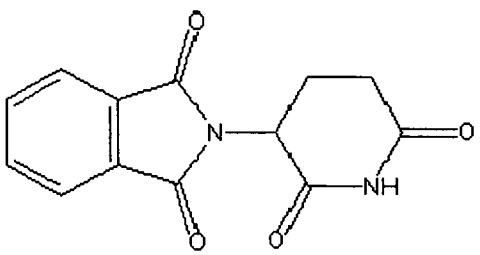
Lenalidomide (Revlimid) is an oral thalidomide derivative developed by Celgene which is a potent inhibitor of TNF-alpha and interleukin-1 beta which is being developed for the treatment of 5q- myelodysplastic syndrome multiple myeloma, chronic lymphocytic leukaemia gliomas, cutaneous T-cell lymphoma and epithelial ovarian cancer.

Lenalidomide (3-(4-amino-1-oxoisoindolin-2-yl)piperidine-2,6-dione) has the following structure:



Thalidomide is a sedative and anti-emetic that became widely recognized as a result of reports of its teratogenic effects, most notably limb deformities in up to 12,000 children born to women who had received thalidomide in Europe and Canada during the 1960s. Celgene has developed and launched thalidomide as an oral TNF-alpha inhibitor (Sold to Pharmion). Extensive clinical evidence has accumulated with regard to the potential antitumor activity of thalidomide in several types of neoplasias, with notable activity in relapsed/refractory multiple myeloma, Waldenstrom's macroglobulinemia (WM) and myelodysplastic syndromes (MDS). There is also evidence of biological activity in acute myeloid leukemia, myelofibrosis with myeloid metaplasia, renal cell carcinoma, malignant gliomas, prostate cancer, Kaposi's sarcoma and colorectal carcinoma.

Thalidomide (1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)isoindoline) has the following structure:



Posology: Thalidomide may be advantageously administered in dosages of 100 to 800 mg/day continuously as tolerated. Lenalidomide may be advantageously administered in 5- to 40-mg doses continuously as tolerated.

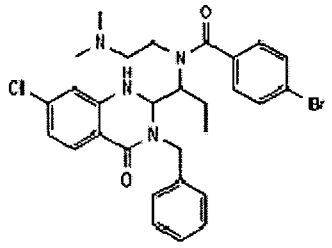
10. Checkpoint targeting agents

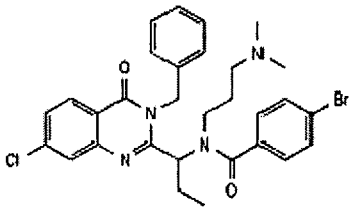
The cell proliferation cycle is a complex process during which the cell first replicates its chromosomes and then undergoes cell division or cytokinesis. At various stages of the cycle, mechanisms exist to prevent further progression through the cycle until all appropriate events have occurred. This ensures the integrity of the DNA of the cell as it progresses through the cycle in the required sequential manner. One such checkpoint is known to occur in mitosis. This is variously referred to as the mitotic or spindle checkpoint. Cells are held at this checkpoint until all chromosomes are appropriately attached to the mitotic spindle via their centrosomes. Defects in this checkpoint lead to either aneuploid phenotypes, typical of cancer cells or an imbalance of chromosomes in daughter cells. Some cancer therapies are known to act by disruption of this checkpoint causing chromosome mis-alignment or premature cytokinesis leading to activation of a checkpoint that results in preferential death of the tumour cell. For example the taxanes and epothilones are classes of agents which cause stabilisation of spindle microtubules preventing the normal spindle contraction process. The vinca alkaloids are another class of agents which act to prevent spindle formation via an action on tubulin the principal protein in the microtubules. Agents which cause DNA damage or disrupt DNA replication including platinum compounds and nucleoside analogues such as 5-FU lead to cell arrest at checkpoints and subsequent cell death. They thus require a functional checkpoint for their therapeutic action.

Further checkpoint targeting agents are those that cause DNA damage or disrupt DNA replication including platinum compounds such as cisplatin and nucleoside analogues such as 5-FU leading to cell arrest at checkpoints and subsequent cell death. In this context a combination of Aurora kinase inhibitors with the platinum compounds and nucleoside analogues would be expected to be beneficial as they could sensitise cells to the cytotoxic effects. Particular platinum compounds and nucleoside analogues are described herein.

Further checkpoint targeting agents that activate, interfere with or modulate the cell cycle checkpoints include polo-like kinase inhibitors (Plks), CHK kinase inhibitors, inhibitors of the BUB kinase family and kinesin inhibitors. Polo-like kinases are important regulators of cell cycle progression during M-phase. Plks are involved in the assembly of the mitotic spindle apparatus and in the activation of CDK/cyclin complexes. Plk1 regulates tyrosine dephosphorylation of CDKs through phosphorylation and activation of Cdc25C. CDK1 activation in turn leads to spindle formation and entry into M phase. The importance of Checkpoint kinases such as Chk1 and Chk2 is described herein.

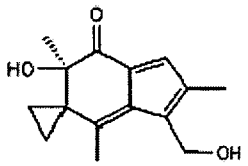
Thus other agents in development which act to disrupt the mitotic checkpoint and therefore could be combined beneficially with the compounds of the invention include polo-like kinase inhibitors (e.g. BI-2536), CHK kinase inhibitors (e.g. Irofulven (a CHK2 inhibitor), 7-hydroxystaurosporine (UCN-01, an inhibitor of both CHK1 and PKC) and PD-321852), inhibitors of the BUB kinase family, and kinesin inhibitors (also known as mitotic kinesin spindle protein (KSP) inhibitors) such as CK0106023, CK-0060339 and SB-743921 (structures shown below).



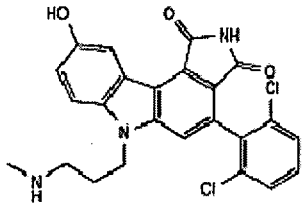


CK0106023, CK-0060339 and SB-743921 can be prepared and used as described in WO 01/30768 and WO 01/98278 and processes analogous thereto.

CHK kinase inhibitors include irofulven, UCN-01 and PD-321852. Irofulven (structure shown) is a semisynthetic compound derived from illudin S, a toxin from the Omphalotus illudens mushroom, for the potential treatment of refractory and relapsed tumors, including ovarian, prostate, hepatocellular, breast, lung and colon cancers, and gliomas. This can be synthesised as described in WO 98/05669 or processes analogous thereto.

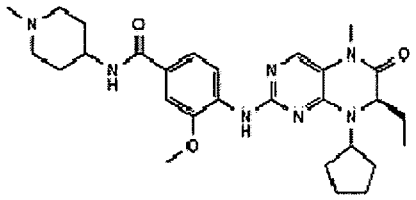


PD-321852, a checkpoint kinase Chk I inhibitor, (structure shown), is being investigated by Pfizer for the potential treatment of cancer.



It can be prepared and used as described in WO 01/53274, WO 01/53268 and in particular WO 03/091255 or processes analogous thereto.

BI-2536 (structure shown below) an inhibitor of the serine-threonine kinase polo-like kinase -1 (PLK-1), for the potential treatment of solid tumors. It can be prepared and used as described in WO2004/076454, WO 2006/018220, WO 2006/018221 and WO 2006/018222 or processes analogous thereto.



In addition, checkpoint targeting agents that arrest cells in G2/M phase could also be combined. Therefore Platinum compounds and CDK inhibitors would be therefore be expected to be beneficial in combination with the combinations of the invention and are thus further Checkpoint Targeting Agents. Particular Platinum compounds and CDK inhibitors are described herein.

Thus, examples of Checkpoint Targeting Agents for use according to the invention include Platinum compounds, nucleoside analogues, CDK inhibitors, Taxanes, Vinca alkaloids, polo-like kinase inhibitors, CHK kinase inhibitors, inhibitors of the BUB kinase family and kinesin inhibitors, in particular Platinum compounds, nucleoside analogues, Taxanes and Vinca alkaloids more particularly checkpoint targeting agents which target the mititoic

checkpoint such as Taxanes and Vinca alkaloids. Particular combinations of the invention include cisplatin or vinblastine or taxol or 5FU, in particular taxol.

Particular examples of Checkpoint Targeting Agents for use according to the invention include polo-like kinase inhibitors, CHK kinase inhibitors, inhibitors of the BUB kinase family and kinesin inhibitors. In one embodiment the optional auxiliary agents for use in the combinations of the invention are selected from BI-2536, Irofulven, 7-hydroxystaurosporine, PD-321852, CK0106023, CK-0060339 and SB-743921.

11. DNA repair inhibitors

DNA repair inhibitors include PARP inhibitors.

Definition: The term "PARP inhibitor" is used herein to define compounds which inhibit or modulate the activity of the family of Poly adenosine diphosphate rbose (poly(ADP-Ribose)) nuclear enzymes, including the ionic, salt, solvate, isomers, tautomers, N-oxides, ester, prodrugs, isotopes and protected forms thereof (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), as described above. They may also be referred to as "DNA repair inhibitors".

Biological activity: PARP inhibitors have a role as chemosensitizing agents (for example by preventing DNA repair after anticancer therapy) and may have a role in enhancing overall patient response to anti-cancer treatments. PARP inhibitors may also act in isolation as anti cancer agents in patients whose tumours have intrinsic deficiencies in DNA repair.

Technical background: The PARP enzyme synthesizes poly(ADP-ribose), a branched polymer that can consists of over 200 ADP-ribose units. The protein acceptors of poly(ADP-ribose) are directly or indirectly involved in maintaining DNA integrity. They include histones, topoisomerases, DNA and RNA polymerases, DNA ligases, and Ca 2'- and Mg 2, -dependent endonucleases. PARP protein is expressed at a high level in many tissues, most notably in the immune system, heart, brain and germ-line cells. Under normal physiological conditions, there is minimal PARP activity. However, DNA damage causes an immediate activation of PARP by up to 500-fold.

PARP is activated by damaged DNA fragments and, once activated, catalyzes the attachment of up to 100 ADP-ribose units to a variety of nuclear proteins, including histones and PARP itself. It is also known that PARP inhibitors, such as 3-amino benzamide, affect overall DNA repair in response, for example, to hydrogen peroxide or ionizing radiation. The pivotal role of PARP in the repair of DNA strand breaks is well established, especially when caused directly by ionizing radiation or, indirectly after enzymatic repair of DNA lesions induced by methylating agents, especially temozolamide, topoisomerases I inhibitors and other chemotherapeutic agents as cisplatin and bleomycin. A variety of studies using knockout mice, trans-dominant inhibition models (over-expression of the DNA- binding domain), antisense and small molecular weight inhibitors have demonstrated the role of PARP in repair and cell survival after induction of DNA damage. The inhibition of PARP enzymatic activity should lead to an enhanced sensitivity of tumor cells towards DNA damaging treatments.

PARP inhibitors have been reported to be effective in radiosensitizing (hypoxic) tumor cells and effective in preventing tumor cells from recovering from potentially lethal and sublethal damage of DNA after radiation therapy, presumably by their ability to prevent DNA strand break rejoining and by affecting several DNA damage signaling pathways. PARP inhibitors have been used to treat cancer. A recent comprehensive review of the state of the art has been published by Li and Zhang in IDrugs 2001, 4(7): 804.

Preferences and specific embodiments: Preferred PARP inhibitors for use in accordance with the invention are selected from Bendamustine (5-[Bis(2-chloroethyl)amino]-1-methyl-2-benzimidazolebutyric acid or α-[1-Methyl-5-[bis(.beta.-chloroethyl)amino]-2-benzimidazolyl]butyric acid), available from Bayer, INO-1001 (Pardex) from Inotek Pharmaceuticals, BSI-201 from BiPar Sciences, AG-014699 from Pfizer, and ONO-2231 (N-[3-(3,4-dihydro-4-oxo-1-phthalazinyl)phenyl]-4-morpholinebutanamide methanesulfonate) from Ono Pharmaceutical.

Posology: The PARP inhibitors are advantageously administered in daily dosages of 20-100mg, for example 80-120 mg/m2 iv over a 30 to 60 min infusion over a 21 day cycle for Bendamustine. – The key PARP inhibitor is a Pfizer product which is in phase III combination trials in metastatic melanoma. It is administered intravenously on days one thru five of a twenty-one day cycle dose ?

12. Inhibitors of G-protein coupled receptors (GPCR)

A preferred GPCR is Atrasentan (3-Pyrrolidinecarboxylic acid, 4-(1,3-benzodioxol-5-yl)-1-[2-(dibutylamino)-2-oxoethyl]-2-(4-methoxyphenyl)-, [2R-(2.alpha.,3.beta.,4.alpha.)]-). Atrasentan, from Abbott Laboratories, is a potent and selective endothelin A receptor antagonist for the treatment of prostate tumors. There is also evidence of biological activity in other cancer types such as glioma, breast tumor, lung tumor, brain tumor, ovary tumor, colorectal tumor and renal tumor.

Posology: Atrasentan may be advantageously administered orally in dosages of e.g. 10mg daily.

Disease-specific anti-cancer agent combinations

Multiple myeloma

Combinations with vincristine, doxorubicin, thalidomide and dexamethasone are particularly suitable for treating multiple myeloma. In addition, combinations with vincristine, doxorubicin and dexamethasone are particularly suitable for treating multiple myeloma.

Particularly suitable for treating multiple myeloma are combinations with: (a) monoclonal antibodies (e.g. those targeting Interleukin 6); (b) proteasome inhibitors (e.g. bortezomib); (c) proteasome inhibitors and corticosteroids (e.g. velcade and dexamethasone); and (d) corticosteroids, alkylating agents and lenolidamide/thalidomide (e.g. prednisolone, melphalan and thalidomide).

Melanoma

Particularly suitable for treating melanoma are combinations with: (a) DNA methylase inhibitors/hypomethylating agents (e.g. temozolamide); (b) alkylating agents (e.g. dacarbazine or fotemustine); and (c) DNA methylase inhibitors/hypomethylating agents (e.g. temozolamide) and DNA repair inhibitors/PARP inhibitors.

Breast Cancer

Particularly suitable for treating breast cancer are combinations with: (a) monoclonal antibodies (e.g. trastuzumab and bevicizamab); (b) monoclonal antibodies (e.g. trastuzumab and bevicizamab) and taxanes; and (c) antimetabolites (e.g. capecitabine) and signalling inhibitors (e.g. lapatinib).

Prostate cancer

Particularly suitable for treating prostate cancer are combinations with hormones and G-protein coupled receptor inhibitors.

Non small cell lung cancer (NSCLC)

Particularly suitable for treating NSCLC are combinations with: (a) platinum compounds and taxanes; and (b) platinum compounds and antimetabolites.

Chronic myeloid leukemia (CML)

In particular chronic myeloid leukemia (CML)) treatment, the two or more anti-cancer agents are independently selected from hydroxyurea, cytarabine, Interferon-alpha and imatinib. Alternatively for cancer (and in particular chronic myeloid leukemia (CML)) treatment, two or more anti-cancer agents independently selected from hydroxyurea, cytarabine, dasatinib, nilotinib and imatinib.

Pharmaceutical Formulations

While it is possible for the active compounds in the combinations of the invention to be administered without any accompanying pharmaceutical excipients or carriers, it is preferable to present them in the form of pharmaceutical compositions (e.g. formulations). As such, they may be formulated for simultaneous or sequential administration.

Where they are intended for sequential administration, they will typically be formulated in separate compositions which may be of the same type or a different type. Thus, for example, the components of the combination may be formulated for delivery by the same route (e.g. both by the oral route or both by injection) or they may be formulated for administration by different routes (e.g. one by the oral route and another by a parenteral route such as by i.v. injection or infusion). In a preferred embodiment the compound 4-(2,6-

dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide and salts therof, particularly acid addition salts such as the methanesulphonic acid, acetic acid and hydrochloric acid salts is administered sequentially (either before or after) or simulatenously with the ancillary compound. Preferably the compound 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide and salts therof, particularly acid addition salts such as the methanesulphonic acid, acetic acid and hydrochloric acid salts is administered using an i.v. formulation as defined herein.

When they are intended for simultaneous administration, they may be formulated together or separately and, as above, may be formulated for administration by the same route or by different routes.

The compositions typically comprise at least one active compound of the combination 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. The compositions may also include 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).

Also included are agents that inhibit bone resorption such as bisphosphonate agents e.g. zoledronate, pamidronate and ibandronate, as well as agents that suppress inflammatory responses (such as dexamethazone, prednisone, and prednisolone). Also included are agents used to reduce blood levels of growth hormone and IGF-I in acromegaly patients such as synthetic forms of the brain hormone somatostatin, which includes octreotide acetate which is a long-acting octapeptide with pharmacologic properties mimicking those of the natural hormone somatostatin. Further included are agents such as leucovorin, which is used as an antidote to drugs that decrease levels of folic acid, or folinic acid it self. In one particular embodiment is the combination of 5FU and leucovorin or 5FU and folinic acid. In addition megestrol acetate can be used for the treatment of side-effects including oedema and thromoembolic episodes.

Therefore in one embodiment the combinations further include an additional agent selected from erythropoietin (EPO), granulocyte macrophage-colony stimulating factor (GM-CSF),

granulocyte-colony stimulating factor (G-CSF), zoledronate, pamidronate, ibandronate, dexamethazone, prednisone, prednisolone, leucovorin, folinic acid and megestrol acetate.

In particular the combinations further include an additional agent selected from erythropoietin (EPO), granulocyte macrophage-colony stimulating factor (GM-CSF), granulocyte-colony stimulating factor (G-CSF), zoledronate, pamidronate, dexamethazone, prednisone, prednisolone, leucovorin, and folinic acid such as erythropoietin (EPO), granulocyte macrophage-colony stimulating factor (GM-CSF) and granulocyte-colony stimulating factor (G-CSF).

Zoledronic acid is available from Novartis under the Tradename Zometa®. It is used in the treatment of bone metastasis in a variety of tumor types and for the treatment of hypercalcemia.

Pamidronate disodium (APD) available from Novartis under the tradename Aredia is a bone-resorption inhibitor and is used in the treatment of moderate or severe hypercalcemia. Pamidronate disodium is for i.v. injection.

Octreotide acetate is available from Novartis as Sandostatin LAR ® (octreotide acetate for injectable suspension) and Sandostatin® (octreotide acetate for injection ampuls or for vials). Octreotide is known chemically as L-Cysteinamide, D-phenylalanyl-L-cysteinyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-N-[2-hydroxy-1-(hydroxy-methyl) propyl]-, cyclic (2, 7)-disulfide; [R-(R*,R*)]. Synthetic forms of the brain hormone somatostatin, such as octreotide, work at the site of the tumour. They bind to sst-2/sst-5 receptors to regulate gastrointestinal hormone secretion and affect tumour growth.

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 combinations of an ancillary compound and a compound of the formula (0) or a sub-group thereof such as formulae (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) 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 (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein 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 lyophilsation 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.

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, or 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).

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.

Where the compounds of the combination of the invention are presented together, they can be formulated together as tablets, capsules, solutions for infusion or injection or any of the other solid or liquid dosage forms described above. For example, where they are formulated together, they may be intimately mixed, or physically separated within the same formulation, for example by virtue of being present in different layers or granules within a tablet, or a separate beads or granules within a capsule. More typically, however, they are formulated separately for separate or concurrent administration.

In one embodiment, the the individual components of the combination may be formulated separately and presented together in the form of a kit, optionally under common outer packaging and optionally with instructions for their use.

More commonly these days, pharmaceutical formulations are prescribed to a patient in "patient packs" containing the whole 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 physicians instructions.

Accordingly, in a further embodiment, the invention provides a package containing separate dosage units, one or more of which contain a compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein, and one or more of which contain an ancillary compound. Dosage units containing a compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein and an ancillary compound have suitable amounts of active ingredient as defined herein. A package contains enough tablets, capsules or the like to treat a patient for a pre-determined period of time, for instance for 2 weeks, 1 month or 3 months.

Methods of Treatment

The combinations containing an ancillary compound and compounds of the formula (0) and sub-groups thereof such as formulae (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof 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/or GSKs (e.g. GSK-3). Examples of such disease states and conditions are set out herein.

The combinations 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.

The compounds of the combination 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, e.g.1, 2, 3, 4, 5, 6, or 7 days, apart where

required), the precise dosage regimen being commensurate with the properties of the therapeutic agent(s). With sequential administration, the delay in administering the second (or additional) active ingredient should not be such as to lose the advantageous benefit of the efficacious effect of the combination of the active ingredients. In addition, the delay in administering the second (or additional) active ingredient is typically timed so as to allow for any adverse side effects of the first compound to subside to an acceptable level before adminstration of the second compound, whilst not losing the advantageous benefit of the efficacious effect of the combination of the active ingredients.

The two or more treatments may be given in individually varying dose schedules and via the same or different routes.

For example, one compound may be administered by the oral route and the other compound administered by parenteral administration such as administration by injection (e.g. i.v.) or infusion. In an alternative, both compounds may be administered by injection or infusion. In a further alternative, both compounds may be given orally. In one particular embodiment, the compound of the formula (I) is administered by injection or infusion and the ancillary compound is adminstered orally.

When administered at different times, the administration of one component of the combination may alternate with or interleaf with administration of the other component or the components of the combination may be administered in sequential blocks of therapy. As indicated above, the administration of the components of the combination may be spaced apart in time, for example by one or more hours, or days, or even weeks, provided that they form part of the same overall treatment.In one embodiment of the invention, the compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein is administered sequentially or simultaneously with the ancillary compound.

In another embodiment of the invention, the compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein is administered sequentially with the ancillary compound in either order.

In a further embodiment, the ancillary compound is administered prior to the compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein.

In another embodiment, the ancillary compound is administered after the compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein.

In another embodiment of the invention, the compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein and the ancillary compound are administered simultaneously.

In another embodiment, the compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein and the ancillary compound are each administered in a therapeutically effective amount with respect to the individual components; in other words, the the compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein and the ancillary compound are administered in amounts that would be therapeutically effective even if the components were administered other than in combination.

In another embodiment, the compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein and the ancillary compound are each administered in a sub-therapeutic amount with respect to the individual components; in other words, the compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein and the ancillary compound are administered in amounts that would be therapeutically ineffective if the components were administered other than in combination.

Preferably, the ancillary compound(s) and the compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein interact in a synergistic or additive manner, and in particular a synergistic manner.

A typical daily dose of the compound of the formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein 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.

An example of a dosage for a 60 kilogram person comprises administering a compound of the formula (I) as defined herein, for example the free base of compound 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide at a starting dosage of 4.5-10.8 mg/60kg/day (equivalent to 75-180ug/kg/day) and subsequently by an efficacious dose of 44-97 mg/60kg/day (equivalent to 0.7-1.6 mg/kg/day) or an efficacious dose of 72-274 mg/60kg/day (equivalent to 1.2-4.6 mg/kg/day). The mg/kg dose would scale pro-rata for any given body weight.

An example of a dosage for the mesylate salt is, at a starting dosage of 5.6-13.5 mg/60 kg/day (equivalent to 93-225 µg/kg/day/person) and subsequently by an efficacious dose of 55-122 mg/60 kg/day (equivalent to 0.9-2.0mg/kg/day/person) or an efficacious dose of 90-345 mg/60 kg/day (equivalent to 1.5-5.8 mg/kg/day/person).

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, the type of composition used, and the timing and frequency of the adinstration of the two components will be commensurate with the nature of the disease or physiological condition being treated and will be at the discretion of the physician.

Accordingly, a person skilled in the art would know through their common general knowledge the dosing regimes and combination therapies to use. It will be appreciated that the preferred method and order of administration and the respective dosage amounts and regimes for each component of the combination will depend on the particular ancillary compound and compounds of formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein being administered, their route of administration, the particular tumour being treated and the particular host being treated. The optimum method and order of administration and dosage amounts and regime can be readily determined by those skilled in the art using conventional methods and in view of the information set out herein.

As described *infra*, the compounds of the formula (I) are administered in combination therapy with one of more other ancillary compounds, for example in the treatment of a particular disease state (for example a neoplastic disease such as a cancer as hereinbefore defined). Examples of suitable ancillary compounds that may be used in the combinations of the invention are described in detail above.

However, the combinations of the invention may also be further combined with other classes of therapeutic agents or treatments that may be administered together (whether concurrently or at different time intervals) with the combinations of the invention, including (but not limited to):

1. hormones, hormone agonists, hormone antagonists and hormone modulating agents (including antiandrogens, antiestrogens and GNRAs);
2. monoclonal antibodies (e.g. monoclonal antibodies to cell surface antigen(s));
3. alkylating agents (including aziridine, nitrogen mustard and nitrosourea alkylating agents);
4. CDK inhibitors;
5. COX-2 inhibitors;
6. HDAC inhibitors;
7. DNA methylase inhibitors;
8. proteasome inhibitors;
9. 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), granulocyte-colony stimulating factor (G-CSF). In other embodiments, the other therapeutic or prophylactic agents can be as described below.

Alternatively, the combinations of the invention may also be further combined with other classes of therapeutic agents or treatments that may be administered together (whether concurrently or at different time intervals) with the combinations of the invention, including (but not limited to):

1. hormones, hormone agonists, hormone antagonists and hormone modulating agents (including antiandrogens, antiestrogens and GNRAs);
2. monoclonal antibodies (e.g. monoclonal antibodies to cell surface antigen(s));
3. camptothecin compounds;
4. antimetabolites;
5. vinca alkaloids;
6. taxanes;
7. platinum compounds;
8. DNA binders and Topo II inhibitors (including anthracycline derivatives);
9. alkylating agents (including aziridine, nitrogen mustard and nitrosourea alkylating agents);
10. a combination of two or more of the foregoing classes (1)-(9).
11. signalling inhibitors (including PKB signalling pathway inhibitors);
12. CDK inhibitors;
13. COX-2 inhibitors;
14. HDAC inhibitors;
15. DNA methylase inhibitors;
16. proteasome inhibitors;
17. a combination of two or more of the foregoing classes (11)-(16);
18. a combination of two or more of the foregoing classes (1)-(17);
19. 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), granulocyte-colony stimulating factor (G-CSF). In other embodiments, the other therapeutic or prophylactic agents can be as described below.

Other therapeutic or prophylactic agents

The compositions may also include 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).

Also included are agents that inhibit bone resorption such as bisphosphonate agents e.g. zoledronate, pamidronate and ibandronate, as well as agents that suppress inflammatory responses (such as dexamethazone, prednisone, and prednisolone). Also included are agents used to reduce blood levels of growth hormone and IGF-I in acromegaly patients such as synthetic forms of the brain hormone somatostatin, which includes octreotide acetate which is a long-acting octapeptide with pharmacologic properties mimicking those of the natural hormone somatostatin. Further included are agents such as leucovorin, which is used as an antidote to drugs that decrease levels of folic acid, or folinic acid it self. In one particular embodiment is the combination of 5FU and leucovorin or 5FU and folinic acid. In addition megestrol acetate can be used for the treatment of side-effects including oedema and thromoembolic episodes.

Therefore in one embodiment the combinations further include an additional agent selected from erythropoietin (EPO), granulocyte macrophage-colony stimulating factor (GM-CSF), granulocyte-colony stimulating factor (G-CSF), zoledronate, pamidronate, ibandronate, dexamethazone, prednisone, prednisolone, leucovorin, folinic acid and megestrol acetate.

In particular the combinations further include an additional agent selected from erythropoietin (EPO), granulocyte macrophage-colony stimulating factor (GM-CSF),

granulocyte-colony stimulating factor (G-CSF), zoledronate, pamidronate, dexamethazone, prednisone, prednisolone, leucovorin, and folinic acid such as erythropoietin (EPO), granulocyte macrophage-colony stimulating factor (GM-CSF) and granulocyte-colony stimulating factor (G-CSF).

Zoledronic acid is available from Novartis under the Tradename Zometa®. It is used in the treatment of bone metastasis in a variety of tumor types and for the treatment of hypercalcemia.

Pamidronate disodium (APD) available from Novartis under the tradename Aredia is a bone-resorption inhibitor and is used in the treatment of moderate or severe hypercalcemia. Pamidronate disodium is for i.v. injection.

Octreotide acetate is available from Novartis as Sandostatin LAR ® (octreotide acetate for injectable suspension) and Sandostatin® (octreotide acetate for injection ampuls or for vials). Octreotide is known chemically as L-Cysteinamide, D-phenylalanyl-L-cysteinyl-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threonyl-N-[2-hydroxy-1-(hydroxy-methyl) propyl]-, cyclic (2, 7)-disulfide; [R-(R*,R*)]. Synthetic forms of the brain hormone somatostatin, such as octreotide, work at the site of the tumour. They bind to sst-2/sst-5 receptors to regulate gastrointestinal hormone secretion and affect tumour growth.

However, the combinations of the invention as defined herein can be further combined and/or administered 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 treatments that may be administered together (whether concurrently or at different time intervals) with the combinations of the invention include but are not limited to:

- Topoisomerase I inhibitors
- Antimetabolites
- Tubulin targeting agents
- DNA binder and topoisomerase II inhibitors
- Alkylating Agents
- Monoclonal Antibodies.
- Anti-Hormones
- Signal Transduction Inhibitors

- Proteasome Inhibitors
- DNA methyl transferases
- Cytokines and retinoids
- Chromatin targeted therapies
- Radiotherapy, and,
- 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). Also included are agents that inhibit bone resorption such as bisphosphonate agents e.g. zoledronate, pamidronate and ibandronate, agents that suppress inflammatory responses (such as dexamethazone, prednisone, and prednisolone) and agents used to reduce blood levels of growth hormone and IGF-I in acromegaly patients such as synthetic forms of the brain hormone somatostatin, which includes octreotide acetate which is a long-acting octapeptide with pharmacologic properties mimicking those of the natural hormone somatostatin. Further included are agents such as leucovorin, which is used as an antidote to drugs that decrease levels of folic acid, or folinic acid it self and agents such as megestrol acetate which can be used for the treatment of side-effects including oedema and thromoembolic episodes.

Each of the compounds present in the combinations of the invention may be given in individually varying dose schedules and via different routes.

Thus, administration of the compound of the formula (I) in combination therapy with one or more ancillary compounds may comprise simultaneous or sequential administration. 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 combinations of the invention may also be administered in conjunction with non-chemotherapeutic treatments such as radiotherapy, photodynamic therapy, gene therapy, surgery and controlled diets.

The combination therapy may therefore involve the formulation of the compound of the formula (I) with one, two, three, four or more other therapeutic agents (including at least one ancillary compound). Such formulations can be, for example, 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 their common general knowledge the dosing regimes and combination therapies to use.

Methods of Diagnosis

Prior to administration of a combination of the invention, 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 kinase(s) and/or GSK (e.g. GSK-3) or treatment with an ancillary compound.

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). 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 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 combinations of CDK inhibitors and ancillary compounds. Patients with mantle cell lymphoma (MCL) could be selected for treatment with the combination 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 combination of the invention.

It has also been found, see Ewart-Toland et al., (Nat Genet. 2003 Aug;34(4):403-12), that individuals forming part of the sub-population possessing the Ile31 variant of the STK gene (the gene for Aurora kinase A) may have an increased susceptibility to certain forms of cancer. Therefore that such individuals suffering from cancer will benefit from the administration of combinations having Aurora kinase inhibiting activity. A patient suffering from, or suspected of suffering from, a cancer may therefore be screened to determine whether he or she forms part of the Ile31 variant sub-population. Thus, the patient may be subjected to a diagnostic test to detect a marker characteristic of over-expression, up-regulation or activation of Aurora kinase. Tumours with activating mutants of Aurora or up-regulation of Aurora including any of the isoforms thereof, may be particularly sensitive to Aurora inhibitors. Tumours may preferentially be screened for up-regulation of Aurora or for Aurora possessing the Ile31 variant prior to treatment (Ewart-Toland et al., Nat Genet. 2003 Aug;34(4):403-12). Ewart-Toland *et al* identified a common genetic variant in STK15 (resulting in the amino acid substitution F31I) that is preferentially amplified and associated with the degree of aneuploidy in human colon tumors. These results are consistent with an important role for the Ile31 variant of STK15 in human cancer susceptibility. In particular, this polymorphism in Aurora A has been suggested to be a genetic modifier fir developing breast carcinoma (Sun *et al*, Carcinogenesis, 2004, 25(11), 2225-2230).

The Aurora A gene maps to the chromosome 20q13 region that is frequently amplified in many cancers e.g. breast, bladder, colon, ovarian, pancreatic. Patients with a tumour that has this gene amplification might be particularly sensitive to treatments targeting Aurora kinase inhibition

Methods of identification and analysis of mutations and up-regulation of protein e.g. Aurora isoforms and chromosome 20q13 amplification are 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.

Prior to administration of the combination of the invention, 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 Flt3, C-abl, PDK1. These techniques may also be used for screening for diseases or conditions caused by the up-regulation or mutants of Flt3, C-abl, PDK1,kinases. These techniques may also be used for screening for diseases or conditions caused by the up-regulation or mutants of VEGFR kinases, include, but are not limited to, standard methods such as reverse-transcriptase polymerase chain reaction (RT-PCR) or in-situ hybridisation.

In addition, mutant forms of, for example, VEGFR2 can be identified by direct sequencing of, for example, tumour biopsies using PCR and methods to sequence PCR products directly as hereinbefore described. The skilled artisan will recognize that all such well-known techniques for detection of the over expression, activation or mutations of the aforementioned proteins could be applicable in the present case.

Abnormal levels of proteins such as VEGFR can be measured using standard enzyme assays, for example, those assays described herein. Activation or overexpression could also be detected in a tissue sample, for example, a tumour tissue, by measuring the tyrosine kinase activity with an assay such as that available from Chemicon International. The tyrosine kinase of interest would be immunoprecipitated from the sample lysate and its activity measured.

Alternative methods for the measurement of the over expression or activation of VEGFR including the isoforms thereof, include the measurement of microvessel density. This can for example be measured using methods described by Orre and Rogers (Int J Cancer 1999 84(2) 101-8). Assay methods also include the use of markers, for example, in the case of

VEGFR these include CD31, CD34 and CD105 (Mineo et al. J Clin Pathol. 2004 57(6) 591-7).

Activating mutations of FLT3 are frequently observed in acute myeloid leukaemia, myelodysplastic syndromes (MDS) and some cases with acute lymphoblastic leukemia (ALL). Cancer patients with activating mutants of FLT3 can be screened for presence of the length mutations or internal tandem duplication mutations as an indication of those most sensitive to inhibitors of FLT3.

Patients with tumours harbouring cells expressing the resisance mutants of BCR-abl e.g. T315I can be identified using the methods described herein.

Therefore in addition the methods described herein could be used to diagnose mutations of activating mutations of FLT3, mutants of C-Abl e.g. T315I.

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 compounds prepared were characterised by liquid chromatography and mass spectroscopy (LC-MS) using the system and operating conditions set out in WO2005/012256 at pages 116 *et seq.*.

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

Examples 1 to 254 of WO2005/012256 at pages 121 to 222 are incorporated herein by reference, so that examples of the preparation of the following compounds are specifically described herein:

- 4-Amino-1H-pyrazole-3-carboxylic acid phenylamide
- 4-Acetylamino-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(2,2,2-Trifluoro-acetylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide

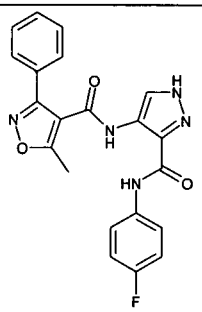
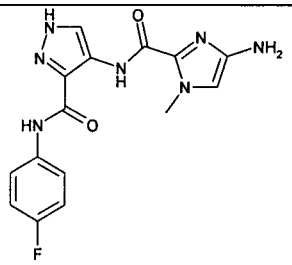
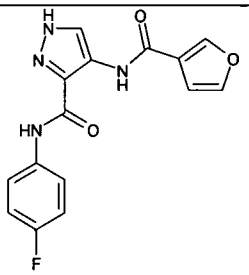
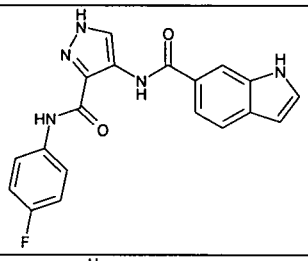
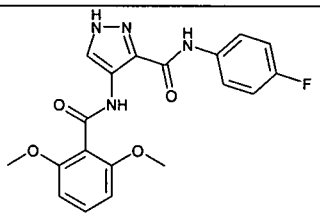
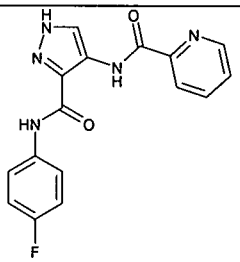
- 4-[(5-Oxo-pyrrolidine-2-carbonyl)-amino]-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-Phenylacetylamino-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(2-1H-Indol-3-yl-acetylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(2-Benzenesulphonyl-acetylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-[2-(5-Amino-tetrazol-1-yl)-acetylamino]-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- N-[3-(4-Fluoro-phenylcarbamoyl)-1H-pyrazol-4-yl]-6-hydroxy-nicotinamide
- 4-[3-(4-Chloro-phenyl)-propionylamino]-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(3-4H-[1,2,4]Triazol-3-yl-propionylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-[2-(1-Methyl-1H-indol-3-yl)-acetylamino]-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-[(1-Hydroxy-cyclopropanecarbonyl)-amino]-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 1-Acetyl-piperidine-4-carboxylic acid [3-(4-fluoro-phenylcarbamoyl)-1H-pyrazol-4-yl]-amide
- 4-[3-(4-Methyl-piperazin-1-yl)-propionylamino]-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(2-1H-Imidazol-4-yl-acetylamino)-1H-pyrazole-3-carboxylic acid (4-fluorophenyl)-amide
- 4-(3-Morpholin-4-yl-propionylamino)-1H-pyrazole-3-carboxylic acid (4-fluorophenyl)-amide
- 4-(3-Piperidin-1-yl-propionylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-Cyclohexylamino-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-Isopropylamino-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(2-Hydroxy-1-methyl-ethylamino)-1H-pyrazole-3-carboxylic acid (4-fluorophenyl)-amide
- 4-(1-Ethyl-propylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide

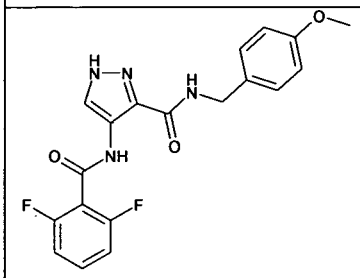
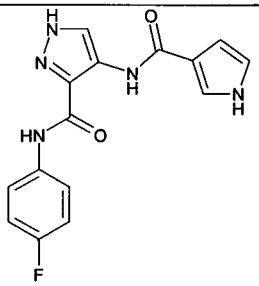
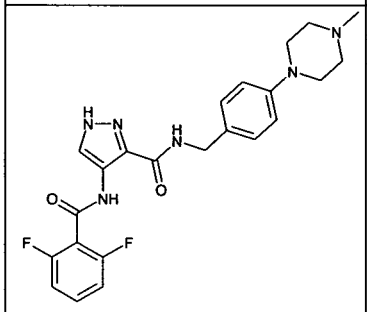
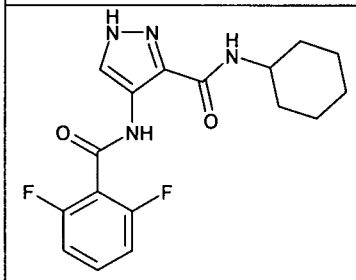
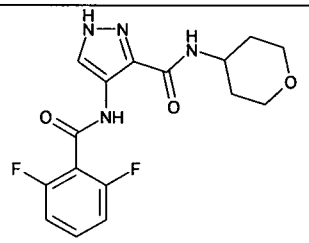
- 4-(3-Chloro-pyrazin-2-ylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(Pyrazin-2-ylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(2-Methoxy-benzoylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-Benzoylamino-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(Cyclohexanecarbonyl-amino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-[(1-Methyl-cyclopropanecarbonyl)-amino]-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(2-Hydroxy-acetylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(2,2-Dimethyl-propionylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(3-Hydroxy-propionylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(2-Fluoro-benzoylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(3-Fluoro-benzoylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(3-Methoxy-benzoylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(4-Nitro-benzoylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-[(3-Methyl-furan-2-carbonyl)-amino]-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-[(Furan-2-carbonyl)-amino]-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-[(3H-Imidazole-4-carbonyl)-amino]-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(4-Fluoro-benzoylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(2,6-Difluoro-benzoylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(3-Nitro-benzoylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 1H-Indole-3-carboxylic acid [3-(4-fluoro-phenylcarbamoyl)-1H-pyrazol-4-yl]-amide
- 4-(4-Hydroxymethyl-benzoylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(3-Methyl-benzoylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(2-Methyl-benzoylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(4-Methyl-benzoylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide

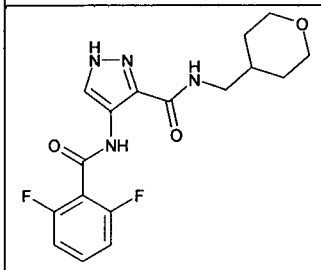
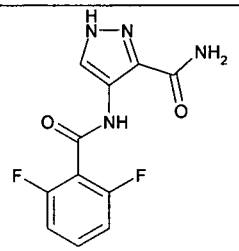
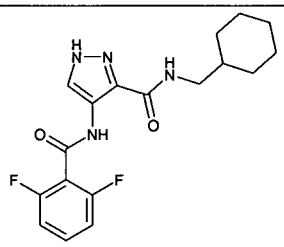
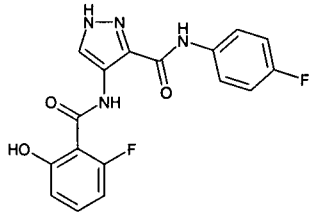
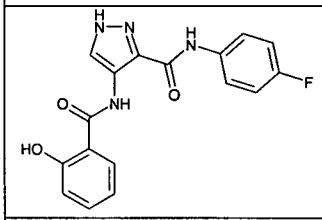
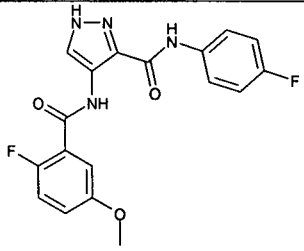
- 4-[(2-Methyl-thiophene-3-carbonyl)-amino]-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- Quinoline-2-carboxylic acid [3-(4-fluoro-phenylcarbamoyl)-1H-pyrazol-4-yl]-amide
- 4-[(Thiophene-3-carbonyl)-amino]-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(2-fluoro-3-methoxy-benzoylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-[2-(2-Pyrrolidin-1-yl-ethoxy)-benzoylamino]-1H-pyrazole-3-carboxylic acid 4-fluorophenylamide
- 4-(2,6-Difluoro-benzoylamino)-1*H*-pyrazole-3-carboxylic acid (1-methyl-piperidin-4-yl)-amide
- 4-(Cyclohexyl-methyl-amino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(Pyridin-2-ylamino)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-[(4-Amino-1-methyl-1H-imidazole-2-carbonyl)-amino]-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-{[4-(2,6-Difluoro-benzoylamino)-1H-pyrazole-3-carbonyl]-amino}-cyclohexanecarboxylic acid
- 4-(2,6-difluoro-benzoylamino)-1H-pyrazole-3-carboxylic acid [5-fluoro-2-(1-methyl-piperidin-4-yloxy)-phenyl]-amide
- 4-(2,6-Difluoro-benzoylamino)-1H-pyrazole-3-carboxylic acid [5-fluoro-2-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-amide
- 4-(4-Methyl-piperazin-1-yl)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-Morpholin-4-yl-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(2,4-Dichloro-phenyl)-1H-pyrazole-3-carboxylic acid 4-(4-methyl-piperazin-1-yl)-benzylamide
- 4-(2,4-Dichloro-phenyl)-1H-pyrazole-3-carboxylic acid 4-methylsulphamoylmethyl-benzylamide
- 4-Phenyl-1H-pyrazole-3-carboxylic acid amide
- 4-phenyl-1H-pyrazole-3-carboxylic acid phenylamide
- 4-Phenyl-1H-pyrazole-3-carboxylic acid 4-(4-methyl-piperazin-1-yl)-benzylamide
- 4-Phenyl-1H-pyrazole-3-carboxylic acid (6-methoxy-pyridin-3-yl) amide
- 4-(3-Benzyloxy-phenyl)-1H-pyrazole-3-carboxylic acid 4-(4-methyl-piperazin-1-yl)-benzylamide

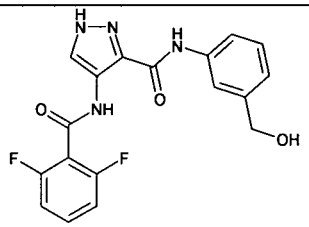
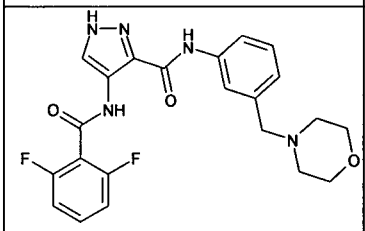
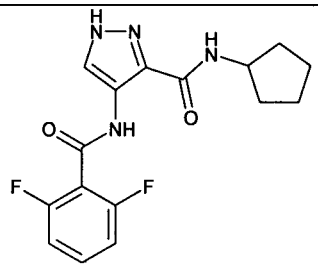
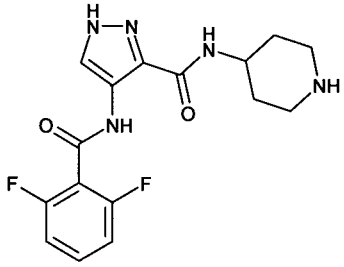
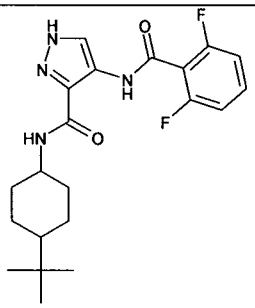
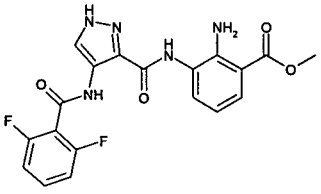
- 4-(3-Hydroxy-phenyl)-1H-pyrazole-3-carboxylic acid 4-(4-methyl-piperazin-1-yl)-benzylamide
- 4-(5-Methyl-3H-imidazol-4-yl)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(2,5-Dimethyl-pyrrol-1-yl)-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(3-Hydroxymethyl-phenyl)-1H-pyrazole-3-carboxylic acid phenylamide
- 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide hydrochloride
- 4-Methanesulfonylamino-1H-pyrazole-3-carboxylic acid (4-fluoro-phenyl)-amide
- 4-(2,6-Difluoro-benzoylamino)-1H-pyrazole-3-carboxylic acid [1-(2-fluoro-ethyl)-piperidin-4-yl]-amide
- 4-(2,6-Dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid (6-chloro-pyridin-3-yl)-amide
- 4-(2,6-Dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid (6-amino-pyridin-3-yl)-amide
- 4-(2,6-Dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid (6-methoxy-pyridin-3-yl)-amide
- 4-[3-Chloro-5-(4-methyl-piperazin-1-yl)-benzoylamino]-1H-pyrazole-3-carboxylic acid cyclohexylamide
- 4-(2,6-Difluoro-benzoylamino)-1H-pyrazole-3-carboxylic acid [1-(2,2-difluoro-ethyl)-piperidin-4-yl]-amide
- 4-[3-(4-Methyl-piperazin-1-yl)-benzoylamino]-1H-pyrazole-3-carboxylic acid cyclohexylamide
- 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide acetic acid salt
- Methanesulphonic acid salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide

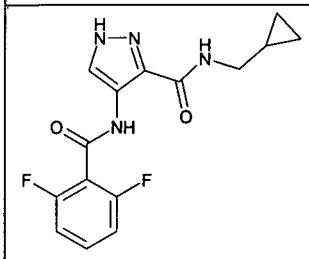
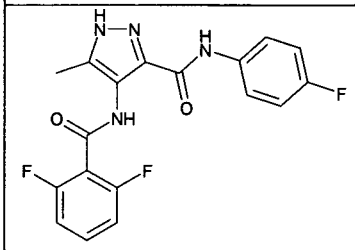
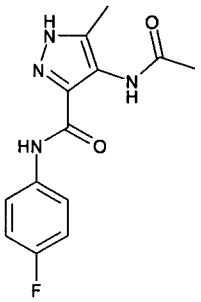
The compounds set out in the Tables below:

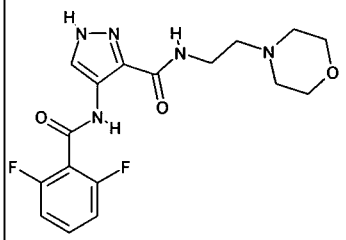
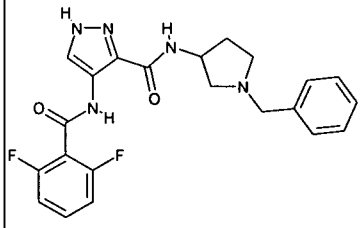
Structure	LCMS
	R_t 3.20 min $[M+H]^+$ 406.07
	R_t 2.35 min m/z 343.72
	R_t 3.51 min m/z 314.62
	R_t 3.79 min m/z 363.67
	R_t 3.68 min m/z 384.69
	R_t 3.61 min m/z 326.10

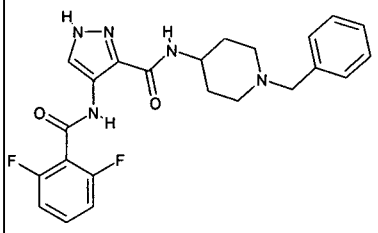
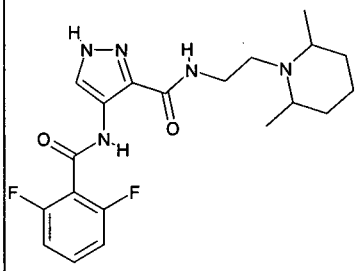
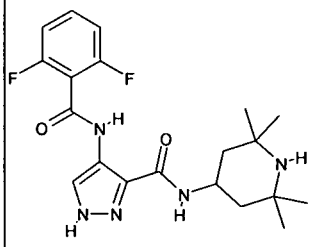
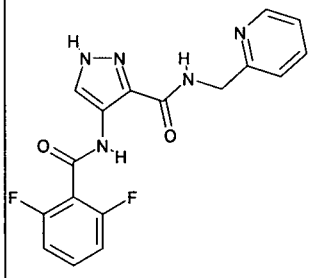
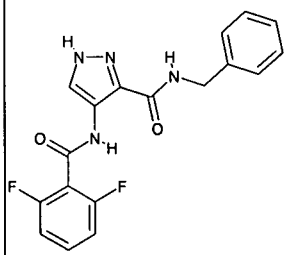
Structure	LCMS
	R_t 3.51 min m/z 387.11
	R_t 3.11 min m/z 313.65
	R_t 2.20 min m/z 455.19
	R_t 3.95 min m/z 349.09
	R_t 2.39 min m/z 351.07

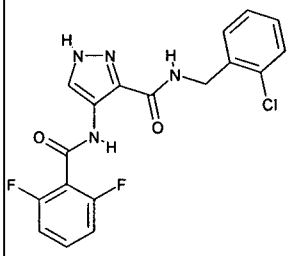
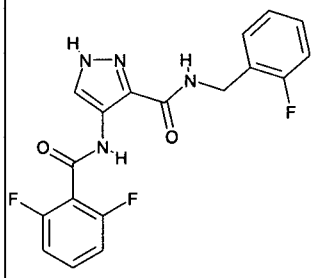
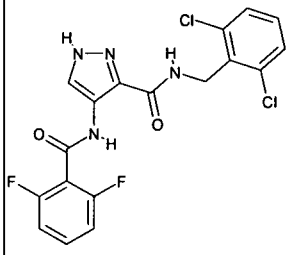
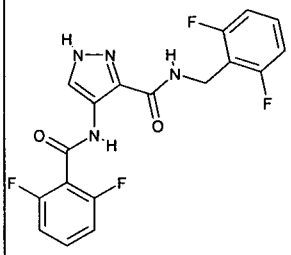
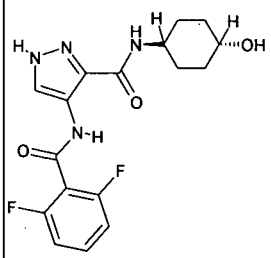
Structure	LCMS
	R_t 2.83 min m/z 365.13
	R_t 2.10 min m/z 266.97
	R_t 3.22 min m/z 363.10
	R_t 4.48 min m/z 358.96
	R_t 3.93 min m/z 340.96
	R_t 4.11 min m/z 373.01

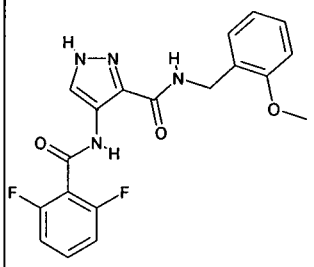
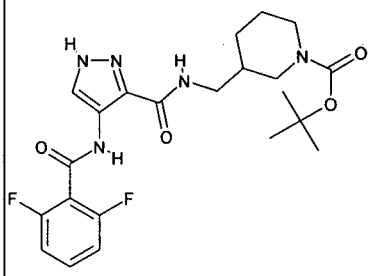
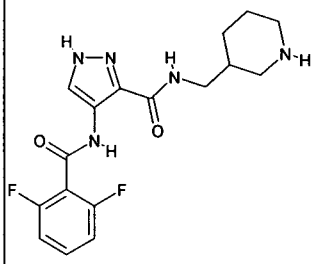
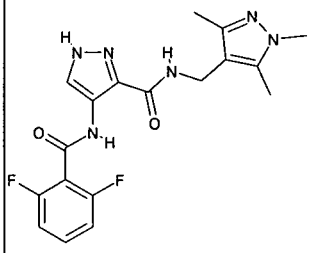
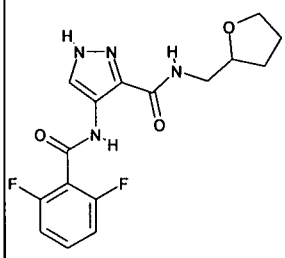
Structure	LCMS
	R_t 2.56 min m/z 373.05
	R_t 1.99 min m/z 442.09
	R_t 3.65 min m/z 335.03
	R_t 1.57 min m/z 350.10
	R_t 5.05 min m/z 405.14
	R_t 2.87 min m/z 416.07

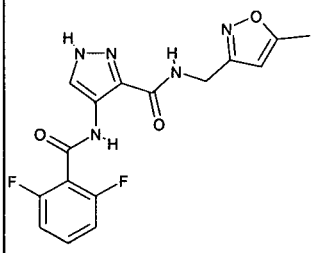
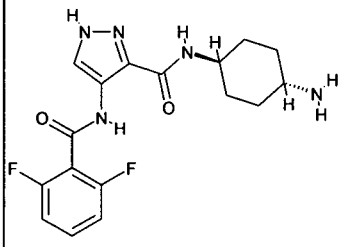
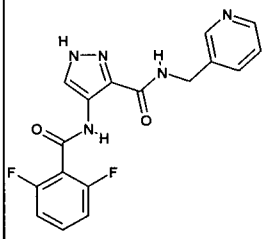
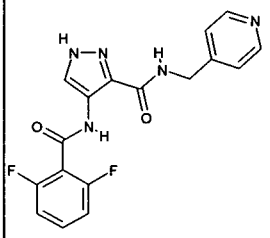
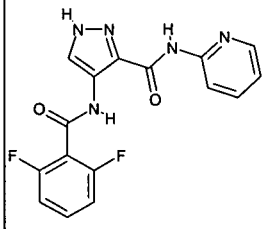
Structure	LCMS
	R_t 3.41 min m/z 321.03
	R_t 3.42 min m/z 375.05
	R_t 2.37 min m/z 277.04

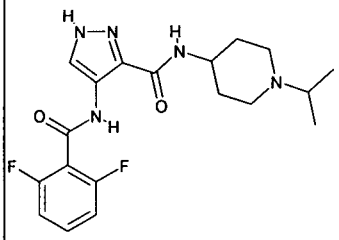
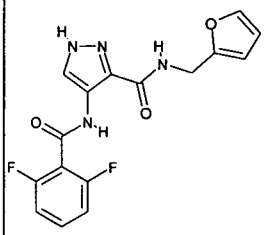
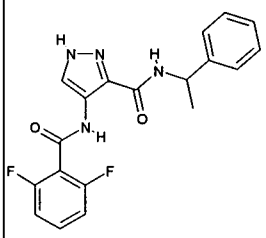
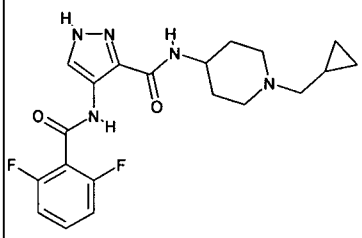
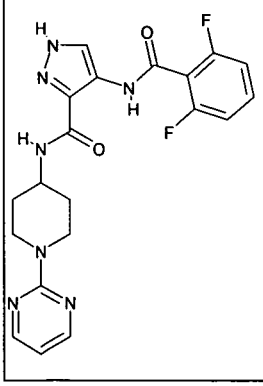
Structure	LCMS
	$[M+H]^+$ 380 R_t 1.42
	$[M+H]^+$ 426 R_t 1.93

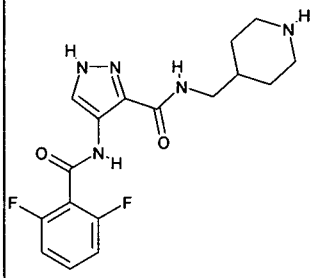
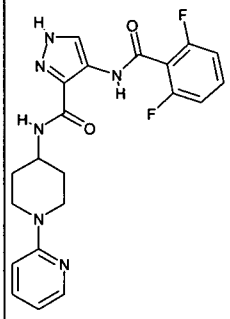
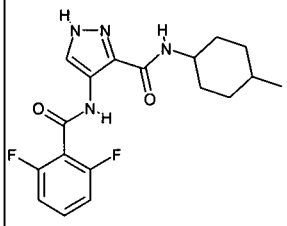
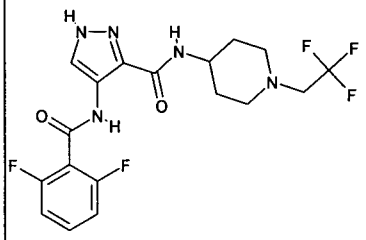
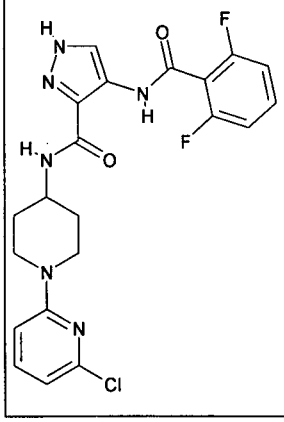
Structure	LCMS
	$[M+H]^+$ 440 R_t 1.87
	$[M+H]^+$ 406 R_t 2.78
	$[M+H]^+$ 406 R_t 2.55
	$[M+H]^+$ 358 R_t 1.98
	$[M+H]^+$ 357 R_t 3.37

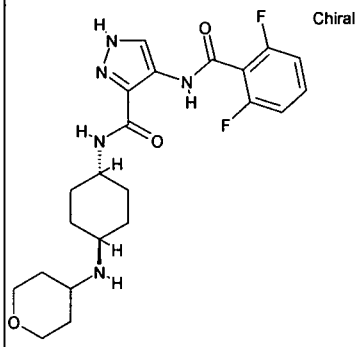
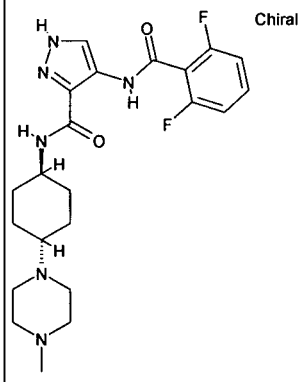
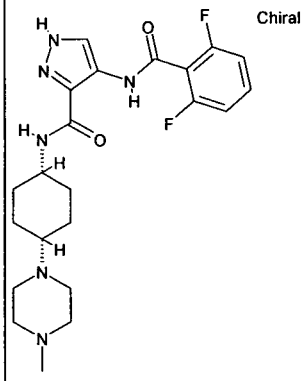
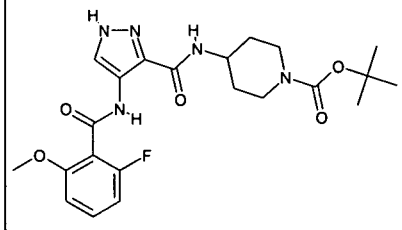
Structure	LCMS
	$[M+H]^+$ 391 R_t 3.16
	$[M+H]^+$ 375 R_t 3.02
	$[M+H]^+$ 425 R_t 3.27
	$[M+H]^+$ 393 R_t 3.01
	$[M+H]^+$ 365 R_t 2.22

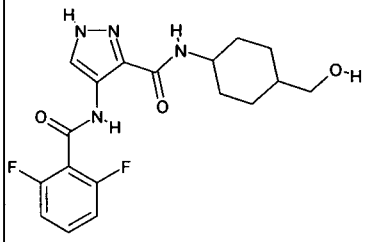
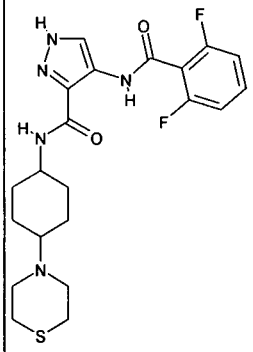
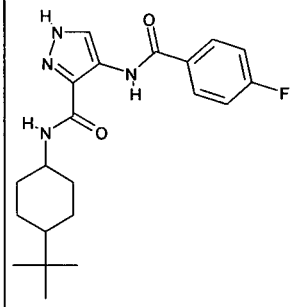
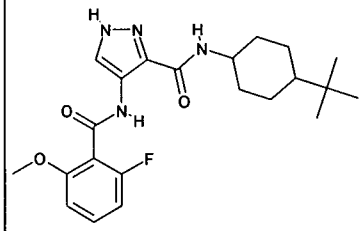
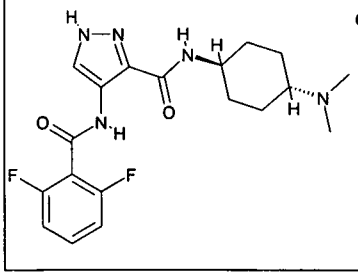
Structure	LCMS
	$[M+H]^+$ 387 R_t 3.05
	$[M+H]^+$ 464 R_t 3.17
	$[M+H]^+$ 364 R_t 1.76
	$[M+H]^+$ 389 R_t 2.36
	$[M+H]^+$ 351 R_t 2.55

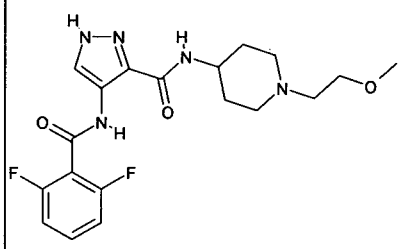
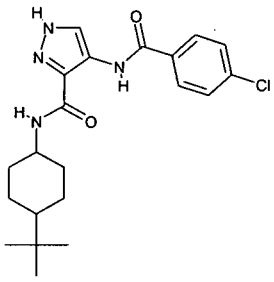
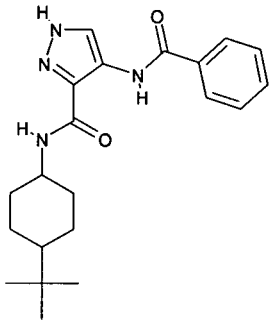
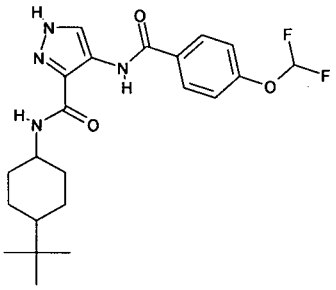
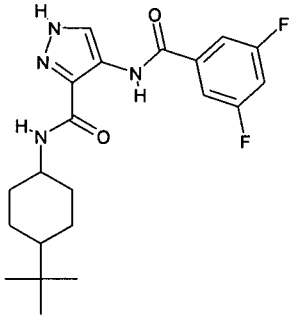
Structure	LCMS
	$[M+H]^+$ 362 R_t 2.63
	$[M+H]^+$ 364 R_t 1.75
	$[M+H]^+$ 358 R_t3.2
	$[M+H]^+$ 358 R_t 1.77
	$[M+H]^+$ 344 R_t 2.71

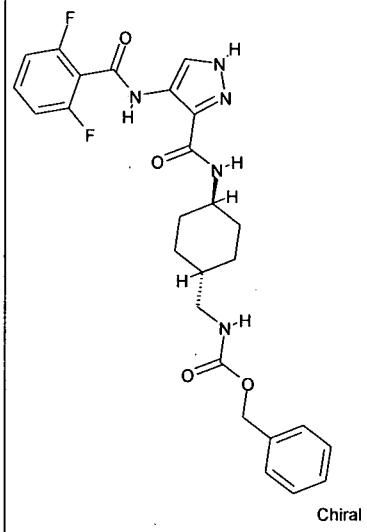
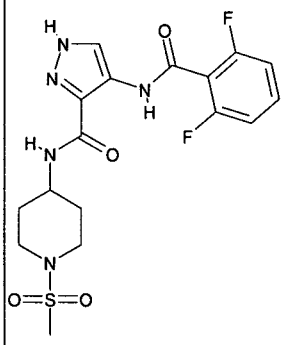
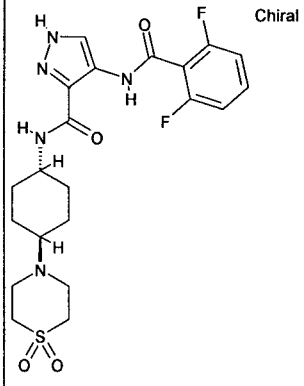
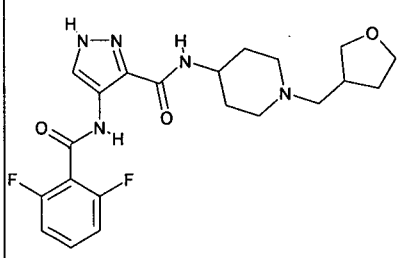
Structure	LCMS
	$[M+H]^+$ 392 R_t 2.57
	$[M+H]^+$ 347 R_t 2.8
	$[M+H]^+$ 371 R_t3.1
	$[M+H]^+$ 404 R_t 2.7
	$[M+H]^+$ 428 R_t 2.63

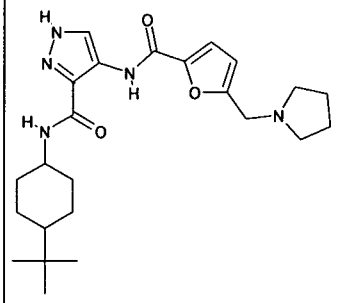
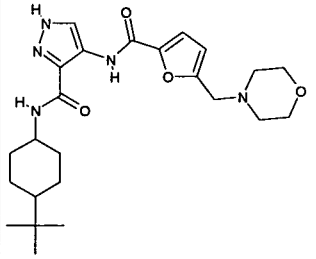
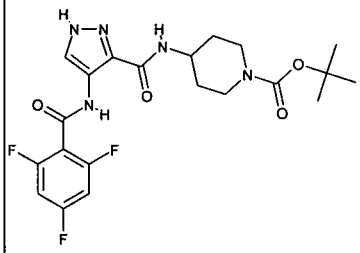
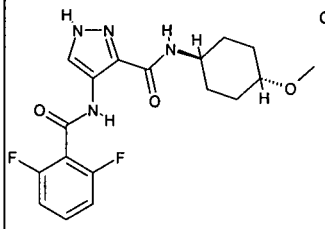
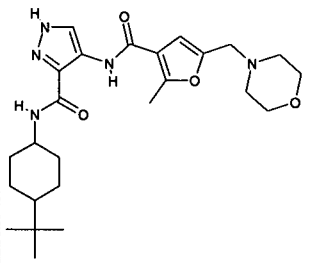
Structure	LCMS
	$[M+H]^+$ 364 R_t 1.75
	$[M+H]^+$ 427 R_t 2.71
	$[M+H]^+$ 363 R_t 3.34
	$[M+H]^+$ 432 R_t 2.63
	$[M+H]^+$ 461 R_t 3.3

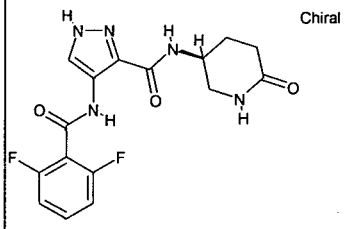
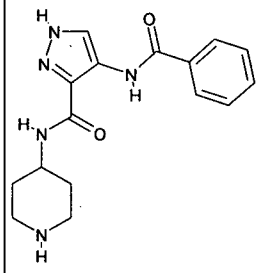
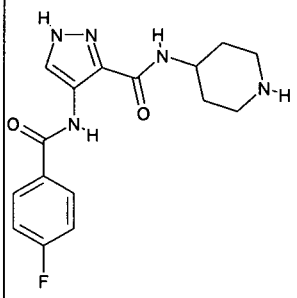
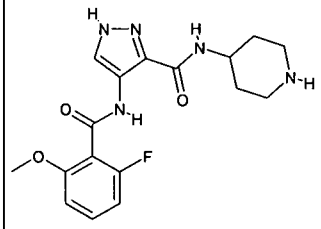
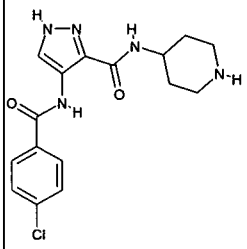
Structure	LCMS
 Chiral	$[M+H]^+$ 448 R_t 1.87
 Chiral	$[M+H]^+$ 447 R_t 1.65
 Chiral	$[M+H]^+$ 447 R_t 1.72
	$[M+H]^+$ 462 R_t 2.97

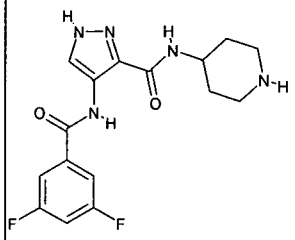
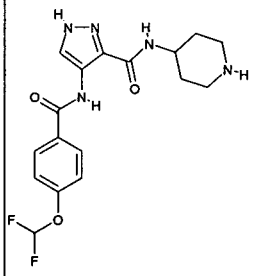
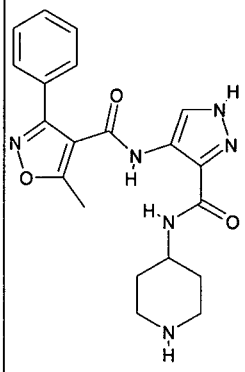
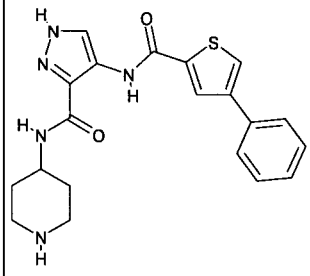
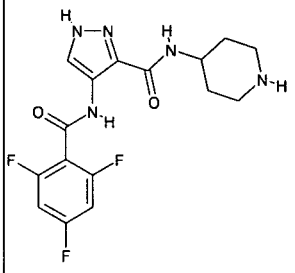
Structure	LCMS
	$[M+H]^+$ 379 R_t 2.45
	$[M+H]^+$ 450 R_t 1.97
	$[M+H]^+$ 387 R_t 3.83
	$[M+H]^+$ 417 R_t 3.65
 Chiral	$[M+H]^+$ 392 R_t 1.85

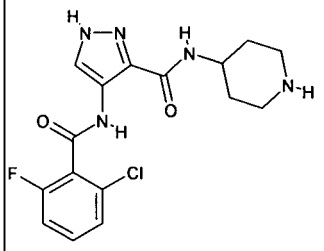
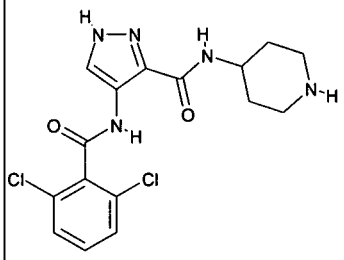
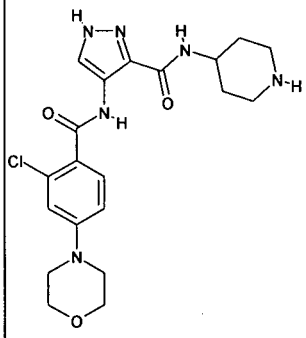
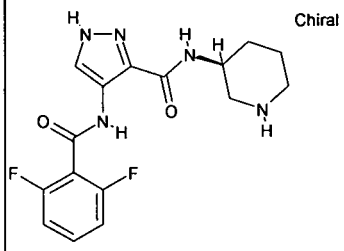
Structure	LCMS
	$[M+H]^+$ 408 R_t 1.82
	$[M+H]^+$ 403 R_t 4.02
	$[M+H]^+$ 369 R_t 3.78
	$[M+H]^+$ 435 R_t 3.83
	$[M+H]^+$ 405 R_t 3.96

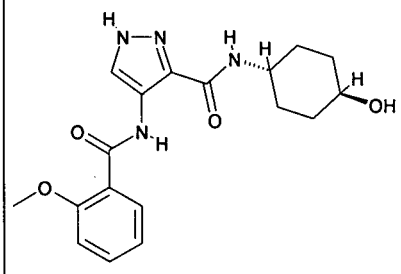
Structure	LCMS
 Chiral	$[M+H]^+$ 512 R_t3.1
	$[M+H]^+$ 428 R_t2.45
 Chiral	$[M+H]^+$ 482 R_t1.96
	$[M+H]^+$ 434 R_t2.3

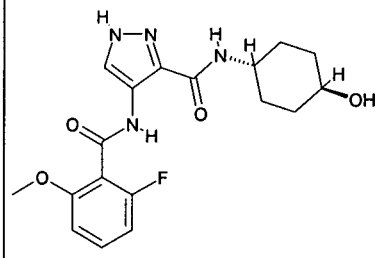
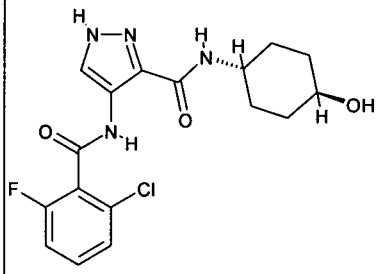
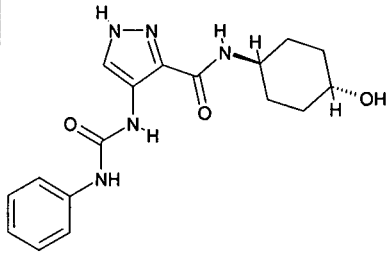
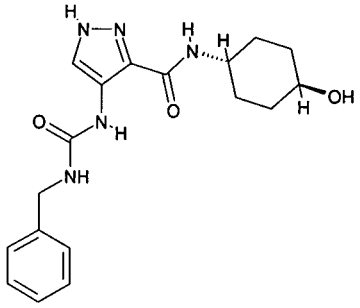
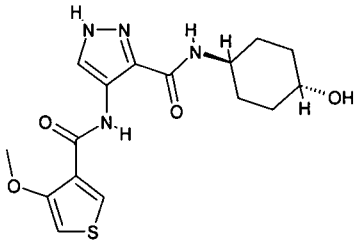
Structure	LCMS
	$[M+H]^+$ 442 R_t 2.39
	$[M+H]^+$ 458 R_t 2.26
	$[M+H]^+$ 468 R_t3.07
 Chiral	$[M+H]^+$ 379 R_t2.6
	$[M+H]^+$ 472 R_t 2.40

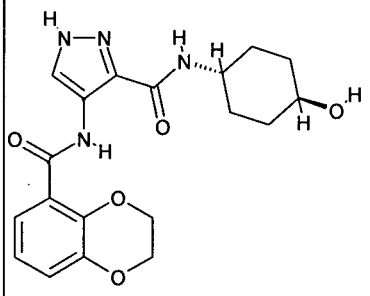
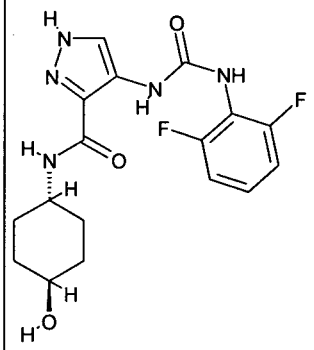
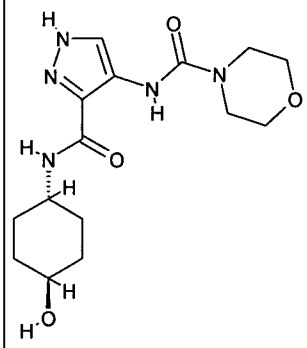
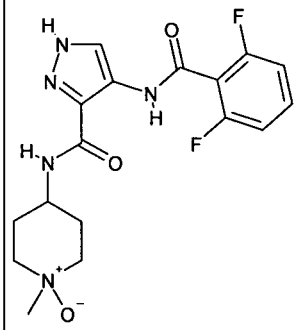
Structure	LCMS
 Chiral	$[M+H]^+$ 364 R_t2.1
	$[M+H]^+$ 314 R_t 1.78
	$[M+H]^+$ 332 R_t 1.89
	$[M+H]^+$ 362 R_t 1.78
	$[M+H]^+$ 348 R_t 2.01

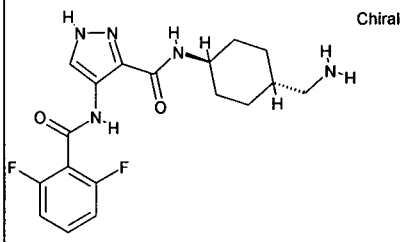
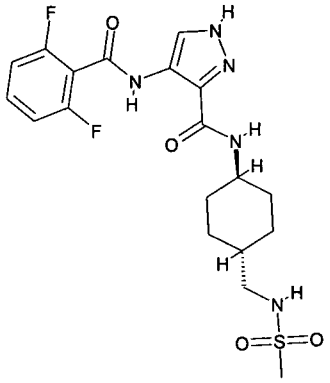
Structure	LCMS
	$[M+H]^+$ 350 R_t 1.97
	$[M+H]^+$ 380 R_t 2.01
	$[M+H]^+$ 395 R_t 1.94
	$[M+H]^+$ 396 R_t 2.11
	$[M+H]^+$ 368 R_t1.76

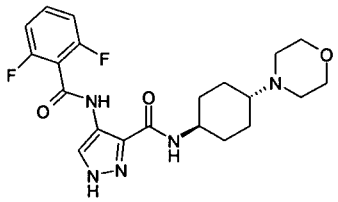
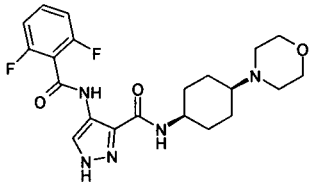
Structure	LCMS
	$[M+H]^+$ 366 R_t 1.78
	$[M+H]^+$ 383 R_t 1.87
	$[M+H]^+$ 433 R_t 1.89
 Chiral	$[M+H]^+$ 350 R_t1.76

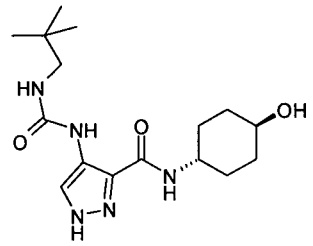
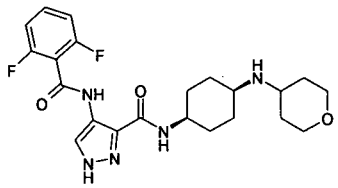
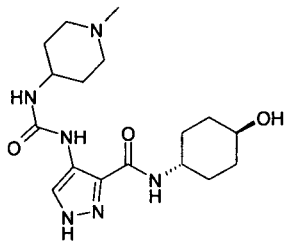
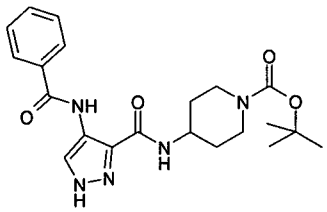
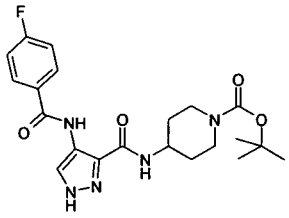
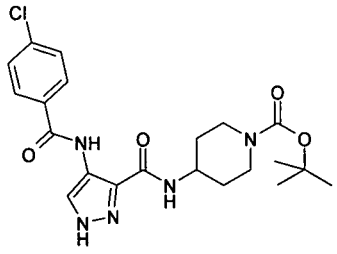
Structure	LCMS
	$[M+H]^+$ 359 R_t 2.29

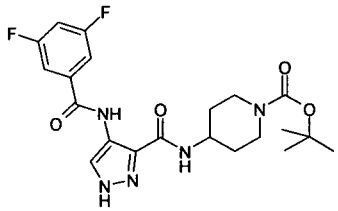
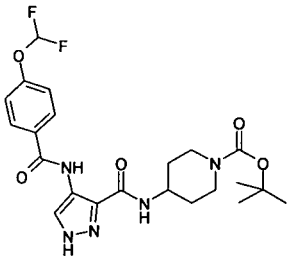
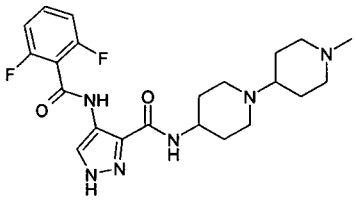
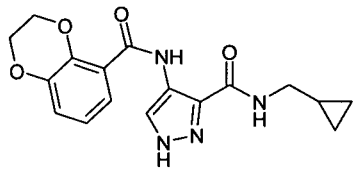
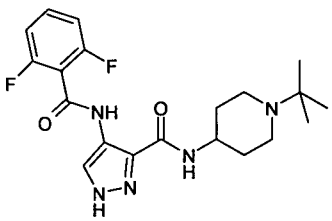
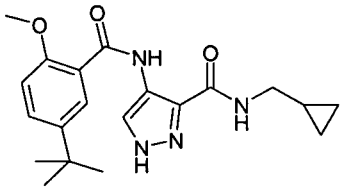
Structure	LCMS
	$[M+H]^+$ 377 R_t 2.22
	$[M+H]^+$ 381 R_t 2.34
	$[M+H]^+$ 344 R_t 2.28
	$[M+H]^+$ 358 R_t 2.22
	$[M+H]^+$ 365 R_t 2.21

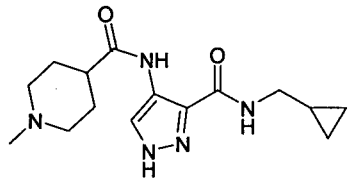
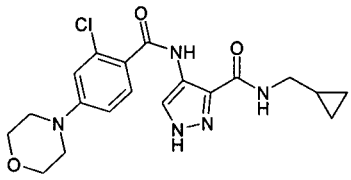
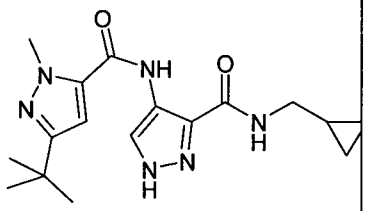
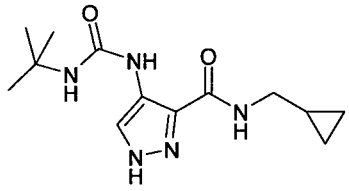
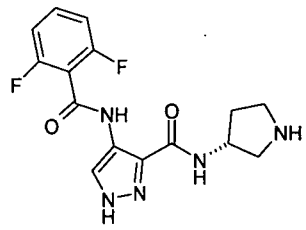
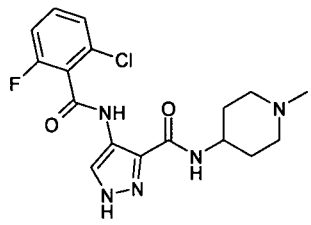
Structure	LCMS
	$[M+H]^+$ 387 R_t 2.29
	$[M+H]^+$ 380 R_t 2.17
	$[M+H]^+$ 338 R_t 1.68
	$[M+H]^+$ 380 R_t1.83

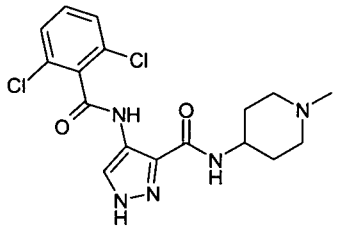
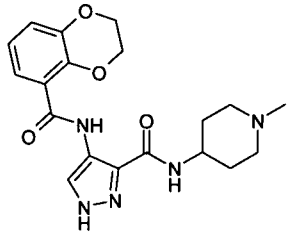
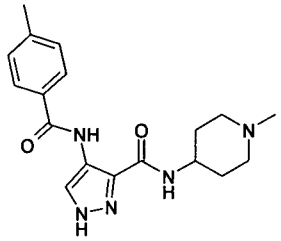
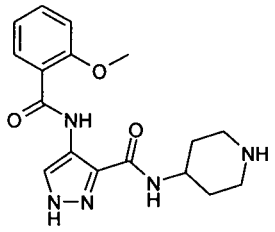
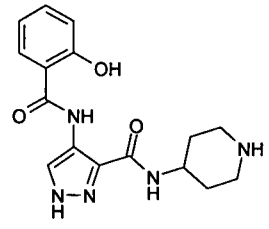
Structure	LCMS
 Chiral	$[M+H]^+$ 378 R_t1.78
	$[M+H]^+$ 456 R_t2.54

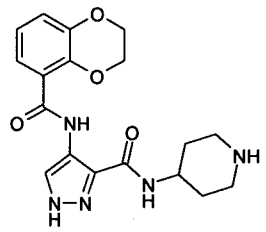
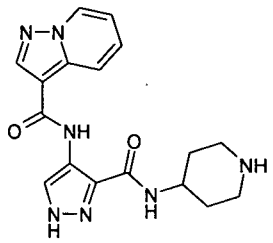
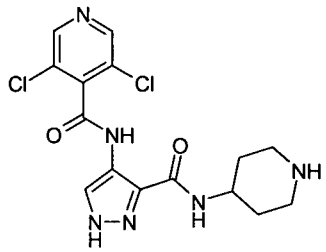
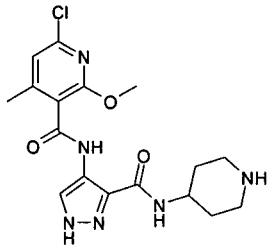
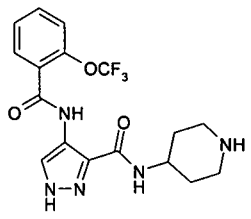
Structure	LCMS
	$[M+H]^+$ 434 R_t 1.97
	$[M+H]^+$ 434 R_t 2.03

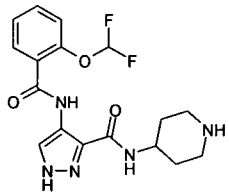
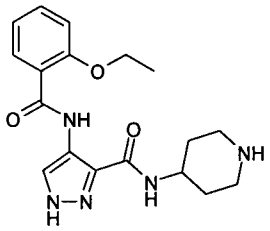
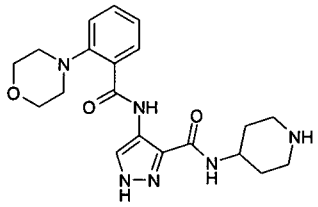
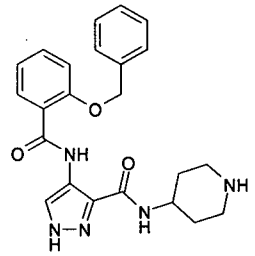
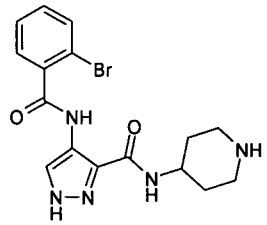
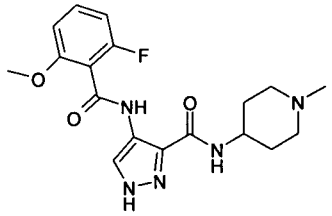
Structure	LCMS
	$[M+H]^+$ 338 R_t 2.28
	$[M+H]^+$ 448 R_t 1.97
	$[M+H]^+$ 365 R_t 0.34
	$[M+H]^+$ 414.13 R_t 3.05
	$[M+H]^+$ 432.12 R_t 3.12
	$[M+H]^+$ 448.06 R_t 3.33

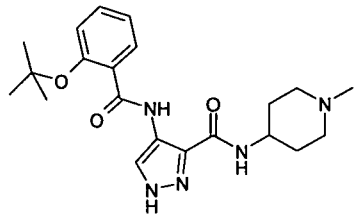
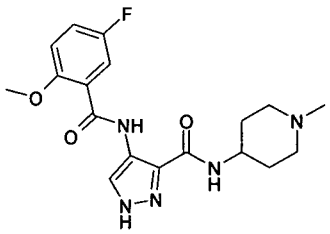
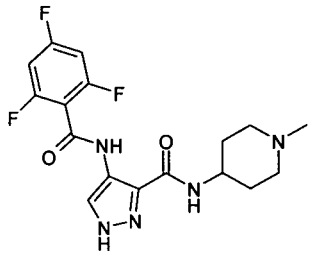
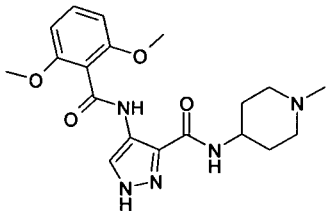
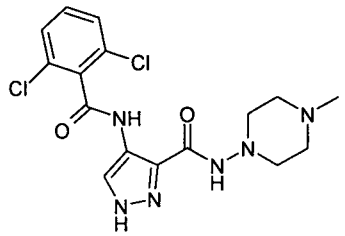
Structure	LCMS
	$[M+H]^+$ 450.08 R_t 3.29
	$[M+H]^+$ 480.05 R_t 3.18
	$[M+H]^+$ 447 R_t 2.01
	$[M+H]^+$ 343.05 R_t 3.38 (polar method)
	$[M+H]^+$ 406 R_t 1.85
	$[M+H]^+$ 371.09 R_t 3.27 (polar method)

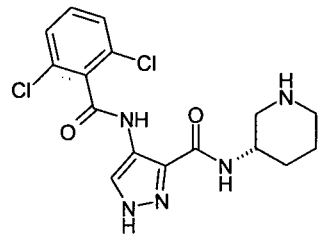
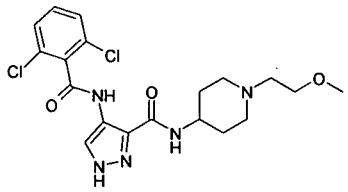
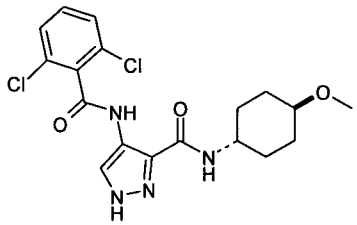
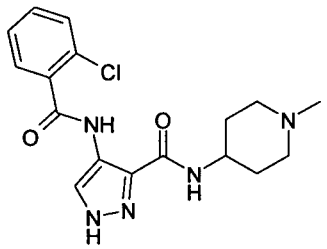
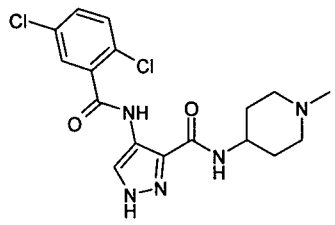
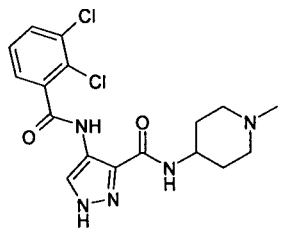
Structure	LCMS
	$[M+H]^+$ 306.06 R_t 1.53
	$[M+H]^+$ 403.98 R_t 2.78
	$[M+H]^+$ 345.05 R_t 3.03
	$[M+H]^+$280.05 R_t 3.75 (basic method)
	$[M+H]^+$ 336 R_t 1.67
	$[M+H]^+$ 380.05 R_t 1.78

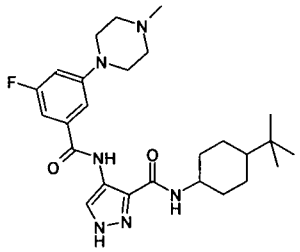
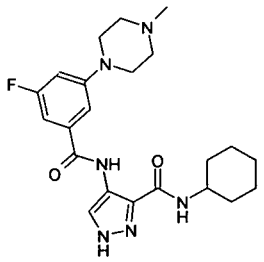
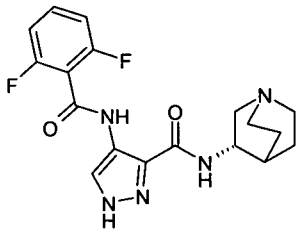
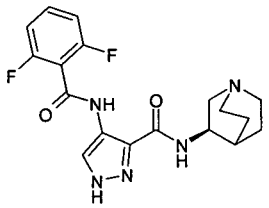
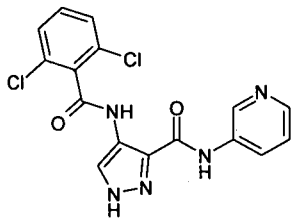
Structure	LCMS
	$[M+H]^+$ 396.02 R_t 1.86
	$[M+H]^+$ 386.10 R_t 1.88
	$[M+H]^+$ 342.10 R_t 1.95
	$[M+H]^+$ = 344 R_t = 1.87
	$[M+H]^+$ = 330 R_t = 1.80

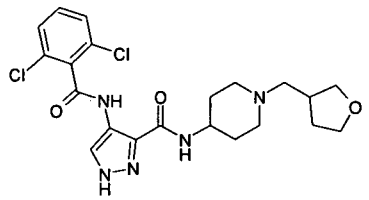
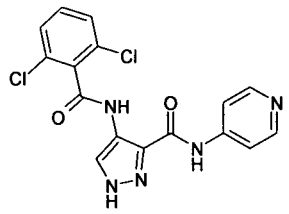
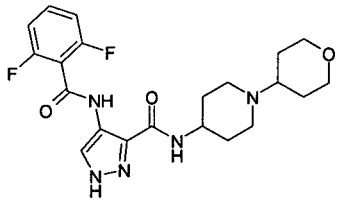
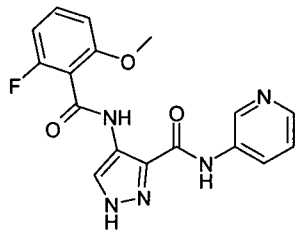
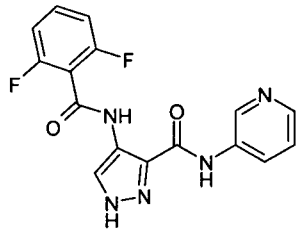
Structure	LCMS
	$[M+H]^+ = 372$ $R_t = 1.87$
	$[M+H]^+ = 354$ $R_t = 1.77$
	$[M+H]^+ = 383 / 385$ $R_t = 1.72$
	$[M+H]^+ = 393 / 395$ $R_t = 1.86$
	$[M+H]^+ = 398$ $R_t = 1.94$

Structure	LCMS
	$[M+H]^+ = 330$ $R_t = 1.80$
	$[M+H]^+ = 358$ $R_t = 1.89$
	$[M+H]^+ = 399$ $R_t = 1.88$
	$[M+H]^+ = 420$ $R_t = 2.13$
	$[M+H]^+ = 392 / 394$ $R_t = 1.84$
	$[M+H]^+$ 376.14 R_t 1.78

Structure	LCMS
	$[M+H]^+$ 400.17 R_t 2.08
	$[M+H]^+$ 376.15 R_t 1.92
	$[M+H]^+$ 382.12 R_t 1.77
	$[M+H]^+$ 388.18 R_t 1.73
	$[M+H]^+$ = 397 / 399 R_t = 1.83

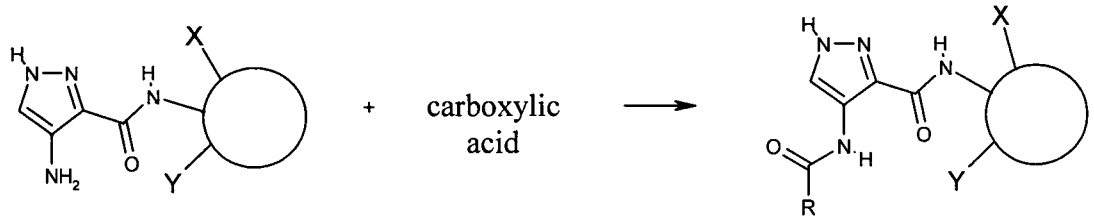
Structure	LCMS
	$[M+H]^+$ 382.02 R_t 1.82
	$[M+H]^+$ 440.22 R_t 1.92
	$[M+H]^+$ 411.20 R_t 2.97
	$[M+H]^+$ 362.11 R_t 1.91
	$[M+H]^+$ 396.08 R_t 2.06
	$[M+H]^+$ 396.06 R_t 2.04

Structure	LCMS
	$[M+H]^+$ 485 R_t 2.59
	$[M+H]^+$ 429 R_t 2.25
	$[M+H]^+$ = 376 R_t = 1.85
	$[M+H]^+$ = 376 R_t = 1.87
	$[M+H]^+$ = 376 / 378 R_t = 2.23

Structure	LCMS
	$[M+H]^+$ = 466 / 468 R_t = 1.98
	$[M+H]^+$ = 376 / 378 R_t = 2.09
	$[M+H]^+$ = 434 R_t = 1.82
	$[M+H]^+$ = 356 R_t = 2.11
	$[M+H]^+$ = 344 R_t = 2.09

EXAMPLE 1

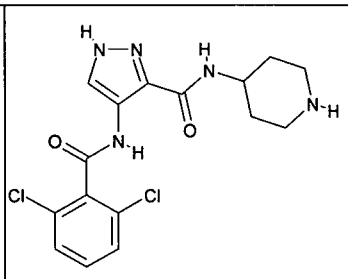
General Procedure A: Preparation of Amide from Amino-Pyrazole



To a stirred solution of the appropriate 4-amino-1H-pyrazole-3-carboxylic acid amide (0.23 mmol), EDAC (52 mg; 0.27 mmol) and HOBt (37 mg; 0.27 mmol) in 5 ml of N,N-dimethylformamide was added the corresponding carboxylic acid (0.25 mmol), and the mixture was then left at room temperature overnight. The reaction mixture was evaporated and the residue purified by preparative LC/MS, to give the product.

General Procedure B: Deprotection of Piperidine Ring Nitrogen by Removal of *tert*-Butoxycarbonyl Group

A product of Procedure B containing a piperidine group bearing an N-*tert*-butoxycarbonyl (t-Boc) protecting group (40 mg) was treated with saturated ethyl acetate/HCl, and stirred at room temperature for 1 hour. A solid precipitated out of the reaction mixture, which was filtered off, washed with ether, and then dried to give 25 mg product (LC/MS: $[M+H]^+$ 364).

Example 1		Procedure A followed by Procedure B	$[M+H]^+$ 383 R_t 1.87

EXAMPLE 2: Preparation of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide hydrochloride

2A. 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 (prepared in a manner analogous to 165B) (5 g; 35.5 mmol) and triethylamine (5.95 ml; 42.6 mmol) in dioxan (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.

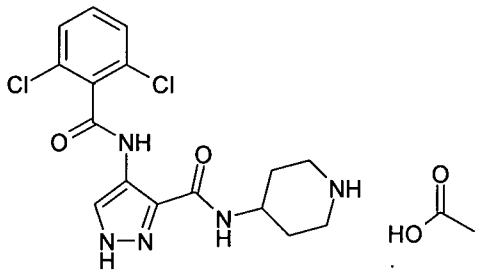
2B. 4-{[4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carbonyl]-amino}-piperidine-1-carboxylic acid *tert*-butyl ester

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.

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

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).

EXAMPLE 3: Preparation of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide acetic acid salt



To a solution of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide hydrochloride salt (Example 2C) 20.6 g, 50 mmol) in water (500 ml) stirring at ambient temperature was added sodium bicarbonate (4.5 g, 53.5 mmol). The mixture was stirred for 1 hour and the solid formed collected by filtration and dried *in vacuo* azeotroping with toluene (x 3) to give the corresponding free base of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide.

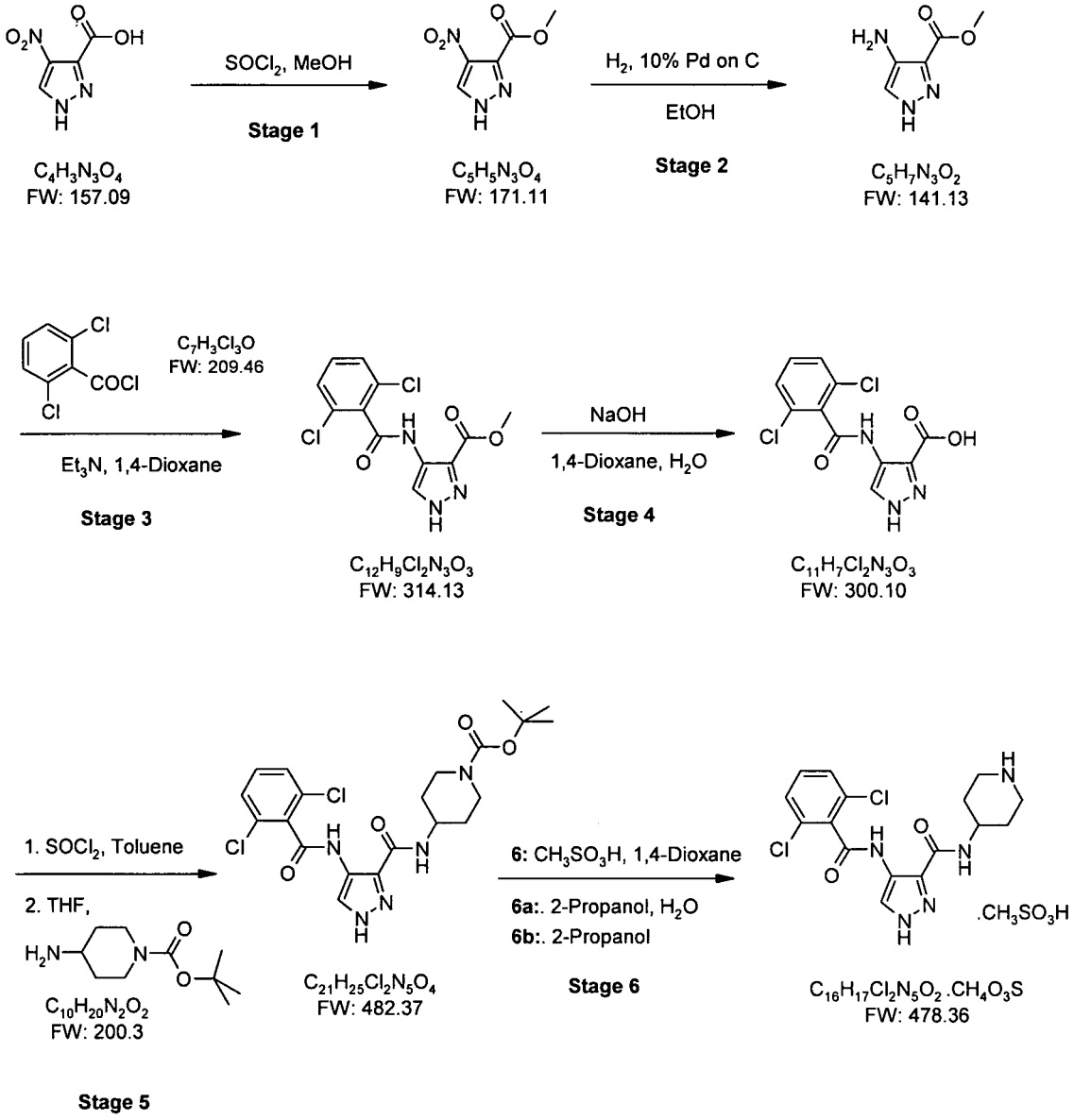
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.20 (s, 1H), 8.30 (s, 1H), 8.25 (d, 1H), 7.60 – 7.50 (m, 3H), 3.70 (m, 1H), 3.00 (d, 2H), 2.50 (m, 2H), 1.70 (d, 2H), 1.50 (m, 2H).

To a stirred suspension of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide (10.0 g, 26.2 mmol) in methanol (150 ml) was added glacial acetic acid (15 ml, 262 mmol) at ambient temperature. After 1 h, a clear solution was obtained which was reduced *in vacuo* azeotroping with toluene (x 2). The residue was then triturated with acetonitrile (2 x 100 ml) and the solid dried *in vacuo* to give 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide acetic acid salt (10.3 g) as a white solid.

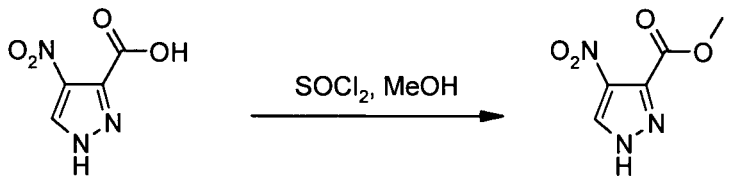
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.20 (s, 1H), 8.40 (d, 1H), 8.35 (s, 1H), 7.60 – 7.50 (m, 3H), 3.85 (m, 1H), 3.00 (d, 2H), 2.60 (t, 2H), 1.85 (s, 3H), 1.70 (d, 2H), 1.55 (m, 2H)

EXAMPLE 4: Synthesis of the methanesulphonic acid salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide

The methane sulphonic acid salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide may be prepared by the synthetic route shown in the Scheme below.



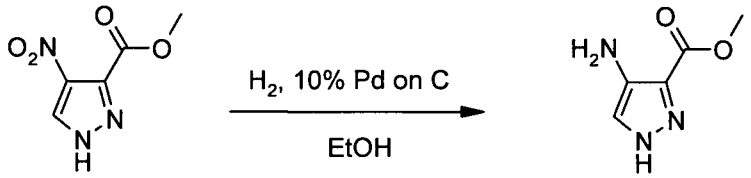
Stage 1: Preparation of 4-nitro-1*H*-pyrazole-3-carboxylic acid methyl ester



A 20L reaction vessel equipped with a digital thermometer and stirrer was charged with 4-nitro-1*H*-pyrazole-3-carboxylic acid (1.117 Kg, 7.11 mol, 1 wt) and methanol (8.950 L, 8 vol). The reaction mixture was stirred under nitrogen, cooled to 0 to 5 °C, thionyl chloride

(0.581 L, 8.0 mol, 0.52 vol) added over 180 minutes and the resultant mixture allowed to warm to and stir at 18 to 22 °C overnight, after which time ^{1}H NMR analysis (d_6-DMSO) indicated reaction completion. The reaction mixture was concentrated under reduced pressure at 40 to 45 °C, the residue treated with toluene and re-concentrated (3x 2.250 L, 3x 2vol) under reduced pressure at 40 to 45 °C to give 4-nitro-1*H*-pyrazole-3-carboxylic acid methyl ester as an off-white solid (1.210 Kg, 99.5%).

Stage 2: Preparation of 4-amino-1*H*-pyrazole-3-carboxylic acid methyl ester

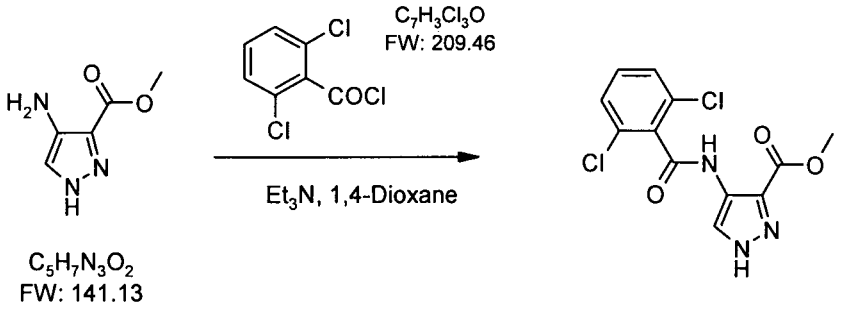


$C_5H_5N_3O_4$
FW: 171.11

$C_5H_7N_3O_2$
FW: 141.13

A 20 L reaction vessel equipped with a digital thermometer and stirrer was charged with palladium on carbon (10% wet paste, 0.170 Kg, 0.14 wt) under nitrogen. In a separate vessel a slurry of 4-nitro-1*H*-pyrazole-3-carboxylic acid methyl ester (1.210 Kg, 7.07 mol, 1 wt) in ethanol (12.10 L, 10 vol) was warmed to 30 to 35 °C to effect dissolution and the solution added to the catalyst under nitrogen. Following a nitrogen-hydrogen purge sequence an atmosphere of hydrogen was introduced and the reaction mixture maintained at 28 to 30 °C until reaction completion (5 to 10 hours) was noted by ^{1}H NMR analysis (d_6-DMSO). Following a purge cycle, the reaction mixture under nitrogen was filtered and the liquors concentrated under reduced pressure to give 4-amino-1*H*-pyrazole-3-carboxylic acid methyl ester (0.987 Kg, 98.9%).

Stage 3: Preparation of 4-(2,6-dichlorobenzoylamino)-1*H*-pyrazole-3-carboxylic acid methyl ester

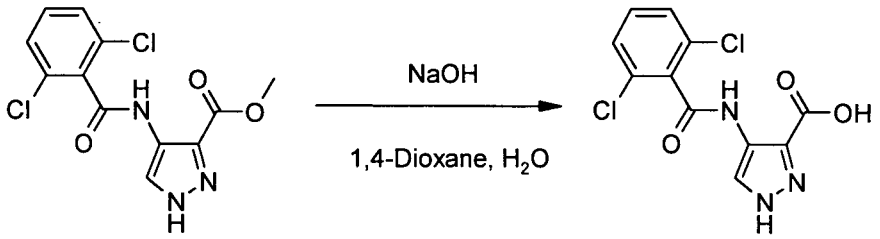


$C_5H_7N_3O_2$
FW: 141.13

$C_{12}H_9Cl_2N_3O_3$
FW: 314.13

A solution of 4-amino-1*H*-pyrazole-3-carboxylic acid methyl ester (0.634 Kg, 4.49 mol, 1 wt) in 1,4-dioxane (8.90 L, 9 vol) under nitrogen was treated with triethylamine (0.761 L, 5.46 mol, 1.2 vol) followed by 2,6-dichlorobenzoyl chloride (0.710 L, 4.96 mol, 0.72 vol) such that the internal temperature was maintained in the range 20 to 25 °C. Residual 2,6-dichlorobenzoyl chloride was washed in with a line rinse of 1,4-dioxane (0.990 L, 1 vol) and the reaction mixture stirred at 18 to 25° C until complete (16 hours) by TLC analysis (eluent: ethyl acetate: heptanes 3:1; $R_{f\ amine}$ 0.25, $R_{f\ product}$ 0.65). The reaction mixture was filtered, the filter-cake washed with 1,4-dioxane (2x 0.990 L, 2x 1 vol) and the combined filtrates (red) progressed to Stage 4 without further isolation.

Stage 4: Preparation of 4-(2,6-dichlorobenzoylamino)-1*H*-pyrazole-3-carboxylic acid



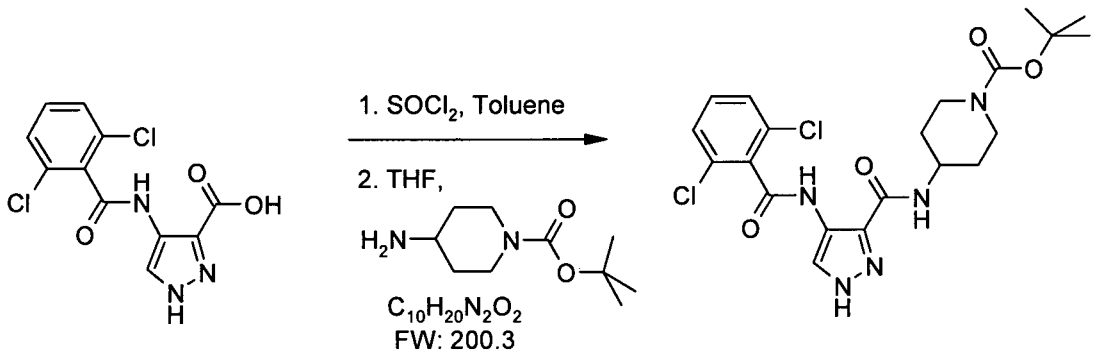
$C_{12}H_9Cl_2N_3O_3$
FW: 314.13

$C_{11}H_7Cl_2N_3O_3$
FW: 300.10

To a solution of sodium hydroxide (0.484 Kg, 12.1 mol) in water (6.05 L) was charged a solution of the Stage 3 ester in one portion: (1.099 Kg, 3.50 mol in 6.00 L). The reaction mixture was stirred to completion at 20 to 25 °C as determined by TLC analysis (eluent: ethyl acetate: heptanes 3:1; $R_{f\ ester}$ 0.65, $R_{f\ Stage\ 4}$ baseline). The reaction mixture was concentrated under reduced pressure at 45 to 50 °C, the oily residue diluted with water (9.90 L) and acidified to pH 1 with concentrated hydrochloric acid such that the temperature was maintained below 30 °C. The resulting precipitate was collected by filtration, washed with water (5.00 L), pulled dry on the filter and subsequently washed with

heptanes (5.00 L). The filter-cake was charged to a 20 L rotary evaporator flask and drying completed azeotropically with toluene (2x 4.50 L) to afford 4-(2,6-dichlorobenzoylamino)-1*H*-pyrazole-3-carboxylic acid as a yellow solid (1.044 Kg, approx. 99.5%).

Stage 5: Preparation of 4-{[4-(2,6-dichlorobenzoylamino)-1*H*-pyrazole-3-carbonyl]amino}piperidine-1-carboxylic acid *tert*-butyl ester



$C_{11}H_7Cl_2N_3O_3$
FW: 300.10

$C_{10}H_{20}N_2O_2$
FW: 200.3

$C_{21}H_{25}Cl_2N_5O_4$
FW: 482.37

Stage 4 product (1.0 wt) and toluene (10.0 vol) were charged to a suitably sized flange flask equipped with a mechanical stirrer, dropping funnel and thermometer. The contents were stirred under nitrogen at 16 to 25 °C and thionyl chloride (0.3 vol) was added slowly. The contents were then heated to 80 to 100 °C and stirred at this temperature until the reaction was judged complete by ^{1}H NMR. Further toluene (up to 10 vol) could be added at this stage if the contents were to become too thick to stir. Once complete, the mixture was cooled to between 40 and 50 °C and then concentrated under vacuum at 45 to 50 °C to dryness. The residue was then azeo-dried with toluene (3x 2.0 vol).

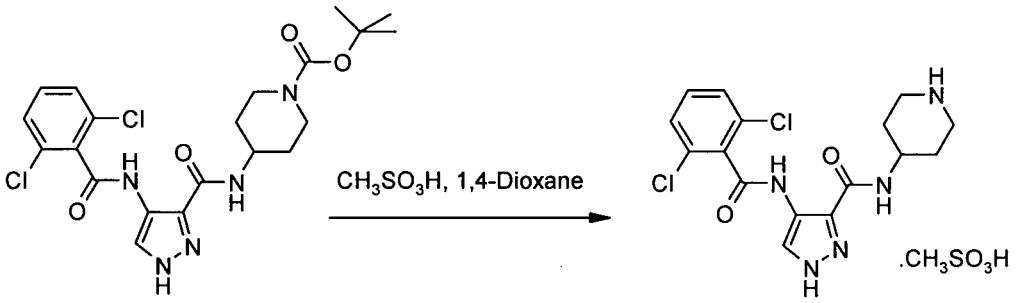
The isolated solid was transferred to a suitably sized flask and tetrahydrofuran (5.0 vol) was charged. The contents were stirred under nitrogen at 16 to 25 °C and triethylamine (0.512 vol) was added. To a separate flask was charged 4-amino-piperidine-1-carboxylic acid *tert-butyl ester* (0.704 wt) and tetrahydrofuran (5.0 vol). The contents were agitated until complete dissolution was achieved and the solution was then charged to the reaction flask, maintaining the temperature between 16 and 30 °C. The reaction mixture was then heated to between 45 and 50 °C and the contents stirred until judged complete by ^{1}H NMR. The contents were then cooled to between 16 and 25 °C and water (5.0 vol) was charged. Mixed heptanes (0.5 vol) were added, the contents were stirred for up to 10 minutes and the layers were separated. The aqueous phase was then extracted with tetrahydrofuran:mixed heptanes [(9:1), 3x 5.0 vol]. The organic phases were combined,

washed with water (2.5 vol) and then concentrated under vacuum at 40 to 45 °C. The residue was azeotroped with toluene (3x 5.0 vol) and concentrated to dryness to yield the crude Stage 5 product.

The solid was then transferred to a suitably sized flask, methanol: toluene [(2.5:97.5), 5.0 vol] was added and the slurry was stirred under nitrogen for 3 to 18 hours. The contents were filtered, the filter-cake was washed with toluene (2x 0.7 vol) and the solid was then dried under vacuum at 40 to 50 °C to yield 4-{[4-(2,6-dichlorobenzoylamino)-1*H*-pyrazole-3-carbonyl]amino}piperidine-1-carboxylic acid *tert*-butyl ester as an off-white solid.

Two batches of Stage 4 product (0.831 kg per batch) were processed in this way to give a total of 2.366 kg (88.6% yield) of 4-{[4-(2,6-dichlorobenzoylamino)-1*H*-pyrazole-3-carbonyl]amino}piperidine-1-carboxylic acid *tert*-butyl ester.

Stage 6: Preparation of 4-(2,6-dichlorobenzoylamino)-1*H*-pyrazole-3-carboxylic acid piperidin-4-ylamide methanesulphonate



$C_{21}H_{25}Cl_2N_5O_4$
FW: 482.37

$C_{16}H_{17}Cl_2N_5O_2.CH_4O_3S$
FW: 478.36

Stage 5 product (1.0 wt) and 1,4-dioxane (30.0 vol) were charged to a suitably sized flange flask equipped with a mechanical stirrer, dropping funnel and thermometer. The contents were stirred under nitrogen and heated to between 80 and 90 °C. Methanesulphonic acid (0.54 vol) was added over 30 to 60 minutes and the contents were then heated to 95 to 105 °C and stirred in this temperature range until the reaction was judged complete by ^1H NMR. Once complete, the contents were cooled to between 20 and 30 °C and the resultant precipitate collected by filtration. The filter-cake was washed with 2-propanol (2x 2.0 vol) and pulled dry on the filter for 3 to 24 hours to give crude 4-(2,6-dichlorobenzoylamino)-1*H*-pyrazole-3-carboxylic acid piperidin-4-ylamide methanesulphonate as a free-flowing off-white solid (80.0 to 120.0 %w/w, uncorrected for impurities or solutes).

Several batches of Stage 5 product were processed in this way and the details of the quantities of starting material and product for each batch are set out in Table 1 below.

Table 1 – Yields from the deprotection step - Stage 6

Batch	Input (g) of (4-{[4-(2,6-Dichloro-benzoylamino)-1*H*-pyrazole-3-carbonyl]amino}-piperidine-1-carboxylic acid *tert*-butyl ester)	Output (g) of [4-(2,6-Dichlorobenzoyl-amino)-1*H*-pyrazole-3-carboxylic acid piperidin-4-ylamide methanesulphonate]	Chemical purity (HPLC % area)
1	590.0	579.6 99.1%th, 98.2%w/w	97.88
2	521.0	532.7 103.1%th, 102.2%w/w	98.09
3	523.8	511.7 98.5%th, 97.7%w/w	98.17
4	518.4	596.3 116.0%th, 115.0%w/w	98.24
5	563.2	600.1 107.4%th, 106.6%w/w	98.16
6	563.1	565.2 101.2%th, 100.4%w/w	98.49
7	560.4	553.9 99.7%th, 98.8%w/w	98.70
8	569.7	560.6 99.2%th, 98.4%w/w	98.41

Stage 6a: Recrystallisation of 4-(2,6-dichlorobenzoylamino)-1*H*-pyrazole-3-carboxylic acid piperidin-4-ylamide methanesulphonate

The product of Stage 6 was recrystallised to ensure that any residual levels of Boc-protected product of Stage 5 were no greater than 0.25%. Four batches of Stage 6 product were recrystallised using the following protocol.

Crude Stage 6 product and 2-propanol (10.0 vol) were charged to a suitably sized flask equipped with a mechanical stirrer, dropping funnel and thermometer. The contents were stirred under nitrogen and heated to between 75 and 85 °C. Water (up to 2.5 vol) was then charged to the contents until a clear solution was obtained. The contents were then cooled to between 40 and 60 °C and concentrated under vacuum at 40 to 50 °C until the reaction volume was reduced by approximately 50%. 2-Propanol (3.0 vol) was charged to the flask and the contents were concentrated at 40 to 50 °C until approximately 3.0 vol of solvent was removed. This process was then repeated twice more with 2-propanol (2x 3.0 vol) and the water content was checked. The resultant slurry was then cooled to between 0 and 5 °C and stirred at this temperature for 1 to 2 hours. The contents were filtered, the filter-cake was washed with 2-propanol (2x 1.0 vol) and then pulled dry on the filter for up to 24 hours. The solid was transferred to drying trays and dried under vacuum at 45 to 50 °C to constant weight to give 4-(2,6-dichlorobenzoylamino)-1*H*-pyrazole-3-carboxylic acid piperidin-4-ylamide methanesulphonate as an off-white solid (60.0 to 100.0% w/w).

The recrystallisation yields for the four batches ranged between 85.6% and 90.4% and the purities of the recrystallised product ranged from 99.29% to 99.39%. A second recrystallisation increased the purity still further.

The 4-(2,6-dichlorobenzoylamino)-1*H*-pyrazole-3-carboxylic acid piperidin-4-ylamide methanesulphonate produced by this route had a melting point (by DSC) of 379.8 °C.

Removal of residual Boc-protected product of Stage 5

In some cases, when the methanesulphonate salt was dissolved in acetate buffer, a fine precipitate consisting of residual traces of the Boc-protected free base was observed. Several techniques may be used for removing or preventing the formation of the precipitate, as set out below.

(a) Filtration

A mixture of the methanesulphonate salt in 200 mM acetate buffer was drawn from a vial into a 20 mL single-use syringe using a sterile needle, and a clinical grade 0.2 µm filter (a Sartorius Minisart sterile single use filter unit) was then attached to the syringe. The

plunger of the syringe was slowly depressed and the filtrate collected in a clean, clear glass vial. The content of the vial was a clear, colourless solution of the methanesulphonate salt free of particulate matter.

(b) Heating in aqueous acid

A mixture of the methanesulphonate salt and methanesulphonic acid (0.4 eq.) in water (10 vol) was heated at 100 °C for 4 hours, and then cooled to 60 °C. Analysis by TLC indicates that the methanesulphonate salt was present as a single component. 2-Propanol (10 vol) was added and the mixture cooled to 40 °C. The mixture was reduced *in vacuo* to approximately 10 volumes, then a further portion of 2-propanol added (10 vol) and the mixture again reduced to 10 volumes. This cycle was repeated a further three times. The mixture was cooled in an ice-bath and the solid formed collected by filtration, washed with 2-propanol (5 vol) and dried *in vacuo* to give the methanesulphonate salt as a white to off-white solid.

(c) Organic-aqueous Extractions

A mixture of the methanesulphonate salt and methanesulphonic acid (0.4 eq.) in water (10 vol) was heated at 100 °C for 3 hours, and then cooled to ambient temperature. To this mixture was added THF-heptane (9:1, 10 vol) and the resultant mixture stirred vigorously to give a solution. The layers were separated and the aqueous phase washed with THF-heptane (9:1, 2 x 10 vol) then ethyl acetate (2 x 10 vol). To the aqueous phase was added 2-propanol (10 vol) and the solution was reduced *in vacuo* to approximately 5 volumes, then a further portion of 2-propanol added (10 vol) and the mixture again reduced to 5 volumes. This cycle was repeated a further three times. The solid formed was collected by filtration, washed with 2-propanol (5 vol) and dried *in vacuo* to give the methanesulphonate salt as a white to off-white solid.

(d) Chromatography

The use of chromatographic techniques may provide a route for removing non-polar impurities from the methanesulphonate salt. The use of reverse-phase methods will be particularly useful.

BIOLOGICAL ACTIVITY

The biological activities of the compounds of (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined in WO 2005/012256 (PCT/GB2004/003179) (and therefore herein also by dint of the incorporation of the relevant subject matter of WO 2005/012256 (PCT/GB2004/003179) by reference, see *infra*) as inhibitors of CDK kinases, GSK-3 kinase and as inhibitors of cell growth are demonstrated by the examples set out below.

EXAMPLE 5: Measurement of CDK2 Kinase Inhibitory Activity (IC_{50})

See Example 246 of WO 2005/012256 at pages 213-214 (which is incorporated herein by reference).

EXAMPLE 6: CDK Selectivity Assays

See Example 247 of WO 2005/012256 at pages 214-215 (which is incorporated herein by reference).

EXAMPLE 7A. Measurement of Activated CDK2/CyclinA Kinase Inhibitory Activity Assay (IC_{50})

See Example 12 of WO 2006/077426 at pages 98-99 (which is incorporated herein by reference).

7B. CDK1/CyclinB Assay.

See Example 248B of WO 2005/012256 at pages 216-217 (which is incorporated herein by reference).

EXAMPLE 8: Assay Procedure for CDK4

See Example 249 of WO 2005/012256 at pages 217 (which is incorporated herein by reference).

EXAMPLE 9: Measurement of inhibitory activity against Glycogen Synthase Kinase-3 (GSK-3)

See Example 251 of WO 2005/012256 at pages 218-219 (which is incorporated herein by reference).

EXAMPLE 10: Anti-proliferative Activity

See Example 15 of WO 2006/077426 at pages 100-101 (which is incorporated herein by reference).

HCT-116 cell line

In assays against the human colon carcinoma cell line HCT 116 (ECACC No. 91091005), the compound of Example 1 has an IC_{50} value of less than 20 μM.

EXAMPLE 11

Assay for therapeutic efficacy

The effect of a compound of formula (0) (Compound I) in combination with an ancillary compound (Compound II) can be assessed using the following technique:

IC_{50} Shift Assay

Cells from human cells lines (e.g. HCT116, U87MG, A549) were seeded onto 96-well tissue culture plates at a concentration of $2.5x10^3$, $6.0 x10^3$, or $4.0 x10^3$ cells/well respectively. Cells were allowed to recover for 48 hours prior to addition of compound(s) or vehicle control (0.35% DMSO) as follows:

Compounds were added concurrent for 96 hours.

Following a total of 96 hours compound incubation, cells were fixed with ice-cold 10% (w/v) trichloroacetic acid for 1 hour on ice and then washed four times with dH_20 using a plate washer (Labsystems Wellwash Ascent) and air-dried. Cells were then stained with 0.4% (w/v) Sulforhodamine B (Sigma) in 1% acetic acid for 20 min at room temperature and then washed four times with 1% (v/v) acetic acid and air-dried before the addition of 10mM Tris buffer to solubilise the dye. Colourmetric product was quantified by reading at Abs490nm on a Wallac Victor^2 plate reader (1420 multilabel counter, Perkin Elmer Life Sciences). The IC_{50} for Compound II in the presence of varying doses of Compound I was determined. Synergy was determined when the IC_{50} shifted down in the presence of sub-effective doses of Compound I. Additivity was determined when the response to Compound II and Compound I together resulted in an effect equivalent to the sum of the two compounds

individually. Antagonistic effects were defined as those causing the IC_{50} to shift upwards, i.e. those where the response to the two compounds was less than the sum of the effect of the two compounds individually.

		Compound I						Compound I					
	Conc	a	b	c	d	e	Control	a	b	c	d	e	Control
Compound II	a												
	b												
	c												
	d												
	e												
	f												
	g												
	Control												

EXAMPLE 12: PHARMACEUTICAL FORMULATIONS

i) Lyophilised formulation I

Aliquots of formulated compound of formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined in WO 2005/012256 (PCT/GB2004/003179) (and therefore herein also by dint of the incorporation of the relevant subject matter of WO 2005/012256 (PCT/GB2004/003179) by reference, see *infra*) 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.

ii) Injectable formulation II

A formulation for i.v. delivery by injection or infusion can be prepared by dissolving the compound of formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein (e.g. in a salt form) in water at 20 mg/ml. The vial is then sealed and sterilised by autoclaving.

iii) Injectable formulation III

A formulation for i.v. delivery by injection or infusion can be prepared by dissolving the compound of formula (0), (I^0), (I), (Ia), (Ib), (II), (III), (IV), (IVa), (Va), (Vb), (VIa), (VIb), (VII) or (VIII) and sub-groups thereof as defined herein (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.

iv) Injectable Formulation IV

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.

(v) Injectable Formulation V

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.

(vi) Subcutaneous Injection Formulation VI

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.

(vii) Tablet Formulation

A tablet composition containing a compound of the formulae (I^0) or (I) or an acid addition salt thereof as defined herein is prepared by mixing 50mg of the compound or its salt with 197mg of lactose (BP) as diluent, and 3mg magnesium stearate as a lubricant and compressing to form a tablet in known manner.

(viii) Capsule Formulation

A capsule formulation is prepared by mixing 100mg of a compound of the formulae (I^0) or (I) or an acid addition salt thereof as defined herein with 100mg lactose and filling the resulting mixture into standard opaque hard gelatin capsules.

(ix) Lyophilised formulation

Aliquots of formulated compound of formulae (I^0) or (I) or an acid addition salt thereof as defined herein 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.

(x) Concentrate for use in i.v. administration

An aqueous buffered solution is prepared by dissolving 4-(2,6-dichlorobenzoylamino)-1*H*-pyrazole-3-carboxylic acid piperidin-4-ylamide methanesulphonate at a concentration of 20 mg/ml in a 0.2M sodium acetate/acetic acid buffer at a pH of 4.6.

The buffered solution is filled, with filtration to remove particulate matter, into a container (such as class 1 glass vials) which is then sealed (e.g. by means of a Florotec stopper) and secured (e.g. with an aluminium crimp). If the compound and formulation are sufficiently stable, the formulation is sterilised by autoclaving at 121 °C for a suitable period of time. If the formulation is not stable to autoclaving, it can be sterilised using a suitable filter and filled under sterile conditions into sterile vials. 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.

EXAMPLE 13: Determination of the crystal structure of 4-(2,6-dichlorobenzoylamino)-1*H*-pyrazole-3-carboxylic acid piperidin-4-ylamide methanesulphonate by X-ray diffraction

See Example 2 and Figure 1 of WO 2006/077426 at pages 81-85 (which is incorporated herein by reference).

EXAMPLE 14: Preparation of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide acetic acid salt

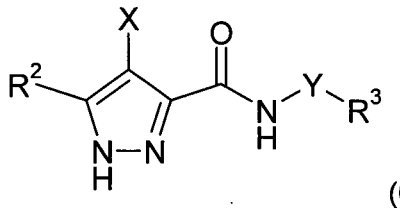
See Example 8 and Figure 2 of WO 2006/077426 at pages 94-95 (which is incorporated herein by reference).

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 combination comprising an ancillary compound and a compound having the formula (0):



(0)

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

wherein

X is a group R^1-A-NR^4- or a 5- or 6-membered carbocyclic or heterocyclic ring;

A is a bond, SO_2, C=O, NR^g(C=O) or O(C=O) wherein R^g is hydrogen or C_{1-4} hydrocarbyl optionally substituted by hydroxy or C_{1-4} alkoxy;

Y is a bond or an alkylene chain of 1, 2 or 3 carbon atoms in length;

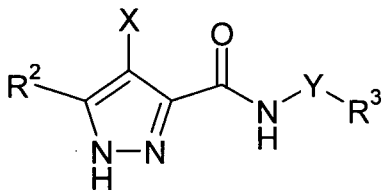
R^1 is hydrogen; a carbocyclic or heterocyclic group having from 3 to 12 ring members; or a C_{1-8} hydrocarbyl group optionally substituted by one or more substituents selected from halogen (e.g. fluorine), hydroxy, C_{1-4} hydrocarbyloxy, amino, mono- or di-C_{1-4} hydrocarbylamino, and carbocyclic or heterocyclic groups having from 3 to 12 ring members, and wherein 1 or 2 of the carbon atoms of the hydrocarbyl group may optionally be replaced by an atom or group selected from O, S, NH, SO, SO_2;

R^2 is hydrogen; halogen; C_{1-4} alkoxy (e.g. methoxy); or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy);

R^3 is selected from hydrogen and carbocyclic and heterocyclic groups having from 3 to 12 ring members; and

R^4 is hydrogen or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy).

2. A combination according to claim 1 comprising an ancillary compound and a compound having the formula (I^0):



(I^0)

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

wherein

X is a group R^1-A-NR^4- or a 5- or 6-membered carbocyclic or heterocyclic ring;

A is a bond, C=O, NR^9(C=O) or O(C=O) wherein R^9 is hydrogen or C_{1-4} hydrocarbyl optionally substituted by hydroxy or C_{1-4} alkoxy;

Y is a bond or an alkylene chain of 1, 2 or 3 carbon atoms in length;

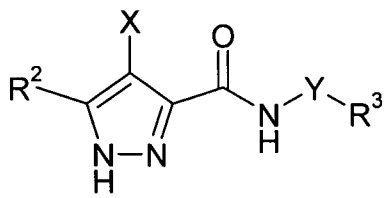
R^1 is hydrogen; a carbocyclic or heterocyclic group having from 3 to 12 ring members; or a C_{1-8} hydrocarbyl group optionally substituted by one or more substituents selected from halogen (e.g. fluorine), hydroxy, C_{1-4} hydrocarbyloxy, amino, mono- or di-C_{1-4} hydrocarbylamino, and carbocyclic or heterocyclic groups having from 3 to 12 ring members, and wherein 1 or 2 of the carbon atoms of the hydrocarbyl group may optionally be replaced by an atom or group selected from O, S, NH, SO, SO_2;

R^2 is hydrogen; halogen; C_{1-4} alkoxy (e.g. methoxy); or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy);

R^3 is selected from hydrogen and carbocyclic and heterocyclic groups having from 3 to 12 ring members; and

R^4 is hydrogen or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy).

3. A combination according to claim 1 comprising an ancillary compound and a compound having the formula (I):



(I)

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

wherein

X is a group R^1-A-NR^4-;

A is a bond, C=O, NR^g(C=O) or O(C=O) wherein R^g is hydrogen or C_{1-4} hydrocarbyl optionally substituted by hydroxy or C_{1-4} alkoxy;

Y is a bond or an alkylene chain of 1, 2 or 3 carbon atoms in length;

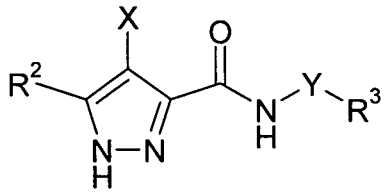
R^1 is hydrogen; a carbocyclic or heterocyclic group having from 3 to 12 ring members; or a C_{1-8} hydrocarbyl group optionally substituted by one or more substituents selected from halogen (e.g. fluorine), hydroxy, C_{1-4} hydrocarbyloxy, amino, mono- or di-C_{1-4} hydrocarbylamino, and carbocyclic or heterocyclic groups having from 3 to 12 ring members, and wherein 1 or 2 of the carbon atoms of the hydrocarbyl group may optionally be replaced by an atom or group selected from O, S, NH, SO, SO_2;

R^2 is hydrogen; halogen; C_{1-4} alkoxy (e.g. methoxy); or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy);

R^3 is selected from hydrogen and carbocyclic and heterocyclic groups having from 3 to 12 ring members; and

R^4 is hydrogen or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy).

4. A combination according to claim 1 comprising an ancillary compound and a compound having the formula (Ia):



(Ia)

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

wherein

X is a group R^1-A-NR^4-;

A is a bond, C=O, NR^g(C=O) or O(C=O) wherein R^g is hydrogen or C_{1-4} hydrocarbyl optionally substituted by hydroxy or C_{1-4} alkoxy;

Y is a bond or an alkylene chain of 1, 2 or 3 carbon atoms in length;

R^1 is a carbocyclic or heterocyclic group having from 3 to 12 ring members; or a C_{1-8} hydrocarbyl group optionally substituted by one or more substituents

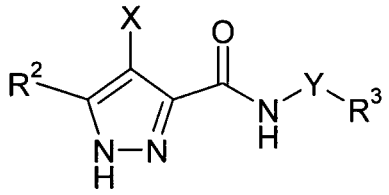
selected from fluorine, hydroxy, C_{1-4} hydrocarbyloxy, amino, mono- or di-C_{1-4} hydrocarbylamino, and carbocyclic or heterocyclic groups having from 3 to 12 ring members, and wherein 1 or 2 of the carbon atoms of the hydrocarbyl group may optionally be replaced by an atom or group selected from O, S, NH, SO, SO_2;

R^2 is hydrogen; halogen; C_{1-4} alkoxy (e.g. methoxy); or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy);

R^3 is selected from hydrogen and carbocyclic and heterocyclic groups having from 3 to 12 ring members; and

R^4 is hydrogen or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy).

5. A combination according to claim 1 comprising an ancillary compound and a compound of the formula (Ib):



(Ib)

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

wherein

X is a group R^1-A-NR^4-;

A is a bond, C=O, NR^9(C=O) or O(C=O) wherein R^9 is hydrogen or C_{1-4} hydrocarbyl optionally substituted by hydroxy or C_{1-4} alkoxy;

Y is a bond or an alkylene chain of 1, 2 or 3 carbon atoms in length;

R^1 is a carbocyclic or heterocyclic group having from 3 to 12 ring members; or a C_{1-8} hydrocarbyl group optionally substituted by one or more substituents selected from fluorine, hydroxy, C_{1-4} hydrocarbyloxy, amino, mono- or di-C_{1-4} hydrocarbylamino, and carbocyclic or heterocyclic groups having from 3 to 12 ring members, and wherein 1 or 2 of the carbon atoms of the hydrocarbyl group may optionally be replaced by an atom or group selected from O, S, NH, SO, SO_2;

R^2 is hydrogen; halogen; C_{1-4} alkoxy (e.g. methoxy); or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy);

R^3 is selected from carbocyclic and heterocyclic groups having from 3 to 12 ring members; and

R^4 is hydrogen or a C_{1-4} hydrocarbyl group optionally substituted by halogen (e.g. fluorine), hydroxyl or C_{1-4} alkoxy (e.g. methoxy).

6. A combination according to claim 5 wherein A is C=O.

7. A combination according to any one of the preceding claims wherein R^4 is hydrogen.

8. A combination according to any one of the preceding claims wherein R^2 is hydrogen or methyl, preferably hydrogen.

9. A combination according to any one of the preceding claims wherein Y is a bond.

10. A combination according to any one of the preceding claims wherein R^1 is a carbocyclic or heterocyclic group having from 3 to 12 ring members (e.g. 5 to 10 ring members).

11. A combination according to claim 10 wherein the carbocyclic and heterocyclic groups are monocyclic.

12. A combination according to claim 11 wherein the monocyclic groups are aryl groups.

13. A combination according to claim 12 wherein the aryl group is a substituted or unsubstituted phenyl group.

14. A combination according to any one of claims 10 to 13 wherein the carbocyclic and heterocyclic groups are substituted by one or more (e.g. 1 or 2 or 3 or 4) substituent groups R^{10} selected from halogen, hydroxy, trifluoromethyl, cyano, nitro, carboxy, amino, mono- or di-C_{1-4} hydrocarbylamino, carbocyclic and heterocyclic groups having from 3 to 12 ring members; a group R^a-R^b wherein R^a is a bond, O, CO, $X^1C(X^2)$, $C(X^2)X^1$, $X^1C(X^2)X^1$, S, SO, SO_2, NR^c, SO_2NR^c or NR^cSO_2; and R^b is selected from hydrogen, carbocyclic and heterocyclic groups having from 3 to 12 ring members, and a C_{1-8} hydrocarbyl group optionally substituted by one or more substituents selected from hydroxy, oxo, halogen, cyano, nitro, carboxy, amino, mono- or di-C_{1-4} hydrocarbylamino, carbocyclic and heterocyclic groups having from

3 to 12 ring members and wherein one or more carbon atoms of the C_{1-8} hydrocarbyl group may optionally be replaced by O, S, SO, SO_2, NR^c, $X^1C(X^2)$, $C(X^2)X^1$ or $X^1C(X^2)X^1$;

R^c is selected from hydrogen and C_{1-4} hydrocarbyl; and

X^1 is O, S or NR^c and X^2 is =O, =S or $=NR^c$.

15. A combination according to claim 14 wherein the substituent groups R^{10} are selected from the group R^{10a} consisting of halogen, hydroxy, trifluoromethyl, cyano, nitro, carboxy, a group R^a-R^b wherein R^a is a bond, O, CO, $X^3C(X^4)$, $C(X^4)X^3$, $X^3C(X^4)X^3$, S, SO, or SO_2, and R^b is selected from hydrogen and a C_{1-8} hydrocarbyl group optionally substituted by one or more substituents selected from hydroxy, oxo, halogen, cyano, nitro, carboxy and monocyclic non-aromatic carbocyclic or heterocyclic groups having from 3 to 6 ring members; wherein one or more carbon atoms of the C_{1-8} hydrocarbyl group may optionally be replaced by O, S, SO, SO_2, $X^3C(X^4)$, $C(X^4)X^3$ or $X^3C(X^4)X^3$; X^3 is O or S; and X^4 is =O or =S.

16. A combination according to claim 15 wherein the substituents are selected from halogen, hydroxy, trifluoromethyl, a group R^a-R^b wherein R^a is a bond or O, and R^b is selected from hydrogen and a C_{1-4} hydrocarbyl group optionally substituted by one or more substituents selected from hydroxyl, halogen (preferably fluorine) and 5 and 6 membered saturated carbocyclic and heterocyclic groups.

17. A combination according to any one of claims 13 to 16 wherein R^1 is a phenyl ring having 1, 2 or 3 substituents located at the 2-, 3-, 4-, 5- or 6-positions around the ring.

18. A combination according to claim 17 wherein the phenyl group is 2-monosubstituted, 3-monosubstituted, 2,6-disubstituted, 2,3-disubstituted, 2,4-disubstituted 2,5-disubstituted, 2,3,6-trisubstituted or 2,4,6-trisubstituted.

19. A combination according to claim 18 wherein the phenyl group is:

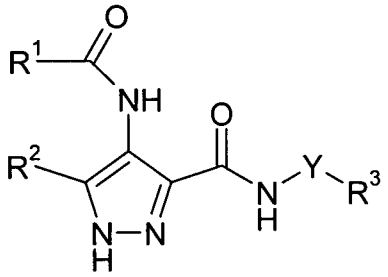
(i) monosubstituted at the 2-position, or disubstituted at positions 2- and 3-, or disubstituted at positions 2- and 6- with substituents selected from fluorine, chlorine and R^a-R^b, where R^a is O and R^b is C_{1-4} alkyl; or

(ii) monosubstituted at the 2-position with a substituent selected from fluorine; chlorine; C_{1-4} alkoxy optionally substituted by one or more fluorine atoms; or

disubstituted at the 2- and 5-positions with substituents selected from fluorine, chlorine and methoxy.

20. A combination according to any one of the preceding claims wherein A is CO and R^1-CO- is selected from the groups listed in Table 1 herein, particularly groups J, AB, AH, AJ, AL, AS, AX, AY, AZ, BA, BB, BD, BH, BL, BQ and BS, and more particularly groups AJ, AX, BQ, BS and BAI, and preferably groups AJ and BQ.

21. A combination according to claim 1 comprising an ancillary compound and a compound having the formula (II):



(II)

wherein R^1, R^2, R^3 and Y are as defined in any one of the preceding claims.

22. A combination according to claim 34 wherein R^1 is selected from:

(i) phenyl optionally substituted by one or more substituents (e.g. 1, 2 or 3) selected from fluorine; chlorine; hydroxy; 5- and 6-membered saturated heterocyclic groups containing 1 or 2 heteroatoms selected from O, N and S, the heterocyclic groups being optionally substituted by one or more C_{1-4} alkyl groups; C_{1-4} hydrocarbyloxy; and C_{1-4} hydrocarbyl; wherein the C_{1-4} hydrocarbyl and C_{1-4} hydrocarbyloxy groups are optionally substituted by one or more substituents chosen from hydroxy, fluorine, C_{1-2} alkoxy, amino, mono and di-C_{1-4} alkylamino, phenyl, halophenyl, saturated carbocyclic groups having 3 to 7 ring members (more preferably 4, 5 or 6 ring members, e.g. 5 or 6 ring members) or saturated heterocyclic groups of 5 or 6 ring members and containing up to 2 heteroatoms selected from O, S and N; or 2, 3-dihydro-benzo[1,4]dioxine; or

(ii) a monocyclic heteroaryl group containing one or two heteroatoms selected from O, S and N; or a bicyclic heteroaryl group containing a single heteroatom selected from O, S and N; the monocyclic and bicyclic heteroaryl groups each being optionally substituted by one or more substituents selected from fluorine; chlorine; C_{1-3} hydrocarbyloxy; and C_{1-3} hydrocarbyl optionally substituted by hydroxy, fluorine,

methoxy or a five or six membered saturated carbocyclic or heterocyclic group containing up to two heteroatoms selected from O, S and N;

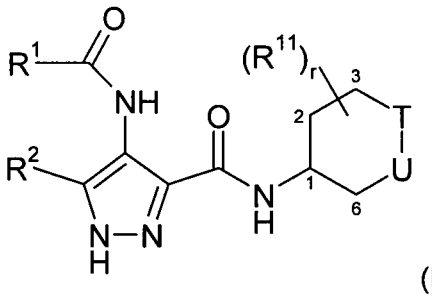
(iii) a substituted or unsubstituted cycloalkyl group having from 3 to 6 ring members; and

(iv) a C_{1-4} hydrocarbyl group optionally substituted by one or more substituents selected from fluorine; hydroxy; C_{1-4} hydrocarbyloxy; amino; mono- or di-C_{1-4} hydrocarbylamino; and carbocyclic or heterocyclic groups having from 3 to 12 ring members, and wherein one of the carbon atoms of the hydrocarbyl group may optionally be replaced by an atom or group selected from O, NH, SO and SO_2.

23. A combination according to claim 22 wherein R^1 is selected from unsubstituted phenyl, 2-fluorophenyl, 2-hydroxyphenyl, 2-methoxyphenyl, 2-methylphenyl, 2-(2-(pyrrolidin-1-yl)ethoxy)-phenyl, 3-fluorophenyl, 3-methoxyphenyl, 2,6-difluorophenyl, 2-fluoro-6-hydroxyphenyl, 2-fluoro-3-methoxyphenyl, 2-fluoro-5-methoxyphenyl, 2-chloro-6-methoxyphenyl, 2-fluoro-6-methoxyphenyl, 2,6-dichlorophenyl and 2-chloro-6-fluorophenyl; and is optionally further selected from 5-fluoro-2-methoxyphenyl.

24. A combination according to claim 23 wherein R^1 is selected from 2,6-difluorophenyl, 2-fluoro-6-methoxyphenyl, 2,6-dichlorophenyl and 2-chloro-6-fluorophenyl.

25. A combination according to claim 1 comprising an ancillary compound and a compound having the formula (IV):



(IV)

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

wherein R^1 and R^2 are as defined in any one of the preceding claims;

an optional second bond may be present between between carbon atoms numbered 1 and 2;

one of U and T is selected from CH_2, CHR^{13}, $CR^{11}R^{13}$, NR^{14}, $N(O)R^{15}$, O and $S(O)_t$; and the other of U and T is selected from , NR^{14}, O, CH_2, CHR^{11}, $C(R^{11})_2$, and C=O;

r is 0, 1, 2, 3 or 4; t is 0, 1 or 2;

R^{11} is selected from hydrogen, halogen (particularly fluorine), C_{1-3} alkyl (e.g. methyl) and C_{1-3} alkoxy (e.g. methoxy);

R^{13} is selected from hydrogen, NHR^{14}, NOH, NOR^{14} and R^a-R^b;

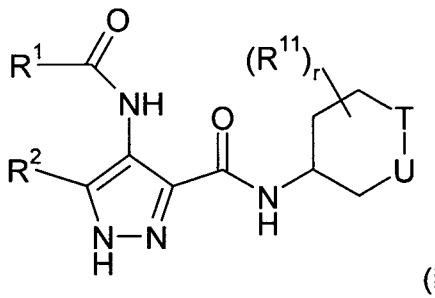
R^{14} is selected from hydrogen and R^d-R^b;

R^d is selected from a bond, CO, $C(X^2)X^1$, SO_2 and SO_2NR^c;

R^a, R^b and R^c are as hereinbefore defined; and

R^{15} is selected from C_{1-4} saturated hydrocarbyl optionally substituted by hydroxy, C_{1-2} alkoxy, halogen or a monocyclic 5- or 6-membered carbocyclic or heterocyclic group, provided that U and T cannot be O simultaneously.

26. A combination according to claim 25 comprising an ancillary compound and a compound having the formula (IVa):



(IVa)

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

wherein one of U and T is selected from CH_2, CHR^{13}, $CR^{11}R^{13}$, NR^{14}, $N(O)R^{15}$, O and $S(O)_t$; and the other of U and T is selected from CH_2, CHR^{11}, $C(R^{11})_2$, and C=O;

r is 0, 1 or 2; t is 0, 1 or 2;

R^{11} is selected from hydrogen and C_{1-3} alkyl;

R^{13} is selected from hydrogen and R^a-R^b;

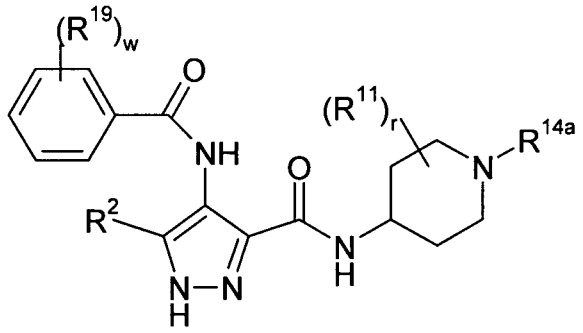
R^{14} is selected from hydrogen and R^d-R^b;

R^d is selected from a bond, CO, $C(X^2)X^1$, SO_2 and SO_2NR^c;

R^{15} is selected from C_{1-4} saturated hydrocarbyl optionally substituted by hydroxy, C_{1-2} alkoxy, halogen or a monocyclic 5- or 6-membered carbocyclic or heterocyclic group; and

R^1, R^2, R^a, R^b and R^c are as defined in any one of the preceding claims.

27. A combination according to claim 26 comprising an ancillary compound and a compound having the formula (Va):



(Va)

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

wherein R^{14a} is selected from hydrogen, C_{1-4} alkyl optionally substituted by fluoro (e.g. methyl, ethyl, n-propyl, i-propyl, butyl and 2,2,2-trifluoroethyl), cyclopropylmethyl, phenyl-C_{1-2} alkyl (e.g. benzyl), C_{1-4} alkoxycarbonyl (e.g.ethoxycarbonyl and t-butyloxycarbonyl), phenyl-C_{1-2} alkoxycarbonyl (e.g. benzyloxycarbonyl), C_{1-2}-alkoxy-C_{1-2} alkyl (e.g. methoxymethyl and methoxyethyl), and C_{1-4} alkylsulphonyl (e.g.methanesulphonyl), wherein the phenyl moieties when present are optionally substituted by one to three substituents selected from fluorine, chlorine, C_{1-4} alkoxy optionally substituted by fluoro or C_{1-2}-alkoxy, and C_{1-4} alkyl optionally substituted by fluoro or C_{1-2}-alkoxy;

w is 0, 1, 2 or 3;

R^2 is hydrogen or methyl, most preferably hydrogen;

R^{11} and r are as defined in any one of claims 82 to 90; and

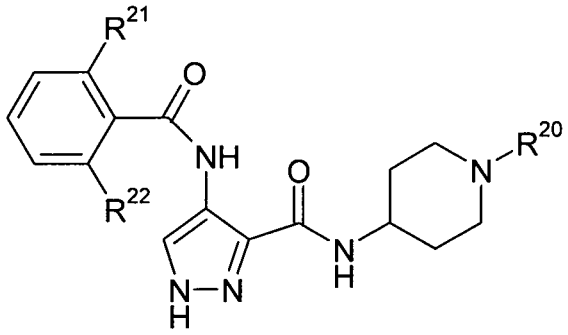
R^{19} is selected from fluorine; chlorine; C_{1-4} alkoxy optionally substituted by fluoro or C_{1-2}-alkoxy; and C_{1-4} alkyl optionally substituted by fluoro or C_{1-2}-alkoxy.

28. A composition according to claim 27 wherein the phenyl ring is disubstituted at positions 2- and 6- with substituents selected from fluorine, chlorine and methoxy.

29. A combination according to any one of claims 25 to 28 wherein R^{11} is hydrogen.

30. A combination according to any one of claims 25 to 29 wherein R^{14a} is hydrogen or methyl.

31. A combination according to claim 30 comprising an ancillary compound and a compound of the formula (VIa):



(VIa)

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

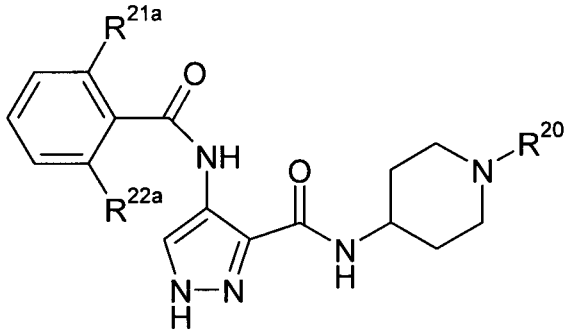
wherein R^{20} is selected from hydrogen and methyl;

R^{21} is selected from fluorine and chlorine; and

R^{22} is selected from fluorine, chlorine and methoxy; or

one of R^{21} and R^{22} is hydrogen and the other is selected from chlorine, methoxy, ethoxy, difluoromethoxy, trifluoromethoxy and benzyloxy.

32. A combination according to claim 31 comprising an ancillary compound and a compound the formula (VIb):



(VIb)

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

wherein R^{20} is selected from hydrogen and methyl;

R^{21a} is selected from fluorine and chlorine; and

R^{22a} is selected from fluorine, chlorine and methoxy.

33. A combination according to claim 32 wherein the compound of the formula (VIb) is selected from:

4-(2,6-difluoro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide;

4-(2,6-difluoro-benzoylamino)-1H-pyrazole-3-carboxylic acid (1-methyl-piperidin-4-yl)-amide;

4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide; and
4-(2-fluoro-6-methoxy-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide.

34. A combination according to claim 33 wherein the compound of the formula (VIb) is 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide.

35. A combination according to any one of the preceding claims wherein the compound of the formula (0) is in the form of a salt.

36. A combination according to claim 34 wherein the 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide is in the form of a salt, preferably an acid addition salt.

37. A combination according to claim 36 wherein the 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide is in the form of a salt selected from the acid addition salts formed with hydrochloric acid, methanesulphonic acid and acetic acid.

38. A combination according to claim 37 wherein the salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide is the salt formed with hydrochloric acid.

39. A combination according to claim 37 wherein the salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide is the salt formed with methanesulphonic acid.

40. A combination according to claim 36 wherein the salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide is the salt formed with acetic acid.

41. A combination according to any one of the preceding claims wherein the ancillary compound and compound of formula (0), (I^0), (I), (Ia), (Ib), (II), (IV), (IVa), (Va), (VIa) or (VIb) are physically associated.

42. The combination of claim 41 wherein the ancillary compound and compound of formula (0), (I^0), (I), (Ia), (Ib), (II), (IV), (IVa), (Va), (VIa) or (VIb) are: (a) in

admixture (for example within the same unit dose); (b) chemically/physicochemically linked (for example by crosslinking, molecular agglomeration or binding to a common vehicle moiety); (c) chemically/physicochemically co-packaged (for example, disposed on or within lipid vesicles, particles (e.g. micro- or nanoparticles) or emulsion droplets); or (d) unmixed but co-packaged or co-presented (e.g. as part of an array of unit doses).

43. The combination of any one of claims 1 to 40 wherein the ancillary compound and compound of formula (0), (I^0), (I), (Ia), (Ib), (II), (IV), (IVa), (Va), (VIa) or (VIb) are non-physically associated.

44. The combination of claim 43 wherein the combination comprises: (a) at least one of the two or more compounds together with instructions for the extemporaneous association of the at least one compound to form a physical association of the two or more compounds; or (b) at least one of the two or more compounds together with instructions for combination therapy with the two or more compounds; or (c) at least one of the two or more compounds together with instructions for administration to a patient population in which the other(s) of the two or more compounds have been (or are being) administered; or (d) at least one of the two or more compounds in an amount or in a form which is specifically adapted for use in combination with the other(s) of the two or more compounds.

45. The combination as defined in any one of the preceding claims in the form of a pharmaceutical pack, kit or patient pack.

46. A combination according to any one of the preceding claims for use in alleviating or reducing the incidence of a disease or condition comprising or arising from abnormal cell growth in a mammal.

47. 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 combination according to any one of claims 1 to 44 in an amount effective in inhibiting abnormal cell growth.

48. 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

combination according to any one of claims 1 to 44 in an amount effective in inhibiting abnormal cell growth.

49. A combination according to any one of claims 1 to 44 for use in inhibiting tumour growth in a mammal.

50. A method of inhibiting tumour growth in a mammal, which method comprises administering to the mammal an effective tumour growth-inhibiting amount of a combination according to any one of claims 1 to 44.

51. A combination according to any one of claims 1 to 44 for use in inhibiting the growth of tumour cells.

52. A method of inhibiting the growth of tumour cells, which method comprises contacting the tumour cells with administering to the mammal an effective tumour cell growth-inhibiting amount of a combination according to any one of claims 1 to 44.

53. A pharmaceutical composition comprising a combination according to any one of claims 1 to 46 and a pharmaceutically acceptable carrier.

54. A combination according to any one of claims 1 to 44 for use in medicine.

55. The use of a combination according to any one of claims 1 to 44 for the manufacture of a medicament for the prophylaxis or treatment of any one of the disease states or conditions disclosed herein.

56. A method for the treatment or prophylaxis of any one of the disease states or conditions disclosed herein, which method comprises administering to a patient (e.g. a patient in need thereof) combination according to any one of claims 1 to 44.

57. A method for alleviating or reducing the incidence of a disease state or condition disclosed herein, which method comprises administering to a patient (e,g, a patient in need thereof) a combination according to any one of claims 1 to 44.

58. A method for the diagnosis and treatment of a cancer in a mammalian patient, which method comprises (i) screening a patient to determine whether a cancer 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 an

ancillary compound; and (ii) where it is indicated that the disease or condition from which the patient is thus susceptible, thereafter administering to the patient a combination according to any one of claims 1 to 44.

59. The use of a combination according to any of the claims 1 to 44 for the manufacture of a medicament for the treatment or prophylaxis of a cancer in a patient who has been screened and has been determined as suffering from, or being at risk of suffering from, a cancer which would be susceptible to treatment with a combination as defined in any one of claims 1 to 44 having activity against cyclin dependent kinase.

60. A method for treating a cancer in a patient comprising administration of a combination according to any one of claims 1 to 44 to said patient in an amount and in a schedule of administration that is therapeutically efficaceous in the treatment of said cancer.

61. A method for preventing, treating or managing cancer in a patient in need thereof, said method comprising administering to said patient a prophylactically or therapeutically effective amount of a combination according to any one of claims 1 to 44.

62. The use of a combination according to any one of claims 1 to 44 for the manufacture of a medicament for use in the production of an anti-cancer effect in a warm-blooded animal such as a human.

63. A pharmaceutical pack, kit or patient pack comprising a combination according to any one of claims 1 to 46.

64. A pharmaceutical pack, kit or patient pack for anticancer therapy comprising an ancillary compound in dosage form and a compound of Formula (0), (I^0), (I), (Ia), (Ib), (II), (IV), (IVa), (Va), (VIa) or (VIb) according to any one of claims 1 to 44, also in dosage form (e.g. wherein the dosage forms are packaged together in common outer packaging).

65. A method for the treatment of a cancer in a warm-blooded animal such as a human, which comprises administering to said animal an effective amount of an ancillary compound sequentially e.g. before or after, or simultaneously with an effective

amount of a compound of Formula (0), (I^0), (I), (Ia), (Ib), (II), (IV), (IVa), (Va), (VIa) or (VIb) as defined in any one of claims 1 to 44.

66. A method of combination cancer therapy in a mammal comprising administering a therapeutically effective amount of an ancillary compound and a therapeutically effective amount of a compound of Formula (0), (I^0), (I), (Ia), (Ib), (II), (IV), (IVa), (Va), (VIa) or (VIb) as defined in any one of claims 1 to 44.

67. A compound of Formula (0), (I^0), (I), (Ia), (Ib), (II), (IV), (IVa), (Va), (VIa) or (VIb) as defined in any one of claims 1 to 44 for use in combination therapy with an ancillary compound.

68. A compound of Formula (0), (I^0), (I), (Ia), (Ib), (II), (IV), (IVa), (Va), (VIa) or (VIb) as defined in any one of claims 1 to 44 for use in combination therapy with an ancillary compound to alleviate or reduce the incidence of a disease or condition comprising or arising from abnormal cell growth in a mammal.

69. A compound of Formula (0), (I^0), (I), (Ia), (Ib), (II), (IV), (IVa), (Va), (VIa) or (VIb) as defined in any one of claims 1 to 44 for use in combination therapy with an ancillary compound to inhibit tumour growth in a mammal.

70. A compound of Formula (0), (I^0), (I), (Ia), (Ib), (II), (IV), (IVa), (Va), (VIa) or (VIb) as defined in any one of claims 1 to 44 for use in combination therapy with an ancillary compound to prevent, treat or manage cancer in a patient in need thereof.

71. A compound of Formula (0), (I^0), (I), (Ia), (Ib), (II), (IV), (IVa), (Va), (VIa) or (VIb) as defined in any one of claims 1 to 44 for use in enhancing or potentiating the response rate in a patient suffering from a cancer where the patient is being treated with an ancillary compound.

72. A method of enhancing or potentiating the response rate in a patient suffering from a cancer where the patient is being treated with an ancillary compound, which method comprises administering to the patient, in combination with the ancillary compound, a compound of Formula (0), (I^0), (I), (Ia), (Ib), (II), (IV), (IVa), (Va), (VIa) or (VIb) as defined in any one of claims 1 to 44.

73. The use of a combination according to any one of claims 1 to 44 for the manufacture of a medicament for any of the medical uses as defined herein.

74. An ancillary compound for use in combination therapy with a compound of formula (0), (I^0), (I), (Ia), (Ib), (II), (IV), (IVa), (Va), (VIa) or (VIb) as defined in any one of claims 1 to 44.

75. The compound of claim 74 wherein the combination therapy comprises treatment, prophylaxis or any of the therapeutic uses as defined herein.

76. Use of an ancillary compound for the manufacture of a medicament for use in the treatment or prophylaxis of a patient undergoing treatment with a compound of formula (0), (I^0), (I), (Ia), (Ib), (II), (IV), (IVa), (Va), (VIa) or (VIb) as defined in any one of claims 1 to 44.

77. Use of a compound of formula (0), (I^0), (I), (Ia), (Ib), (II), (IV), (IVa), (Va), (VIa) or (VIb) as defined in any one of claims 1 to 44 for the manufacture of a medicament for use in the treatment or prophylaxis of a patient undergoing treatment with an ancillary compound.

78. The combination of any one of the preceding claims within the ancillary compounds is selected from: (a) epothilones; (b) aurora inhibitors; (c) Hsp90 inhibitors; (d) tyrosine kinase inhibitors; (e) EGF antibodies; (f) decitabine and azacytidine DNA methyl transferase inhibitors; (g) cytokines and cytokine activating agents; (h) retinoids and rexinoids; (i) selective immunoresponse modulators; (j) checkpoint targeting agents; (k) DNA repair inhibitors; (l) inhibitors of G-protein coupled receptor inhibitors; and (m) a combination of two or more of the foregoing classes (a) to (l).

79. The combination of any one of the preceding claims within the ancillary compounds is selected from one or more: aurora inhibitor, HSP-90 inhibitor, an epothilone, a tyrosine kinase inhibitor or a EGF antibody.

80. The invention of any one of the preceding claims wherein the ancillary compound comprises an Aurora inhibitor.

81. The invention of claim 78 wherein the Aurora inhibitor is selected from AZD1152, MK0457 (VX-680), PHA-739358, MLN-8054, and MP-235.

82. The invention of claim 79 wherein the Aurora inhibitor is VX-680.

83. The invention of any one of claims 1 to 77 wherein the ancillary compound comprises an Hsp90 inhibitor.

84. The invention of claim 81 wherein the HSP90 inhibitor is selected from herbimycin, geldanamycin (GA), 17-AAG e.g. Kos-953 and CNF-1010, 17-DMAG (Kos-1022), and IPI-504.

85. The invention of any one of claims 1 to 77 wherein the ancillary compound comprises an epothilone.

86. The invention of claim 83 wherein the epothiline is selected from ixabepilone, patupilone, BMS-310705, KOS-862 and ZK-EPO.

87. The invention of any one of claims 1 to 77 wherein the ancillary compound comprises a tyrosine kinase inhibitor.

88. The invention of claim 83 wherein the tyrosine kinase inhibitor is selected from dasatinib, lapatinib, nilotinib, vandetanib, vatalinib and CHIR-258

89. The invention of any one of claims 1 to 77 wherein the ancillary compound comprises panitumumab.

90. The combination of any one of the preceding claims within the ancillary compounds is selected from: (a) epothilones; (b) aurora inhibitors; (c) Hsp90 inhibitors; (d) tyrosine kinase inhibitors; (e) EGF antibodies; (f) decitabine and azacytidine DNA methyl transferase inhibitors; and (m) a combination of two or more of the foregoing classes (a) to (l).

91. The invention of any one of claims 1 to 77 wherein the ancillary compound comprises thalidomide or lenalidomide.

92. The invention of any one of the preceding claims wherein the compound of formula (0), (I^0), (I), (Ia), (Ib), (II), (IV), (IVa), (Va), (VIa) or (VIb) as defined in any one of claims 1 to 44 is the methane sulphonic acid salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide.

93. The invention of claim 85 wherein the the methane sulphonic acid salt of 4-(2,6-dichloro-benzoylamino)-1H-pyrazole-3-carboxylic acid piperidin-4-ylamide is in crystalline form.