Fluoro substituted Omega-Carboxyaryl Diphenyl Urea for the Treatment and Prevention of Diseases and Conditions

Field of the Invention

This invention relates to novel compounds, pharmaceutical compositions containing such compounds and the use of those compounds or compositions for treating diseases and conditions mediated by abnormal VEGFR, PDGFR, raf, p38, and/or flt-3 kinase signaling, either alone or in combination with anti-cancer agents.

Background of the Invention

Activation of the ras signal transduction pathway indicates a cascade of events that have a profound impact on cellular proliferation, differentiation, and transformation. Raf kinase, a downstream effector of ras, is recognized as a key mediator of these signals from cell surface receptors to the cell nucleus (Lowy, D. R.; Willumsen, B. M. *Ann. Rev. Biochem.* **1993**, *62*, 851; Bos, J. L. *Cancer Res.* **1989**, *49*, 4682). It has been shown that inhibiting the effect of active ras by inhibiting the raf kinase signaling pathway by administration of deactivating antibodies to raf kinase or by co-expression of dominant negative raf kinase or dominant negative MEK, the substrate of raf kinase, leads to the reversion of transformed cells to the normal growth phenotype (see: Daum et al. *Trends Biochem. Sci.* **1994**, *19*, 474-80; Fridman et al. *J. Biol. Chem.* **1994**, *269*, 30105-8. Kolch et al. (*Nature* **1991**, *349*, 426-28) have further indicated that inhibition of raf expression by antisense RNA blocks cell proliferation in membrane-associated oncogenes. Similarly, inhibition of raf kinase (by antisense oligodeoxynucleotides) has been correlated *in vitro* and *in vivo* with inhibition of the growth of a variety of human tumor types (Monia et al., *Nat. Med.* **1996**, *2*, 668-75).

To support progressive tumor growth beyond the size of 1-2 mm^3, it is recognized that tumor cells require a functional stroma, a support structure consisting of fibroblast, smooth muscle cells, endothelial cells, extracellular matrix proteins, and soluble factors (Folkman, J., *Semin. Oncol.* **2002**. *29*(6 Suppl 16), 15-8). Tumors induce the formation of stromal tissues through the secretion of soluble growth factors such as PDGF and transforming growth factor-beta (TGF-beta), which in turn

stimulate the secretion of complimentary factors by host cells such as fibroblast growth factor (FGF), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF). These stimulatory factors induce the formation of new blood vessels, or angiogenesis, which brings oxygen and nutrients to the tumor and allows it to grow and provides a route for metastasis. It is believed some therapies directed at inhibiting stroma formation will inhibit the growth of epithelial tumors from a wide variety of histological types. (George, D. *Semin. Oncol.* **2001**. *28*(5 Suppl 17), 27-33; Shaheen, R.M., et al., *Cancer Res.* **2001**, *61*(4), 1464-8; Shaheen, R.M., et al. *Cancer Res.* **1999**, *59*(21), 5412-6). However, because of the complex nature and the multiple growth factors involved in angiogenesis process and tumor progression, an agent targeting a single pathway may have limited efficacy. It is desirable to provide treatment against a number of key signaling pathways utilized by tumors to induce angiogenesis in the host stroma. These include PDGF, a potent stimulator of stroma formation (Ostman, A. and C.H. Heldin, *Adv. Cancer Res.* **2001**, *80*, 1-38), FGF, a chemo-attractant and mitogen for fibroblasts and endothelial cells, and VEGF, a potent regulator of vascularization.

PDGF is a key regulator of stromal formation, which is secreted by many tumors in a paracrine fashion and is believed to promote the growth of fibroblasts, smooth muscle and endothelial cells, promoting stroma formation and angiogenesis. PDGF was originally identified as the v-sis oncogene product of the simian sarcoma virus (Heldin, C.H., et al., *J. Cell. Sci. Suppl.* **1985**, *3*, 65-76). The growth factor is made up of two peptide chains, referred to as A or B chains which share 60% homology in their primary amino acid sequence. The chains are disulfide cross linked to form the 30 kDa mature protein composed of either AA, BB or AB homo- or heterodimmers. PDGF is found at high levels in platelets, and is expressed by endothelial cells and vascular smooth muscle cells. In addition, the production of PDGF is up regulated under low oxygen conditions such as those found in poorly vascularized tumor tissue (Kourembanas, S., et al., *Kidney Int.* **1997**, *51*(2), 438-43). PDGF binds with high affinity to the PDGF receptor, a 1106 amino acid 124 kDa transmembrane tyrosine kinase receptor (Heldin, C.H., A. Ostman, and L. Ronnstrand, *Biochim. Biophys. Acta* **1998**, 1378(1), 79-113). PDGFR is found as homo- or heterodimer chains which have 30% homology overall in their amino acid sequence and 64% homology between their kinase domains (Heldin, C.H., et al.. *Embo J.* **1988**, *7*(5), 1387-93). PDGFR is a member of a family of tyrosine kinase

receptors with split kinase domains that includes VEGFR-2 (KDR), VEGFR-3 (flt-4), c-kit, and flt-3. The PDGF receptor is expressed primarily on fibroblasts, smooth muscle cells, and pericytes and to a lesser extent on neurons, kidney mesangial, Leydig, and Schwann cells of the central nervous system. Upon binding to the receptor, PDGF induces receptor dimerization and undergoes auto- and trans-phosphorylation of tyrosine residues which increase the receptors' kinase activity and promotes the recruitment of downstream effectors through the activation of SH2 protein binding domains. A number of signaling molecules form complexes with activated PDGFR including PI-3-kinase, phospholipase C-gamma, src and GAP (GTPase activating protein for p21-ras) (Soskic, V., et al. *Biochemistry* **1999**, *38*(6), 1757-64). Through the activation of PI-3-kinase, PDGF activates the Rho signaling pathway inducing cell motility and migration, and through the activation of GAP, induces mitogenesis through the activation of p21-ras and the MAPK signaling pathway.

In adults, it is believed the major function of PDGF is to facilitate and increase the rate of wound healing and to maintain blood vessel homeostasis (Baker, E.A. and D.J. Leaper, *Wound Repair Regen.* **2000**, *8*(5), 392-8, and Yu, J., A. Moon, and H.R. Kim, *Biochem. Biophys. Res. Commun.* **2001**, *282*(3), 697-700). PDGF is found at high concentrations in platelets and is a potent chemoattractant for fibroblast, smooth muscle cells, neutrophils and macrophages. In addition to its role in wound healing PDGF is known to help maintain vascular homeostasis. During the development of new blood vessels, PDGF recruits pericytes and smooth muscle cells that are needed for the structural integrity of the vessels. PDGF is thought to play a similar role during tumor neovascularization. As part of its role in angiogenesis PDGF controls interstitial fluid pressure, regulating the permeability of vessels through its regulation of the interaction between connective tissue cells and the extracellular matrix. Inhibiting PDGFR activity can lower interstitial pressure and facilitate the influx of cytotoxics into tumors improving the anti-tumor efficacy of these agents (Pietras, K., et al. *Cancer Res.* **2002**, *62*(19), 5476-84; Pietras, K., et al. *Cancer Res.* **2001**, *61*(7), 2929-34).

PDGF can promote tumor growth through either the paracrine or autocrine stimulation of PDGFR receptors on stromal cells or tumor cells directly, or through the amplification of the receptor or activation of the receptor by recombination. Over expressed PDGF can transform human melanoma cells and keratinocytes (Forsberg,

K., et al. *Proc. Natl. Acad. Sci. U S A.* **1993**, *90*(2), 393-7; Skobe, M. and N.E. Fusenig, *Proc. Natl. Acad. Sci. U S A.* **1998**, *95*(3), 1050-5), two cell types that do not express PDGF receptors, presumably by the direct effect of PDGF on stroma formation and induction of angiogenesis. This paracrine stimulation of tumor stroma is also observed in carcinomas of the colon, lung, breast, and prostate (Bhardwaj, B., et al. *Clin. Cancer Res.* **1996**, *2*(4), 773-82; Nakanishi, K., et al. *Mod. Pathol.* **1997**, *10*(4), 341-7; Sundberg, C., et al. *Am. J. Pathol.* **1997**, *151*(2), 479-92; Lindmark, G., et al. *Lab. Invest.* **1993**, *69*(6), 682-9; Vignaud, J.M., et al, *Cancer Res.* **1994**, *54*(20), 5455-63) where the tumors express PDGF, but not the receptor. The autocrine stimulation of tumor cell growth, where a large faction of tumors analyzed express both the ligand PDGF and the receptor, has been reported in glioblastomas (Fleming, T.P., et al. *Cancer Res.* **1992**, *52*(16), 4550-3), soft tissue sarcomas (Wang, J., M.D. Coltrera, and A.M. Gown, *Cancer Res.* **1994**, *54*(2), 560-4) and cancers of the ovary (Henriksen, R., et al. *Cancer Res.* **1993**, *53*(19), 4550-4), prostate (Fudge, K., C.Y. Wang, and M.E. Stearns, *Mod. Pathol.* **1994**, *7*(5), 549-54), pancreas (Funa, K., et al. *Cancer Res.* **1990**, *50*(3), 748-53) and lung (Antoniades, H.N., et al., *Proc. Natl. Acad. Sci. U S A* **1992**, *89*(9), 3942-6). Ligand independent activation of the receptor is found to a lesser extent but has been reported in chronic myelomonocytic leukemia (CMML) where the a chromosomal translocation event forms a fusion protein between the Ets-like transcription factor TEL and the PDGF receptor. In addition, activating mutations in PDGFR have been found in gastrointestinal stromal tumors in which c-kit activation is not involved (Heinrich, M.C., et al., *Science* **2003**, *9*, 9).

Another major regulator of angiogenesis and vasculogenesis in both embryonic development and some angiogenic-dependent diseases is vascular endothelial growth factor (VEGF; also called vascular permeability factor, VPF). VEGF represents a family of isoforms of mitogens existing in homodimeric forms due to alternative RNA splicing. The VEGF isoforms are highly specific for vascular endothelial cells (for reviews, see: Farrara et al. *Endocr. Rev.* **1992**, *13*, 18; Neufield et al. *FASEB J.* **1999**, *13*, 9).

VEGF expression is induced by hypoxia (Shweiki et al. *Nature* **1992**, *359*, 843), as well as by a variety of cytokines and growth factors, such as interleukin-1, interleukin-6, epidermal growth factor and transforming growth factor. To date, VEGF and the VEGF family members have been reported to bind to one or more of three transmembrane receptor tyrosine kinases (Mustonen et al. *J. Cell Biol.* **1995**,

129, 895), VEGF receptor-1 (also known as flt-1 (fms-like tyrosine kinase-1)), VEGFR-2 (also known as kinase insert domain containing receptor (KDR); the murine analogue of VEGFR-2 is known as fetal liver kinase-1 (flk-1)), and VEGFR-3 (also known as flt-4). VEGFR-2 and flt-1 have been shown to have different signal transduction properties (Waltenberger et al. *J. Biol. Chem.* **1994**, *269*, 26988); Park et al. *Oncogene* **1995**, *10*, 135). Thus, VEGFR-2 undergoes strong ligand-dependant tyrosine phosphorylation in intact cells, whereas flt-1 displays a weak response. Thus, binding to VEGFR-2 is believed to be a critical requirement for induction of the full spectrum of VEGF-mediated biological responses.

In vivo, VEGF plays a central role in vasculogenesis, and induces angiogenesis and permeabilization of blood vessels. Deregulated VEGF expression contributes to the development of a number of diseases that are characterized by abnormal angiogenesis and/or hyperpermeability processes. It is believed that regulation of the VEGF-mediated signal transduction cascade by some agents can provide a useful control of abnormal angiogenesis and/or hyperpermeability processes. Tumorigenic cells within hypoxic regions of tumors respond by stimulation of VEGF production, which triggers activation of quiescent endothelial cells to stimulate new blood vessel formation. (Shweiki et al. *Proc. Nat'l. Acad. Sci.* **1995**, *92*, 768). In addition, VEGF production in tumor regions where there is no angiogenesis may proceed through the ras signal transduction pathway (Grugel et al. *J. Biol. Chem.* **1995**, *270*, 25915; Rak et al. *Cancer Res.* **1995**, *55*, 4575). *In situ* hybridization studies have demonstrated VEGF mRNA is strongly upregulated in a wide variety of human tumors, including lung (Mattern et al. *Br. J. Cancer* **1996**, *73*, 931), thyroid (Viglietto et al. *Oncogene* **1995**, *11*, 1569), breast (Brown et al. *Human Pathol.* **1995**, *26*, 86), gastrointestinal tract (Brown et al. *Cancer Res.* **1993**, *53*, 4727; Suzuki et al. *Cancer Res.* **1996**, *56*, 3004), kidney and bladder (Brown et al. *Am. J. Pathol.* **1993**, 143I, 1255), ovary (Olson et al. *Cancer Res.* **1994**, *54*, 1255), and cervical (Guidi et al. *J. Nat'l Cancer Inst.* **1995**, *87*, 12137) carcinomas, as well as angiosarcoma (Hashimoto et al. Lab. Invest. 1995, 73, 859) and several intracranial tumors (Plate et al. *Nature* **1992**, *359*, 845; Phillips et al. *Int. J. Oncol.* **1993**, *2*, 913; Berkman et al. *J. Clin. Invest.* **1993**, *91*, 153). Neutralizing monoclonal antibodies to VEGFR-2 have been shown to be efficacious in blocking tumor angiogenesis (Kim et al. *Nature* **1993**, *362*, 841; Rockwell et al. *Mol. Cell. Differ.* **1995**, *3*, 315).

Overexpression of VEGF, for example under conditions of extreme hypoxia, can lead to intraocular angiogenesis, resulting in hyperproliferation of blood vessels, leading eventually to blindness. Such a cascade of events has been observed for a number of retinopathies, including diabetic retinopathy, ischemic retinal-vein occlusion, and retinopathy of prematurity (Aiello et al. *New Engl. J. Med.* **1994**, *331*, 1480; Peer et al. *Lab. Invest.* **1995**, *72*, 638), and age-related macular degeneration (AMD; see, Lopez et al. *Invest. Opththalmol. Vis. Sci.* **1996**, *37*, 855).

In rheumatoid arthritis (RA), the in-growth of vascular pannus may be mediated by production of angiogenic factors. Levels of immunoreactive VEGF are high in the synovial fluid of RA patients, while VEGF levels were low in the synovial fluid of patients with other forms of arthritis of with degenerative joint disease (Koch et al. *J. Immunol.* **1994**, *152*, 4149). The angiogenesis inhibitor AGM-170 has been shown to prevent neovascularization of the joint in the rat collagen arthritis model (Peacock et al. *J. Exper. Med.* **1992**, *175*, 1135).

Increased VEGF expression has also been shown in psoriatic skin, as well as bullous disorders associated with subepidermal blister formation, such as bullous pemphigoid, erythema multiforme, and dermatitis herpetiformis (Brown et al. *J. Invest. Dermatol.* **1995**, *104*, 744).

The vascular endothelial growth factors (VEGF, VEGF-C, VEGF-D) and their receptors (VEGFR-2, VEGFR-3) are not only key regulators of tumor angiogenesis, but also lymphangiogenesis. VEGF, VEGF-C and VEGF-D are expressed in most tumors, primarily during periods of tumor growth and, often at substantially increased levels. VEGF expression is stimulated by hypoxia, cytokines, oncogenes such as ras, or by inactivation of tumor suppressor genes (McMahon, G. *Oncologist* **2000**, *5*(Suppl. 1), 3-10; McDonald, N.Q.; Hendrickson, W.A. *Cell* **1993**, *73*, 421-424)

The biological activities of the VEGFs are mediated through binding to their receptors. VEGFR-3 (also called flt-4) is predominantly expressed on lymphatic endothelium in normal adult tissues. VEGFR-3 function is needed for new lymphatic vessel formation, but not for maintenance of the pre-existing lymphatics. VEGFR-3 is also upregulated on blood vessel endothelium in tumors. Recently VEGF-C and VEGF-D, ligands for VEGFR-3, have been identified as regulators of lymphangiogenesis in mammals. Lymphangiogenesis induced by tumor-associated lymphangiogenic factors could promote the growth of new vessels into the tumor,

providing tumor cells access to systemic circulation. Cells that invade the lymphatics could find their way into the bloodstream via the thoracic duct. Tumor expression studies have allowed a direct comparison of VEGF-C, VEGF-D and VEGFR-3 expression with clinicopathological factors that relate directly to the ability of primary tumors to spread (e.g., lymph node involvement, lymphatic invasion, secondary metastases, and disease-free survival). In many instances, these studies demonstrate a statistical correlation between the expression of lymphangiogenic factors and the ability of a primary solid tumor to metastasize (Skobe, M. et al. *Nature Med.* **2001**, *7*(2), 192-198; Stacker, S.A. et al.. *Nature Med.* **2001**, *7*(2), 186-191; Makinen, T. et al. *Nature Med.* **2001**, *7*(2), 199-205; Mandriota, S.J. et al. *EMBO J.* **2001**, *20*(4), 672-82; Karpanen, T. et al. *Cancer Res.* **2001**, *61*(5), 1786-90; Kubo, H. et al. *Blood* **2000**, *96*(2), 546-53).

Hypoxia appears to be an important stimulus for VEGF production in malignant cells. Activation of p38 MAP kinase is required for VEGF induction by tumor cells in response to hypoxia (Blaschke, F. et al. *Biochem. Biophys. Res. Commun.* **2002**, *296*, 890-896; Shemirani, B. et al. *Oral Oncology* **2002**, *38*, 251-257). In addition to its involvement in angiogenesis through regulation of VEGF secretion, p38 MAP kinase promotes malignant cell invasion, and migration of different tumor types through regulation of collagenase activity and urokinase plasminogen activator expression (Laferriere, J. et al. *J. Biol. Chem.* **2001**, *276*, 33762–33772; Westermarck, J. et al. *Cancer Res.* **2000**, *60*, 7156–7162; Huang, S. et al. *J. Biol. Chem.* **2000**, *275*, 12266–12272; Simon, C. et al. *Exp. Cell Res.* **2001**, *271*, 344–355).

Inhibition of the mitogen-activated protein kinase (MAPK) p38 has been shown to inhibit both cytokine production (e.g., TNF, IL-1, IL-6, IL-8) and proteolytic enzyme production (e.g., MMP-1, MMP-3) *in vitro* and/or *in vivo*. The mitogen activated protein (MAP) kinase p38 is involved in IL-1 and TNF signaling pathways (Lee, J. C.; Laydon, J. T.; McDonnell, P. C.; Gallagher, T. F.; Kumar, S.; Green, D.; McNulty, D.; Blumenthal, M. J.; Heys, J. R.; Landvatter, S. W.; Stricker, J. E.; McLaughlin, M. M.; Siemens, I. R.; Fisher, S. M.; Livi, G. P.; White, J. R.; Adams, J. L.; Yound, P. R. *Nature* **1994**, *372*, 739).

Clinical studies have linked tumor necrosis factor (TNF) production and/or signaling to a number of diseases including rheumatoid arthritis (Maini. *J. Royal Coll.*

Physicians London **1996**, *30*, 344). In addition, excessive levels of TNF have been implicated in a wide variety of inflammatory and/or immunomodulatory diseases, including acute rheumatic fever (Yegin et al. *Lancet* **1997**, *349*, 170), bone resorption (Pacifici et al. *J. Clin. Endocrinol. Metabol.* **1997**, *82*, 29), postmenopausal osteoporosis (Pacifici et al. *J. Bone Mineral Res.* **1996**, *11*, 1043), sepsis (Blackwell et al. *Br. J. Anaesth.* **1996**, *77*, 110), gram negative sepsis (Debets et al. *Prog. Clin. Biol. Res.* **1989**, *308*, 463), septic shock (Tracey et al. *Nature* **1987**, *330*, 662; Girardin et al. *New England J. Med.* **1988**, *319*, 397), endotoxic shock (Beutler et al. *Science* **1985**, *229*, 869; Ashkenasi et al. *Proc. Nat'l. Acad. Sci. USA* **1991**, *88*, 10535), toxic shock syndrome, (Saha et al. *J. Immunol.* **1996**, *157*, 3869; Lina et al. *FEMS Immunol. Med. Microbiol.* **1996**, *13*, 81), systemic inflammatory response syndrome (Anon. *Crit. Care Med.* **1992**, *20*, 864), inflammatory bowel diseases (Stokkers et al. *J. Inflamm.* **1995-6**, *47*, 97) including Crohn's disease (van Deventer et al. *Aliment. Pharmacol. Therapeu.* **1996**, *10 (Suppl. 2)*, 107; van Dullemen et al. *Gastroenterology* **1995**, *109*, 129) and ulcerative colitis (Masuda et al. *J. Clin. Lab. Immunol.* **1995**, *46*, 111), Jarisch-Herxheimer reactions (Fekade et al. *New England J. Med.* **1996**, *335*, 311), asthma (Amrani et al. *Rev. Malad. Respir.* **1996**, *13*, 539), adult respiratory distress syndrome (Roten et al. *Am. Rev. Respir. Dis.* **1991**, *143*, 590; Suter et al. *Am. Rev. Respir. Dis.* **1992**, *145*, 1016), acute pulmonary fibrotic diseases (Pan et al. *Pathol. Int.* **1996**, *46*, 91), pulmonary sarcoidosis (Ishioka et al. *Sarcoidosis Vasculitis Diffuse Lung Dis.* **1996**, *13*, 139), allergic respiratory diseases (Casale et al: *Am. J. Respir. Cell Mol. Biol.* **1996**, *15*, 35), silicosis (Gossart et al. *J. Immunol.* **1996**, *156*, 1540; Vanhee et al. *Eur. Respir. J.* **1995**, *8*, 834), coal worker's pneumoconiosis (Borm et al. *Am. Rev. Respir. Dis.* **1988**, *138*, 1589), alveolar injury (Horinouchi et al. *Am. J. Respir. Cell Mol. Biol.* **1996**, *14*, 1044), hepatic failure (Gantner et al. *J. Pharmacol. Exp. Therap.* **1997**, *280*, 53), liver disease during acute inflammation (Kim et al. *J. Biol. Chem.* **1997**, *272*, 1402), severe alcoholic hepatitis (Bird et al. *Ann. Intern. Med.* **1990**, *112*, 917), malaria (Grau et al. *Immunol. Rev.* **1989**, *112*, 49; Taverne et al. *Parasitol. Today* **1996**, *12*, 290) including Plasmodium falciparum malaria (Perlmann et al. *Infect. Immunit.* **1997**, *65*, 116) and cerebral malaria (Rudin et al. *Am. J. Pathol.* **1997**, *150*, 257), non-insulin-dependent diabetes mellitus (NIDDM; Stephens et al. *J. Biol. Chem.* **1997**, *272*, 971; Ofei et al. *Diabetes* **1996**, *45*, 881), congestive heart failure (Doyama et al. *Int. J. Cardiol.* **1996**, *54*, 217; McMurray et al. *Br. Heart J.* **1991**, *66*, 356), damage following heart disease (Malkiel

et al. *Mol. Med. Today* **1996**, *2*, 336), atherosclerosis (Parums et al. *J. Pathol.* **1996**, *179*, A46), Alzheimer's disease (Fagarasan et al. *Brain Res.* **1996**, *723*, 231; Aisen et al. *Gerontology* **1997**, *43*, 143), acute encephalitis (Ichiyama et al. *J. Neurol.* **1996**, *243*, 457), brain injury (Cannon et al. *Crit. Care Med.* **1992**, *20*, 1414; Hansbrough et al. *Surg. Clin. N. Am.* **1987**, *67*, 69; Marano et al. *Surg. Gynecol. Obstetr.* **1990**, *170*, 32), multiple sclerosis (M.S.; Coyle. *Adv. Neuroimmunol.* **1996**, *6*, 143; Matusevicius et al. *J. Neuroimmunol.* **1996**, *66*, 115) including demyelation and oligiodendrocyte loss in multiple sclerosis (Brosnan et al. *Brain Pathol.* **1996**, *6*, 243), advanced cancer (MucWierzgon et al. *J. Biol. Regulators Homeostatic Agents* **1996**, *10*, 25), lymphoid malignancies (Levy et al. *Crit. Rev. Immunol.* **1996**, *16*, 31), pancreatitis (Exley et al. *Gut* **1992**, *33*, 1126) including systemic complications in acute pancreatitis (McKay et al. *Br. J. Surg.* **1996**, *83*, 919), impaired wound healing in infection inflammation and cancer (Buck et al. *Am. J. Pathol.* **1996**, *149*, 195), myelodysplastic syndromes (Raza et al. *Int. J. Hematol.* **1996**, *63*, 265), systemic lupus erythematosus (Maury et al. *Arthritis Rheum.* **1989**, *32*, 146), biliary cirrhosis (Miller et al. *Am. J. Gasteroenterolog.* **1992**, *87*, 465), bowel necrosis (Sun et al. *J. Clin. Invest.* **1988**, *81*, 1328), psoriasis (Christophers. *Austr. J. Dermatol.* **1996**, *37*, S4), radiation injury (Redlich et al. *J. Immunol.* **1996**, *157*, 1705), and toxicity following administration of monoclonal antibodies such as OKT3 (Brod et al. *Neurology* **1996**, *46*, 1633). TNF levels have also been related to host-versus-graft reactions (Piguet et al. *Immunol. Ser.* **1992**, *56*, 409) including ischemia reperfusion injury (Colletti et al. *J. Clin. Invest.* **1989**, *85*, 1333) and allograft rejections including those of the kidney (Maury et al. *J. Exp. Med.* **1987**, *166*, 1132), liver (Imagawa et al. *Transplantation* **1990**, *50*, 219), heart (Bolling et al. *Transplantation* **1992**, *53*, 283), and skin (Stevens et al. *Transplant. Proc.* **1990**, *22*, 1924), lung allograft rejection (Grossman et al. *Immunol. Allergy Clin. N. Am.* **1989**, *9*, 153) including chronic lung allograft rejection (obliterative bronchitis; LoCicero et al. *J. Thorac. Cardiovasc. Surg.* **1990**, *99*, 1059), as well as complications due to total hip replacement (Cirino et al. *Life Sci.* **1996**, *59*, 86). TNF has also been linked to infectious diseases (review: Beutler et al. *Crit. Care Med.* **1993**, *21*, 5423; Degre. *Biotherapy* **1996**, *8*, 219) including tuberculosis (Rook et al. *Med. Malad. Infect.* **1996**, *26*, 904), Helicobacter pylori infection during peptic ulcer disease (Beales et al. *Gastroenterology* **1997**, *112*, 136), Chaga's disease resulting from Trypanosoma cruzi infection (Chandrasekar et al. *Biochem. Biophys. Res. Commun.* **1996**, *223*, 365), effects of Shiga-like toxin resulting from E. coli

infection (Harel et al. *J. Clin. Invest.* **1992**, *56*, 40), the effects of enterotoxin A resulting from Staphylococcus infection (Fischer et al. *J. Immunol.* **1990**, *144*, 4663), meningococcal infection (Waage et al. *Lancet* **1987**, 355; Ossege et al. *J. Neurolog. Sci.* **1996**, *144*, 1), and infections from Borrelia burgdorferi (Brandt et al. *Infect. Immunol.* **1990**, *58*, 983), Treponema pallidum (Chamberlin et al. *Infect. Immunol.* **1989**, *57*, 2872), cytomegalovirus (CMV; Geist et al. *Am. J. Respir. Cell Mol. Biol.* **1997**, *16*, 31), influenza virus (Beutler et al. *Clin. Res.* **1986**, *34*, 491a), Sendai virus (Goldfield et al. *Proc. Nat'l. Acad. Sci. USA* **1989**, *87*, 1490), Theiler's encephalomyelitis virus (Sierra et al. *Immunology* **1993**, *78*, 399), and the human immunodeficiency virus (HIV; Poli. *Proc. Nat'l. Acad. Sci. USA* **1990**, *87*, 782; Vyakaram et al. *AIDS* **1990**, *4*, 21; Badley et al. *J. Exp. Med.* **1997**, *185*, 55).

A number of diseases are thought to be mediated by excess or undesired matrix-destroying metalloprotease (MMP) activity or by an imbalance in the ratio of the MMPs to the tissue inhibitors of metalloproteinases (TIMPs). These include osteoarthritis (Woessner et al. *J. Biol. Chem.* **1984**, *259*, 3633), rheumatoid arthritis (Mullins et al. *Biochim. Biophys. Acta* **1983**, *695*, 117; Woolley et al. *Arthritis Rheum.* **1977**, *20*, 1231; Gravallese et al. *Arthritis Rheum.* **1991**, 34, 1076), septic arthritis (Williams et al. *Arthritis Rheum.* **1990**, *33*, 533), tumor metastasis (Reich et al. *Cancer Res.* **1988**, *48*, 3307; Matrisian et al. *Proc. Nat'l. Acad. Sci., USA* **1986**, *83*, 9413), periodontal diseases (Overall et al. *J. Periodontal Res.* **1987**, *22*, 81), corneal ulceration (Burns et al. *Invest. Opthalmol. Vis. Sci.* **1989**, *30*, 1569), proteinuria (Baricos et al. *Biochem. J.* **1988**, *254*, 609), coronary thrombosis from atherosclerotic plaque rupture (Henney et al. *Proc. Nat'l. Acad. Sci., USA* **1991**, *88*, 8154), aneurysmal aortic disease (Vine et al. *Clin. Sci.* **1991**, *81*, 233), birth control (Woessner et al. *Steroids* **1989**, *54*, 491), dystrophobic epidermolysis bullosa (Kronberger et al. *J. Invest. Dermatol.* **1982**, *79*, 208), degenerative cartilage loss following traumatic joint injury, osteopenias mediated by MMP activity, tempero mandibular joint disease, and demyelating diseases of the nervous system (Chantry et al. *J. Neurochem.* **1988**, *50*, 688).

Because inhibition of p38 leads to inhibition of TNF production and MMP production, it is believed inhibition of mitogen activated protein (MAP) kinase p38 enzyme can provide an approach to the treatment of the above listed diseases including osteoporosis and inflammatory disorders such as rheumatoid arthritis and

COPD (Badger, A. M.; Bradbeer, J. N.; Votta, B.; Lee, J. C.; Adams, J. L.; Griswold, D. E. *J. Pharm. Exper. Ther.* **1996**, *279*, 1453).

Hypoxia appears to be an important stimulus for VEGF production in malignant cells. Activation of p38 kinase is required for VEGF induction by tumor cells in response to hypoxia (Blaschke, F. et al. *Biochem. Biophys. Res. Commun.* **2002**, *296*, 890-896; Shemirani, B. et al. *Oral Oncology* **2002**, *38*, 251-257). In addition to its involvement in angiogenesis through regulation of VEGF secretion, p38 kinase promotes malignant cell invasion, and migration of different tumor types through regulation of collagenase activity and urokinase plasminogen activator expression (Laferriere, J. et al. *J. Biol. Chem.* **2001**, *276*, 33762–33772; Westermarck, J. et al. *Cancer Res.* **2000**, *60*, 7156–7162; Huang, S. et al. *J. Biol. Chem.* **2000**, *275*, 12266–12272; Simon, C. et al. *Exp. Cell Res.* **2001**, *271*, 344–355). Therefore, inhibition of p38 kinase is also expected to impact tumor growth by interfering with signaling cascades associated with both angiogenesis and malignant cell invasion.

Certain ureas have been described as having activity as serine-threonine kinase and/or as tyrosine kinase inhibitors. In particular, the utility of certain ureas as an active ingredient in pharmaceutical compositions for the treatment of cancer, angiogenesis disorders, inflammatory disorders, has been demonstrated.

For cancer and angiogenesis, see:

Smith et al., *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2775-2778.

Lowinger et al., *Clin. Cancer Res.* **2000**, *6*(suppl.), 335.

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Hilger et al., Book of Abstracts, 38th ASCO Meeting, Orlando, FL, USA, abstract 1916.

Moore et al., Book of Abstracts, 38th ASCO Meeting, Orlando, FL, USA, abstract 1816.

Strumberg et al., Book of Abstracts, 38th ASCO Meeting, Orlando, FL, USA, abstract 121.

For p38 mediated diseases, including inflammatory disorders, see:

Redman et al., *Bioorg. Med. Chem. Lett.* **2001**, *11*, 9-12.

Dumas et al., *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2047-2050.

Dumas et al., *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2051-2054.

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Dumas et al., *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1559-1562.

Regan et al., *J. Med. Chem.* **2002**, *45*, 2994-3008.

Pargellis et al., *Nature Struct. Biol.* **2002**, *9*(4), 268-272.

Madwed J. B., Book of Abstracts, Protein Kinases: Novel Target Identification and Validation for Therapeutic Development, San Diego, CA, USA, March 2002.

Pargellis C. et al., *Curr. Opin. Invest. Drugs* **2003**, *4*, 566-571.

Branger J. et al., *J. Immunol.* **2002**, *168*, 4070-4077.

Branger J. et al., *Blood* **2003**, *101*, 4446-4448.

Omega-Carboxyaryl diphenyl ureas are disclosed in WO00/42012, published: July 20, 2000, WO00/41698, published: July 20, 2000, the following published U.S. applications:

US2002-0165394-A1, published November 7, 2002,

US2001-003447-A1, published October 25, 2001,

US2001-0016659-A1, published August 23, 2001,

US2002-013774-A1, published September 26, 2002,

and copending U.S. applications:

09/758,547, filed January 12, 2001,

09/889,227, filed July 12, 2001,

09/993,647, filed November 27, 2001,

10/042,203, filed January 11, 2002 and

10/071,248, filed February 11, 2002,

Description of the Invention

It has been discovered that the omega-carboxyaryl diphenyl urea of Formula I below, which has a 2-fluoro-4-(2-(N-methylcarbamoyl)-4-pyridyloxy)phenylene group bound to urea is a potent inhibitor raf kinase, VEGFR kinase, p38 kinase, and PDGFR kinase, which are all molecular targets of interest for the treatment and prevention of osteoporosis, inflammatory disorders, hyper-proliferatrive disorders, and angiogenesis disorders, including cancer.

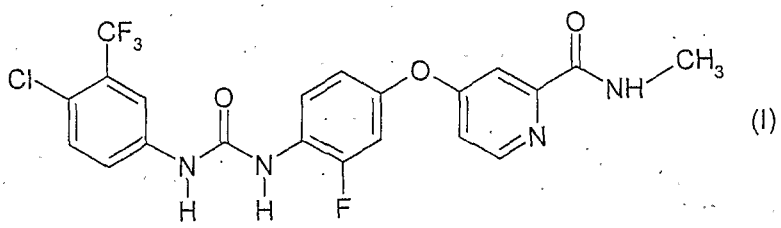
The present invention provides, e.g.,

(i) a novel compound of Formula (I), salts, prodrugs, and metabolites thereof,

(ii) pharmaceutical compositions containing such compound, and

(iii) use of this compound or compositions for treating diseases and conditions mediated by raf, VEGFR, PDGFR, flt-3, and p38, either as a sole agent or in combination with cytotoxic therapies.

The compound of the Formula I below, salts, prodrugs and metabolites thereof is collectively referred to as the "compounds of the invention". Formula I is as follows:



(I)

The metabolites of the compound of this invention include oxidized derivatives of Formula I wherein one or more of the urea nitrogens are substituted with a hydroxy group. The metabolites of the compound of this invention also include analogs where the methylamide group of the compound of Formula I is hydroxylated then de-methylated by metabolic degradation. The metabolites of the compound of this invention further include oxidized derivatives where the pyridine nitrogen atom is in the N-oxide form (e.g. carries a hydroxy substituent) leading to those structures referred to in the art as 1-oxo-pyridine and 1-hydroxy-pyridine.

Where the plural form of the word compounds, salts, and the like, is used herein, this is taken to mean also a single compound, salt, or the like.

The use of pharmaceutically acceptable salts of the compounds of Formula I is also within the scope of this invention. The term "pharmaceutically acceptable salt" refers to a relatively non-toxic, inorganic or organic acid addition salt of a compound of the present invention. For example, see S. M. Berge, et al. "Pharmaceutical Salts," *J. Pharm. Sci.* **1977**, *66*, 1-19.

Representative salts of the compound of this invention include the conventional non-toxic salts, for example, from inorganic or organic acids by means well known in the art. For example, such acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cinnamate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, itaconate, lactate, maleate, mandelate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, sulfonate, tartrate, thiocyanate, tosylate, and undecanoate.

The salts or prodrugs of the compounds of Formula I may contain one or more asymmetric centers. Asymmetric carbon atoms may be present in the (R) or (S) configuration or (R,S) configuration. Substituents on a ring may also be present in either cis or trans form. It is intended that all such configurations (including enantiomers and diastereomers), are included within the scope of the present invention. Preferred isomers are those with the configuration which produces the more desirable biological activity. Separated, pure or partially purified isomers or racemic mixtures of the compounds of this invention are also included within the scope of the present invention. The purification of said isomers and the separation of said isomeric mixtures can be accomplished by standard techniques known in the art.

The particular process to be utilized in the preparation of the compound used in this embodiment of the invention is described in Example 1. Salt forms of the compound of Formula (I) are described in Examples 2, 3, and 4.

Methods of use

The present invention provides compounds which are capable of modulating one or more signal transduction pathways involving raf, VEGFR, PDGFR, p38, and/or flt-3 kinases. Raf is an important signaling molecule involved in the regulation of a number of key cellular processes, including cell growth, cell survival and invasion. It is a member of the Ras/raf/MEK/ERK pathway. This pathway is present in most tumor cells. VEGFR, PDGFR, and flt-3 are transmembrane receptor molecules which, when stimulated by an appropriate ligand, trigger the Ras/raf/MEK/ERK cell signaling pathway, leading to a cascade of cellular events. Each of these receptor molecules have tyrosine kinase activity.

The VEGFR receptors are stimulated by vascular endothelial growth factors (VEGF), and are important control points in the regulation of endothelial cell development and function. The PDGF-beta receptor regulates cell proliferation and survival in a number of cell types, including mesenchymal cells. Flt-3 is a receptor for the FL ligand. It is structurally similar to c-kit, and modulates the growth of pluripotent haemopoietic cells, influencing the development of T-cells, B-cells, and dendritic cells.

Any gene or isoform of raf, VEGFR, PDGFR, p38, and/or flt-3 can be modulated in accordance with present invention, including both wild-type and mutant forms. Raf or raf-1 kinase is a family of serine/threonine kinases which comprise at least three family members, a-raf, b-raf, and c-raf or raf-1. See, e.g., Dhillon and Kolch, Arch. *Biochem. Biophys.* **2002**, *404*, 3-9. C-raf and b-raf are preferred targets for compounds of the present invention. Activating b-raf mutations (e.g., V599E mutant) have been identified in various cancers, including melanoma, and the compounds described herein can be utilized to inhibit their activity.

By the term "modulate", it is meant that the functional activity of the pathway (or a component of it) is changed in comparison to its normal activity in the absence of the compound. This effect includes any quality or degree of modulation, including, increasing, agonizing, augmenting, enhancing, facilitating, stimulating, decreasing, blocking, inhibiting, reducing, diminishing, antagonizing, etc.

The compounds of the present invention can also modulate one or more of the following processes, including, but not limited to, e.g., cell growth (including, e.g., differentiation, cell survival, and/or proliferation), tumor cell growth (including, e.g., differentiation, cell survival, and/or proliferation), tumor regression, endothelial cell growth (including, e.g., differentiation, cell survival, and/or proliferation), angiogenesis (blood vessel growth), lymphangiogenesis (lymphatic vessel growth), and/or hematopoiesis (e.g., T- and B-cell development, dendritic cell development, etc.).

While not wishing to be bound by any theory or mechanism of action, it has been found that compounds of the present invention possess the ability to modulate kinase activity. The methods of the present invention, however, are not limited to any particular mechanism or how the compounds achieve their therapeutic effect. By the term "kinase activity", it is meant a catalytic activity in which a gamma-phosphate from adenosine triphosphate (ATP) is transferred to an amino acid residue (e.g., serine, threonine, or tyrosine) in a protein substrate. A compound can modulate kinase activity, e.g., inhibiting it by directly competing with ATP for the ATP-binding pocket of the kinase, by producing a conformational change in the enzyme's structure that affects its activity (e.g., by disrupting the biologically-active three-dimensional structure), etc.

Kinase activity can be determined routinely using conventional assay methods. Kinase assays typically comprise the kinase enzyme, substrates, buffers, and components of a detection system. A typical kinase assay involves the reaction of a protein kinase with a peptide substrate and an ATP, such as ^{32}P-ATP, to produce a phosphorylated end-product (for instance, a phosphoprotein when a peptide substrate is used). The resulting end-product can be detected using any suitable method. When radioactive ATP is utilized, a radioactively labeled phosphoprotein can be separated from the unreacted gamma-^{32}P-ATP using an affinity membrane or gel electrophoresis, and then visualized on the gel using autoradiography or detected with a scintillation counter. Non-radioactive methods can also be used. Methods can utilize an antibody which recognizes the phosphorylated substrate, e.g., an anti-phosphotyrosine antibody. For instance, kinase enzyme can incubated with a substrate in the presence of ATP and kinase buffer under conditions which are effective for the enzyme to phosphorylate the substrate. The reaction mixture can be separated, e.g., electrophoretically, and then phosphorylation of the substrate can be

measured, e.g., by Western blotting using an anti-phosphotyrosine antibody. The antibody can be labeled with a detectable label, e.g., an enzyme, such as HRP, avidin or biotin, chemiluminescent reagents, etc. Other methods can utilize ELISA formats, affinity membrane separation, fluorescence polarization assays, luminescent assays, etc.

An alternative to a radioactive format is time-resolved fluorescence resonance energy transfer (TR-FRET). This method follows the standard kinase reaction, where a substrate, e.g., biotinylated poly(GluTyr), is phosphorylated by a protein kinase in the presence of ATP. The end-product can then detected with a europium chelate phosphospecific antibody (anti-phosphotyrosine or phosphoserine/threonine), and streptavidin-APC, which binds the biotinylated substrate. These two components are brought together spatially upon binding, and energy transfer from the phosphospecific antibody to the acceptor (SA-APC) produces fluorescent readout in the homogeneous format.

The compounds of the present invention can be used to treat and/or prevent any disease or condition mediated by one or more cellular signal transduction pathways involving raf, VEGFR, PDGFR, p38, and/or flt-3 kinases. The term "treating" is used conventionally, e.g., the management or care of a subject for the purpose of combating, alleviating, reducing, relieving, improving the condition of, etc., of a disease or disorder. The compounds can also be described as being used to prevent and/or treat diseases and/or condition mediated by the signaling molecules. The term "mediated" indicates, e.g., that the signaling molecule is part of the pathway which is aberrant or disturbed in the disease and/or condition.

Diseases and conditions that can be treated include any of those mentioned above and below, as well as:

Raf associated diseases include, e.g., cell-proliferation disorders, cancer, tumors, etc.;

VEGFR-2 associated diseases include, e.g., cancer, tumor growth, inflammatory disease, rheumatoid arthritis, retinopathy, psoriasis, glomerulonephritis, asthma, chronic bronchitis, atherosclerosis, transplant rejection, conditions involving angiogenesis, etc.;

VEGFR-3 associated diseases include, e.g., cancer, corneal disease, inflamed cornea (e.g., Hamrah, *Am. J. Path.* **2003**, *163*, 57-68), corneal transplantation

(Cursiefen et al., *Cornea* **2003**, *22*, 273-81), lymphatic hyperplasia, conditions involving lymphangiogenesis, etc.;

PDGFR-beta associated diseases include, e.g., diseases or conditions characterized by cell proliferation, cell matrix production, cell movement, and/or extracellular matrix production. Specific examples, include, e.g., tumors, malignancies, cancer, metastasis, chronic myeloid leukemia, inflammation, renal disease, diabetic nephropathy, mesangial proliferative glomerulonephritis, fibrotic conditions, atherosclerosis; restenosis, hypertension-related arteriosclerosis, venous bypass graft arteriosclerosis, scleroderma, interstitial pulmonary diseases, synovial disorders, arthritis, leukemias, lymphomas, etc;

Flt-3 associated diseases include, e.g., immune-related disorders, blood cell disorders, conditions involving hematopoietic cell development (e.g., T-cells, B-cells, dendritic cells, cancer, anemia, HIV, acquired immune deficiency syndrome, etc.

p38 associated diseases include inflammatory disorders, immunomodulatory disorders, and other disorders that have been linked to abnormal cytokine production, especially TNF-alpha, or abnormal MMP activity. These disorders include, but are not limited to, rheumatoid arthritis, COPD, osteoporosis, Crohn's disease and psoriasis.

In addition, compounds of the present invention can be used to treat conditions and disorders disclosed in U.S. Pat. No. 6,316,479, e.g., glomerular sclerosis, interstitial nephritis, interstitial pulmonary fibrosis, atherosclerosis, wound scarring and scleroderma.

The compounds of this invention also have a broad therapeutic activity to treat or prevent the progression of a broad array of diseases, such as inflammatory conditions, coronary restenosis, tumor-associated angiogenesis, atherosclerosis, autoimmune diseases, inflammation, certain kidney diseases associated with proliferation of glomerular or mesangial cells, and ocular diseases associated with retinal vessel proliferation. psoriasis, hepatic cirrhosis, diabetes, atherosclerosis, restenosis, vascular graft restenosis, in-stent stenosis, angiogenesis, ocurlar diseases, pulmonary fibrosis, obliterative bronchiolitis, glomerular nephritis, rheumatoid arthritis.

The present invention also provides for treating, preventing, modulating, etc., one or more of the following conditions in humans and/or other mammals: retinopathy, including diabetic retinopathy, ischemic retinal-vein occlusion, retinopathy of prematurity and age related macular degeneration; rheumatoid arthritis,

psoriasis, or bullous disorder associated with subepidermal blister formation, including bullous pemphigoid, erythema multiforme, or dermatitis herpetiformis, rheumatic fever, bone resorption, postmenopausal osteoperosis, sepsis, gram negative sepsis, septic shock, endotoxic shock, toxic shock syndrome, systemic inflammatory response syndrome, inflammatory bowel disease (Crohn's disease and ulcerative colitis), Jarisch-Herxheimer reaction, asthma, adult respiratory distress syndrome, acute pulmonary fibrotic disease, pulmonary sarcoidosis, allergic respiratory disease, silicosis, coal worker's pneumoconiosis, alveolar injury, hepatic failure, liver disease during acute inflammation, severe alcoholic hepatitis, malaria (Plasmodium falciparum malaria and cerebral malaria), non-insulin-dependent diabetes mellitus (NIDDM), congestive heart failure, damage following heart disease, atherosclerosis, Alzheimer's disease, acute encephalitis, brain injury, multiple sclerosis (demyelation and oligiodendrocyte loss in multiple sclerosis), advanced cancer, lymphoid malignancy, pancreatitis, impaired wound healing in infection, inflammation and cancer, myelodysplastic syndromes, systemic lupus erythematosus, biliary cirrhosis, bowel necrosis, radiation injury/ toxicity following administration of monoclonal antibodies, host-versus-graft reaction (ischemia reperfusion injury and allograft rejections of kidney, liver, heart, and skin), lung allograft rejection (obliterative bronchitis), or complications due to total hip replacement, ad an infectious disease selected from tuberculosis, Helicobacter pylori infection during peptic ulcer disease, Chaga's disease resulting from Trypanosoma cruzi infection, effects of Shiga-like toxin resulting from E. coli infection, effects of enterotoxin A resulting from Staphylococcus infection, meningococcal infection, and infections from Borrelia burgdorferi, Treponema pallidum, cytomegalovirus, influenza virus, Theiler's encephalomyelitis virus, and the human immunodeficiency virus (HIV), papilloma, blastoglioma, Kaposi's sarcoma, melanoma, lung cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, astrocytoma, head cancer, neck cancer, bladder cancer, breast cancer, colorectal cancer, thyroid cancer, pancreatic cancer, gastric cancer, hepatocellular carcinoma, leukemia, lymphoma, Hodgkin's disease, Burkitt's disease, arthritis, rheumatoid arthritis, diabetic retinopathy, angiogenesis, restenosis, in-stent restenosis, vascular graft restenosis, pulmonary fibrosis, hepatic cirrhosis, atherosclerosis, glomerulonophritis, diabetic nephropathy, thrombic micoangiopathy syndromes, transplant rejection, psoriasis, diabetes, wound healing, inflammation, and neurodegenerative diseases. hyperimmune disorders, hemangioma, myocardial

angiogenesis, coronary and cerebral collateral vascularization, ischemia, corneal disease, rubeosis, neovascular glaucoma, macular degeneration retinopathy of prematurity, wound healing, ulcer Helicobacter related diseases, fractures, endometriosis, a diabetic condition, cat scratch fever, thyroid hyperplasia, asthma or edema following burns, trauma, chronic lung disease, stroke, polyps, cysts, synovitis, chronic and allergic inflammation, ovarian hyperstimulation syndrome, pulmonary and cerebral edema, keloid, fibrosis, cirrhosis, carpal tunnel syndrome, adult respiratory distress syndrome, ascites, an ocular condition, a cardiovascular condition, Crow-Fukase (POEMS) disease, Crohn's disease, glomerulonophritis, osteoarthritis, multiple sclerosis, graft rejection, Lyme disease, sepsis, von Hippel Lindau disease, pemphigoid, Paget's disease, polycystic kidney disease, sarcoidosis, throiditis, hyperviscosity syndrome, Osler-Weber-Rendu disease, chronic occlusive pulmonary disease, radiation, hypoxia, preeclampsia, menometrorrhagia, endometriosis, infection by Herpes simplex, ischemic retinopathy, corneal angiogenesis, Herpes Zoster, human immunodeficiency virus, parapoxvirus, protozoa, toxoplasmosis, and tumor-associated effusions and edema.

The compounds of this invention can possess more than one of the mentioned activities, and therefore can target a plurality of signal transduction pathways. Thus, these compounds can achieve therapeutic and prophylactic effects which normally are only obtained when using a combination of different compounds. For instance, the ability to inhibit both new vessel formation (e.g., associated with VEGFR-2 and VEGFR-3 function) (e.g., blood and/or lymph) and cell-proliferation (e.g., associated with raf and PDGFR-beta function) using a single compound is especially beneficial in the treatment of cancer, and other cell-proliferation disorders that are facilitated by neo-vascularization. Thus, the present invention relates specifically to compounds which possess at least anti-cell proliferation and anti-angiogenic (i.e., inhibits angiogenesis) activity. Any disorder or condition that would benefit from inhibiting vessel growth and cell proliferation can be treated in accordance with the present invention. Using a single compound is also advantageous because its range of activities can be more precisely defined.

As indicated above, the present invention relates to methods of treating and/or preventing diseases and conditions; and/or modulating one or more of the pathways, polypeptides, genes, diseases, conditions, etc., associated with raf, VEGFR, PDGFR, p38, and/or flt-3. These methods generally involve administering effective amounts of

compounds of the present invention, where an effective amount is the quantity of the compound which is useful to achieve the desired result. Compounds can be administered in any effective form by any effective route, as discussed in more detail below.

Methods include modulating tumor cell proliferation, including inhibiting cell proliferation. The latter indicates that the growth and/or differentiation of tumor cells is reduced, decreased, diminished, slowed, etc. The term "proliferation" includes any process which relates to cell growth and division, and includes differentiation and apoptosis. As discussed above, raf kinases play a key role in the activation of the cytoplasmic signaling cascade involved in cell proliferation, differentiation, and apoptosis. For example, studies have found that inhibiting c-raf by anti-sense oligonucleotides can block cell proliferation (see above). Any amount of inhibition is considered therapeutic.

Included in the methods of the present invention is a method for using the compound described above (Compound of Formula I), including salts, prodrugs, metabolites (oxidized derivatives) and compositions thereof, to treat mammalian hyper-proliferative disorders comprising administering to a mammal, including a human in need thereof, an amount of a compound of this invention, pharmaceutically acceptable salt, prodrug, metabolite (oxidized derivative), and composition thereof, which is effective to treat the disorder. Hyper-proliferative disorders include but are not limited to solid tumors, such as cancers of the breast, respiratory tract, brain, reproductive organs, digestive tract, urinary tract, eye, liver, skin, head and neck, thyroid, parathyroid and their distant metastases. Those disorders also include lymphomas, sarcomas, and leukemias.

Any tumor or cancer can be treated, including, but not limited to, cancers having one or more mutations in raf, ras, and/or flt-3, as well as any upstream or downstream member of the signaling pathways of which they are a part. As discussed earlier, a cancer can be treated with a compound of the present invention irrespective of the mechanism which is responsible for it. Cancers of any organ can be treated, including cancers of, but are not limited to, e.g., colon, pancreas, breast, prostate, bone, liver, kidney, lung, testes, skin, pancreas, stomach, colorectal cancer, renal cell carcinoma, hepatocellular carcinoma, melanoma, etc.

Examples of breast cancer include, but are not limited to, invasive ductal carcinoma, invasive lobular carcinoma, ductal carcinoma in situ, and lobular carcinoma in situ.

Examples of cancers of the respiratory tract include, but are not limited to, small-cell and non-small-cell lung carcinoma, as well as bronchial adenoma and pleuropulmonary blastoma.

Examples of brain cancers include, but are not limited to, brain stem and hypophtalmic glioma, cerebellar and cerebral astrocytoma, medulloblastoma, ependymoma, as well as neuroectodermal and pineal tumor.

Tumors of the male reproductive organs include, but are not limited to, prostate and testicular cancer. Tumors of the female reproductive organs include, but are not limited to, endometrial, cervical, ovarian, vaginal, and vulvar cancer, as well as sarcoma of the uterus.

Tumors of the digestive tract include, but are not limited to, anal, colon, colorectal, esophageal, gallbladder, gastric, pancreatic, rectal, small-intestine, and salivary gland cancers.

Tumors of the urinary tract include, but are not limited to, bladder, penile, kidney, renal pelvis, ureter, and urethral cancers.

Eye cancers include, but are not limited to, intraocular melanoma and retinoblastoma.

Examples of liver cancers include, but are not limited to, hepatocellular carcinoma (liver cell carcinomas with or without fibrolamellar variant), cholangiocarcinoma (intrahepatic bile duct carcinoma), and mixed hepatocellular cholangiocarcinoma.

Skin cancers include, but are not limited to, squamous cell carcinoma, Kaposi's sarcoma, malignant melanoma, Merkel cell skin cancer, and non-melanoma skin cancer.

Head-and-neck cancers include, but are not limited to, laryngeal, hypopharyngeal, nasopharyngeal, and/or oropharyngeal cancers, and lip and oral cavity cancer.

Lymphomas include, but are not limited to, AIDS-related lymphoma, non-Hodgkin's lymphoma, cutaneous T-cell lymphoma, Hodgkin's disease, and lymphoma of the central nervous system.

Sarcomas include, but are not limited to, sarcoma of the soft tissue, osteosarcoma, malignant fibrous histiocytoma, lymphosarcoma, and rhabdomyosarcoma.

Leukemias include, but are not limited to, acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic myelogenous leukemia, and hairy cell leukemia.

In addition to inhibiting the proliferation of tumor cells, compounds of the present invention can also cause tumor regression, e.g., a decrease in the size of a tumor, or in the extent of cancer in the body.

The present invention also relates to methods of modulating angiogenesis and/or lymphangiogenesis in a system comprising cells, comprising administering to the system an effective amount of a compound described herein. A system comprising cells can be an *in vivo* system, such as a tumor in a patient, isolated organs, tissues, or cells, *in vitro* assays systems (CAM, BCE, etc), animal models (e.g., in vivo, subcutaneous, cancer models), hosts in need of treatment (e.g., hosts suffering from diseases having angiogenic and/or lymphangiogenic component, such as cancer), etc.

Inappropriate and ectopic expression of angiogenesis can be deleterious to an organism. A number of pathological conditions are associated with the growth of extraneous blood vessels. These include, e.g., diabetic retinopathy, neovascular glaucoma, psoriasis, retrolental fibroplasias, angiofibroma, inflammation, etc. In addition, the increased blood supply associated with cancerous and neoplastic tissue, encourages growth, leading to rapid tumor enlargement and metastasis. Moreover, the growth of new blood and lymph vessels in a tumor provides an escape route for renegade cells, encouraging metastasis and the consequence spread of the cancer.

Useful systems for measuring angiogenesis and/or lymphangiogenesis, and inhibition thereof, include, e.g., neovascularization of tumor explants (e.g., U.S. Pat. Nos. 5,192,744; 6,024,688), chicken chorioallantoic membrane (CAM) assay (e.g., Taylor and Folkman, *Nature* **1982**, *297*, 307-312; Eliceiri et al., *J. Cell Biol.* **1998**, *140*, 1255-1263), bovine capillary endothelial (BCE) cell assay (e.g., U.S. Pat. No. 6,024,688; Polverini, P. J. et al., *Methods Enzymol.* **1991**, *198*, 440-450), migration assays, and HUVEC (human umbilical cord vascular endothelial cell) growth inhibition assay (e.g., U.S. Pat. No. 6,060,449), and use of the rabbit ear model (e.g., Szuba et al., *FASEB J.* **2002**, *16*(14), 1985-7).

Modulation of angiogenesis can be determined by any other method. For example, the degree of tissue vascularity is typically determined by assessing the number and density of vessels present in a given sample. For example, microvessel density (MVD) can be estimated by counting the number of endothelial clusters in a high-power microscopic field, or detecting a marker specific for microvascular endothelium or other markers of growing or established blood vessels, such as CD31 (also known as platelet-endothelial cell adhesion molecule or PECAM). A CD31 antibody can be employed in conventional immunohistological methods to immunostain tissue sections as described by, e.g., U.S. Pat. No. 6,017,949; Dellas et al., *Gyn. Oncol.* **1997**, *67*, 27-33; and others. Other markers for angiogenesis, include, e.g., Vezf1 (e.g., Xiang et al., *Dev. Bio.* **1999**, *206*, 123-141), angiopoietin, Tie-1, and Tie-2 (e.g., Sato et al., *Nature* **1995**, *376*, 70-74).

Additionally, the present invention relates to methods of screening patients to determine their sensitivity to compounds of the present invention. For example, the invention relates to methods of determining whether a condition can be modulated by a compound disclosed herein, comprising measuring the expression or activity of raf, VEGFR-2, VEGFR-3, PDGFR-beta, p38, and/or flt-3 in a sample comprising cells or a cell extract, wherein said sample has been obtained from a cell or subject having said condition. When the results of the determination indicate that one or more of the mentioned genes (and/or polypeptides which they encode) differ from the normal state, this identifies the condition as being treatable with a compound of the present invention, i.e., whereby said disorder or condition can be modulated by the compound when said expression or activity is increased in said condition as compared to a normal control. The method can further comprise a step of comparing the expression in a sample with a normal control, or expression in a sample obtained from normal or unaffected tissue. Comparing can be done manually, against a standard, in an electronic form (e.g., against a database), etc. The normal control can be a standard sample that is provided with the assay; it can be obtained from adjacent, but unaffected, tissue from the same patient; or, it can be pre-determined values, etc. Gene expression, protein expression (e.g., abundance in a cell), protein activity (e.g., kinase activity), etc., can be determined.

For instance, a biopsy from a cancer patient can be assayed for the presence, quantity, and/or activity of raf, VEGFR-2, VEGFR-3, PDGFR-beta, p38, and/or flt-3. Increased expression or activity of one or more of these can indicate that the cancer

can be targeted for treatment by a compound of the present invention. For example, as described in the examples below, raf activity can be monitored by its ability to initiate the cascade leading to ERK phosphorylation (i.e., raf/MEK/ERK), resulting in phospho-ERK. Increased phospho-ERK levels in a cancer specimen shows that its raf activity is elevated, suggesting the use of compounds of the present invention to treat it.

Measuring expression includes determining or detecting the amount of the polypeptide present in a cell or shed by it, as well as measuring the underlying mRNA, where the quantity of mRNA present is considered to reflect the quantity of polypeptide manufactured by the cell. Furthermore, the genes for raf, VEGFR-2, VEGFR-3, PDGFR-beta, p38, and/or Flt-3 can be analyzed to determine whether there is a gene defect responsible for aberrant expression or polypeptide activity.

Polypeptide detection can be carried out by any available method, e.g., by Western blots, ELISA, dot blot, immunoprecipitation, RIA, immunohistochemistry, etc. For instance, a tissue section can be prepared and labeled with a specific antibody (indirect or direct and visualized with a microscope. Amount of a polypeptide can be quantitated without visualization, e.g., by preparing a lysate of a sample of interest, and then determining by ELISA or Western the amount of polypeptide per quantity of tissue. Antibodies and other specific binding agents can be used. There is no limitation on how detection is performed.

Assays can be utilized which permit quantification and/or presence/absence detection of a target nucleic acid (e.g., genes, mRNA, etc., for raf, VEGFR, PDGFR, p38, and/or flt-3) in a sample. Assays can be performed at the single-cell level, or in a sample comprising many cells, where the assay is "averaging" expression over the entire collection of cells and tissue present in the sample. Any suitable assay format can be used, including, but not limited to, e.g., Southern blot analysis, Northern blot analysis, polymerase chain reaction ("PCR") (e.g., Saiki et al., *Science* **1988**, *241*, 53; U.S. Pat. Nos. 4,683,195, 4,683,202, and 6,040,166; PCR Protocols: A Guide to Methods and Applications, Innis et al., eds., Academic Press, New York, 1990), reverse transcriptase polymerase chain reaction ("RT-PCR"), anchored PCR, rapid amplification of cDNA ends ("RACE") (e.g., Schaefer in Gene Cloning and Analysis: Current Innovations, Pages 99-115, 1997), ligase chain reaction ("LCR") (EP 320 308), one-sided PCR (Ohara et al., *Proc. Natl. Acad. Sci.* **1989**, *86*, 5673-5677), indexing methods (e.g., U.S. Pat. No. 5,508,169), in situ hybridization, differential

display (e.g., Liang et al., *Nucl. Acid. Res.* **1993**, 21, 3269 3275; U.S. Pat. Nos. 5,262,311, 5,599,672 and 5,965,409; WO97/18454; Prashar and Weissman, Proc. Natl. Acad. Sci., 93:659-663, and U.S. Pat. Nos. 6,010,850 and 5,712,126; Welsh et al., Nucleic Acid Res., 20:4965-4970, 1992, and U.S. Pat. No. 5,487,985) and other RNA fingerprinting techniques, nucleic acid sequence based amplification ("NASBA") and other transcription based amplification systems (e.g., U.S. Pat. Nos. 5,409,818 and 5,554,527; WO 88/10315), polynucleotide arrays (e.g., U.S. Pat. Nos. 5,143,854, 5,424,186; 5,700,637, 5,874,219, and 6,054,270; PCT WO 92/10092; PCT WO 90/15070), Qbeta Replicase (PCT/US87/00880), Strand Displacement Amplification ("SDA"), Repair Chain Reaction ("RCR"), nuclease protection assays, subtraction-based methods, Rapid-Scan, etc. Additional useful methods include, but are not limited to, e.g., template-based amplification methods, competitive PCR (e.g., U.S. Pat. No. 5,747,251), redox-based assays (e.g., U.S. Pat. No. 5,871,918), Taqman-based assays (e.g., Holland et al., *Proc. Natl. Acad, Sci.* **1991**, *88*, 7276-7280; U.S. Pat. Nos. 5,210,015 and 5,994,063), real-time fluorescence-based monitoring (e.g., U.S. Pat. 5,928,907), molecular energy transfer labels (e.g., U.S. Pat. Nos. 5,348,853, 5,532,129, 5,565,322, 6,030,787, and 6,117,635; Tyagi and Kramer, Nature Biotech., 14:303-309, 1996). Any method suitable for single cell analysis of gene or protein expression can be used, including in situ hybridization, immunocytochemistry, MACS, FACS, flow cytometry, etc. For single cell assays, expression products can be measured using antibodies, PCR, or other types of nucleic acid amplification (e.g., Brady et al., *Methods Mol. & Cell. Biol.* **1990**, *2*, 17-25; Eberwine et al., *Proc. Natl. Acad. Sci.* **1992**, *89*, 3010-3014; U.S. Pat. No. 5,723,290). These and other methods can be carried out conventionally, e.g., as described in the mentioned publications.

Activity of raf, VEGFR-2, VEGFR-3, PDGFR-beta, p38, and/or flt-3 can be assessed routinely, e.g., as described in the examples below, or using standard assays for kinase activity.

The present invention also provides methods of assessing the efficacy of a compound of the present invention in treating a disorder, comprising one or more of the following steps in any effective order, e.g., administering an amount of a compound, measuring the expression or activity of raf, VEGFR-2, VEGFR-3, PDGFR-beta, p38, and/or flt-3 (see above), determining the effect of said compound on said expression or activity. For instance, biopsy samples can be removed from patients who have been treated with a compound of the present invention, and then

assayed for the presence and/or activity of the mentioned signaling molecules. Similarly, as discussed above, decreases in the levels of phospho-ERK in the cancer tissue (e.g., compared to normal tissue or before treatment) indicate that the compound is exerting *in vivo* efficacy and a therapeutic effect. The method can be used to determine appropriate dosages and dosing regimens, e.g., how much compound to administer and at what frequency to administer it. By monitoring its effect on the signaling molecules in the tissue, the clinician can determine the appropriate treatment protocol and whether it is achieving the desired effect, e.g., on modulating or inhibiting the signal transduction pathway.

Compounds of the present invention also can be used as markers to determine the presence and quantity of raf, VEGFR-2, VEGFR-3, PDGFR-beta, p38, and/or flt-3, in a sample comprising a biological material. This comprises one or more of the following steps in any effective order: (i) contacting said sample comprising a biological material with a compound of the present invention, and (ii) determining whether said compound binds to said material. The compound can be labeled, or it can be used as a competitor to a labeled compound, such as labeled-ATP.

The invention also provides methods for treating, preventing, modulating, etc., diseases and conditions in mammals comprising administering a compound of this invention with another modulator of the signal transduction pathway comprising, but not limited to raf, VEGFR, PDGFR, p38, and/or flt-3. These can be present in the same composition or in separate formulations or dosage units. Administration can be the same or different routes, and can be simultaneous or sequential.

The following publications relate to VEGFR-3 modulation and are incorporated herein for their description of disease states mediated by VEGFR-3 and assays to determine such activity.

WO95/33772	Alitalo, et. al.
WO95/33050	Charnock-Jones, et. al..
WO96/39421	Hu, et. al.
WO98/33917	Alitalo, et. al.
WO02/057299	Alitalo, et. al.
WO02/060950	Alitalo, et. al.

WO02/081520	Boesen, et. al.

The following publications relate to VEGFR-2 modulation and are incorporated herein for their description of disease states mediated by VEGFR-2 and assays to determine such activity.

EP0882799	Hanai, et. al.
EP1167384	Ferraram, et, al.
EP1086705	Sato, et. al.
EP11300032	Tesar, et. al.
EP1166798	Haberey, et. al.
EP1166799	Haberey, et. al.
EP1170017	Maini, et. al.
EP1203827	Smith
WO02/083850	Rosen, et. al.

The following publications relate to flt-3 modulation and are incorporated herein for their description of disease states mediated by flt-3 and assays to determine such activity.

2002/0034517	Brasel, et. al.
2002/0107365	Lyman, et. al.
2002/0111475	Graddis, et. al.
EP0627487	Beckermann, et. al.
WO9846750	Bauer, et. al.
WO9818923	McWherter, et. al.
WO9428391	Beckermann, et al.
WO9426891	Birnbaum, et. al.

The following patents and publication relate to PDGF/PDGFR modulation and are incorporated herein for their description of the disease states mediated by PDGFR-beta and assays to determine such activity.

5,094,941	Hart, et. al.

5,371,205	Kelly, et. al.
5,418,135	Pang
5,444,151	Vassbotn, et. al.
5,468,468	LaRochelle, et. al.
5,567,584	Sledziewski, et. al.
5,618,678	Kelly, et. al.
5,620,687	Hart, et. al.
5,648,076	Ross, et. al.
5,668,264	Janjic, et. al.
5,686,572	Wolf, et. al.
5,817,310	Ramakrishnan, et. al.
5,833,986	LaRochelle, et. al.
5,863,739	LaRochelle, et. al.
5,872,218	Wolf, et. al.
5,882,644	Chang, et. al.
5,891,652	Wolf, et. al.
5,976,534	Hart, et. al.
5,990,141	Hirth, et. al.
6,022,854	Shuman
6,043,211	Williams, et. al.
6,110,737	Escobedo, et. al.
6,207,816B1	Gold, et. al.
6,228,600B1	Matsui, et. al.
6,229,002B1	Janjic, et. al.
6,316,603B1	McTigue, et. al.
6,372,438B1	Williams, et. al.
6,403,769B1	La Rochelle, et. al.
6,440,445B1	Nowak, et. al.
6,475,782B1	Escobedo, et. al.
WO02/083849	Rosen, et. al.
WO02/083704	Rosen, et. al.
WO02/081520	Boesen, et. al.
WO02/079498	Thomas, et. al.
WO02/070008	Rockwell, et. al.

WO09959636	Sato, et. al.
WO09946364	Cao, et. al.
WO09940118	Hanai, et. al.
WO9931238	Yabana, et. al.
WO9929861	Klagsbrun, et. al.
WO9858053	Kendall, et. al.
WO9851344	Maini, et. al.
WO9833917	Alitalo, et. al.
WO9831794	Matsumoto, et. al.
WO9816551	Ferrara, et. al.
WO9813071	Kendall, et al.
WO9811223	Martiny-Baron, et. al.
WO9744453	Chen, et. al.
WO9723510	Plouet, et. al.
WO9715662	Stinchcomb, et. al.
WO9708313	Ferrara, et. al.
WO9639515	Cao, et. al.
WO9623065	Smith, et. al.
WO9606641	Fleurbaaij, et. al.
WO9524473	Cao, et. al.
WO9822316	Kyowa
WO9521868	Rockwell, et. al.
WO02/060489	Xia, et. al.

PDGFR-beta

EP0869177	Matsui, et. al.
WO09010013	Matsui, et. al.
WO9737029	Matsui, et. al.

PDGFR-alpha

EP1000617	Lammers, et. al.
EP0869177	Matsui, et. al.

Pharmaceutical compositions based on the compounds of the present invention

This invention also relates to pharmaceutical compositions containing a compound of the present invention and pharmaceutically acceptable salts thereof. These compositions can be utilized to achieve the desired pharmacological effect by administration to a patient in need thereof. A patient, for the purpose of this invention, is a mammal, including a human, in need of treatment for the particular condition or disease. Therefore, the present invention includes pharmaceutical compositions which are comprised of a pharmaceutically acceptable carrier and a pharmaceutically effective amount of a compound, or salt thereof, of the present invention. The term "pharmaceutically acceptable carrier" is meant as any carrier which is relatively non-toxic and innocuous to a patient at concentrations consistent with effective activity of the active ingredient so that any side effects ascribable to the carrier do not vitiate the beneficial effects of the active ingredient. A pharmaceutically effective amount of compound is that amount which produces a result or exerts an influence on the particular condition being treated. The compound of the present invention can be administered with pharmaceutically-acceptable carriers well known in the art using any effective conventional dosage unit forms, including immediate, slow and timed release preparations, orally, parenterally, topically, nasally, ophthalmically, optically, sublingually, rectally, vaginally, and the like.

For oral administration, the compound can be formulated into solid or liquid preparations such as capsules, pills, tablets, troches, lozenges, melts, powders, solutions, suspensions, or emulsions, and may be prepared according to methods known to the art for the manufacture of pharmaceutical compositions. The solid unit dosage forms can be a capsule which can be of the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers such as lactose, sucrose, calcium phosphate, and corn starch.

In another embodiment, the compounds of this invention may be tableted with conventional tablet bases such as lactose, sucrose and cornstarch in combination with binders such as acacia, corn starch or gelatin, disintegrating agents intended to assist the break-up and dissolution of the tablet following administration such as potato

starch, alginic acid, corn starch, and guar gum, gum tragacanth, acacia, lubricants intended to improve the flow of tablet granulation and to prevent the adhesion of tablet material to the surfaces of the tablet dies and punches, for example talc, stearic acid, or magnesium, calcium or zinc stearate, dyes, coloring agents, and flavoring agents such as peppermint, oil of wintergreen, or cherry flavoring, intended to enhance the aesthetic qualities of the tablets and make them more acceptable to the patient. Suitable excipients for use in oral liquid dosage forms include dicalcium phosphate and diluents such as water and alcohols, for example, ethanol, benzyl alcohol, and polyethylene alcohols, either with or without the addition of a pharmaceutically acceptable surfactant, suspending agent or emulsifying agent. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance tablets, pills or capsules may be coated with shellac, sugar or both.

Dispersible powders and granules are suitable for the preparation of an aqueous suspension. They provide the active ingredient in admixture with a dispersing or wetting agent, a suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example those sweetening, flavoring and coloring agents described above, may also be present.

The pharmaceutical compositions of this invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil such as liquid paraffin or a mixture of vegetable oils. Suitable emulsifying agents may be (1) naturally occurring gums such as gum acacia and gum tragacanth, (2) naturally occurring phosphatides such as soy bean and lecithin, (3) esters or partial esters derived form fatty acids and hexitol anhydrides, for example, sorbitan monooleate, (4) condensation products of said partial esters with ethylene oxide, for example, polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavoring agents.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil such as, for example, arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent such as, for example, beeswax, hard paraffin, or cetyl alcohol. The suspensions may also contain one or more preservatives, for example, ethyl or n-propyl p-hydroxybenzoate; one or more coloring agents; one or more flavoring agents; and one

or more sweetening agents such as sucrose or saccharin.

Syrups and elixirs may be formulated with sweetening agents such as, for example, glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, and preservative, such as methyl and propyl parabens and flavoring and coloring agents.

The compounds of this invention may also be administered parenterally, that is, subcutaneously, intravenously, intraocularly, intrasynovially, intramuscularly, or interperitoneally, as injectable dosages of the compound in a physiologically acceptable diluent with a pharmaceutical carrier which can be a sterile liquid or mixture of liquids such as water, saline, aqueous dextrose and related sugar solutions, an alcohol such as ethanol, isopropanol, or hexadecyl alcohol, glycols such as propylene glycol or polyethylene glycol, glycerol ketals such as 2,2-dimethyl-1,1-dioxolane-4-methanol, ethers such as poly(ethylene glycol) 400, an oil, a fatty acid, a fatty acid ester or, a fatty acid glyceride, or an acetylated fatty acid glyceride, with or without the addition of a pharmaceutically acceptable surfactant such as a soap or a detergent, suspending agent such as pectin, carbomers, methycellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agent and other pharmaceutical adjuvants.

Illustrative of oils which can be used in the parenteral formulations of this invention are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, sesame oil, cottonseed oil, corn oil, olive oil, petrolatum and mineral oil. Suitable fatty acids include oleic acid, stearic acid, isostearic acid and myristic acid. Suitable fatty acid esters are, for example, ethyl oleate and isopropyl myristate. Suitable soaps include fatty acid alkali metal, ammonium, and triethanolamine salts and suitable detergents include cationic detergents, for example dimethyl dialkyl ammonium halides, alkyl pyridinium halides, and alkylamine acetates; anionic detergents, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates; non-ionic detergents, for example, fatty amine oxides, fatty acid alkanolamides, and poly(oxyethylene-oxypropylene)s or ethylene oxide or propylene oxide copolymers; and amphoteric detergents, for example, alkyl-beta-aminopropionates, and 2-alkylimidazoline quarternary ammonium salts, as well as mixtures.

The parenteral compositions of this invention will typically contain from about 0.5% to about 25% by weight of the active ingredient in solution. Preservatives and

buffers may also be used advantageously. In order to minimize or eliminate irritation at the site of injection, such compositions may contain a non-ionic surfactant having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulation ranges from about 5% to about 15% by weight. The surfactant can be a single component having the above HLB or can be a mixture of two or more components having the desired HLB.

Illustrative of surfactants used in parenteral formulations are the class of polyethylene sorbitan fatty acid esters, for example, sorbitan monooleate and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol.

The pharmaceutical compositions may be in the form of sterile injectable aqueous suspensions. Such suspensions may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents such as, for example, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, sodium alginate, gum tragacanth and gum acacia; dispersing or wetting agents which may be a naturally occurring phosphatide such as lecithin, a condensation product of an alkylene oxide with a fatty acid, for example, polyoxyethylene stearate, a condensation product of ethylene oxide with a long chain aliphatic alcohol, for example, heptadeca-ethyleneoxycetanol, a condensation product of ethylene oxide with a partial ester derived form a fatty acid and a hexitol such as polyoxyethylene sorbitol monooleate, or a condensation product of an ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride, for example polyoxyethylene sorbitan monooleate.

The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent. Diluents and solvents that may be employed are, for example, water, Ringer's solution, isotonic sodium chloride solutions and isotonic glucose solutions. In addition, sterile fixed oils are conventionally employed as solvents or suspending media. For this purpose, any bland, fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid can be used in the preparation of injectables.

A composition of the invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritation excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in

the rectum to release the drug. Such material is, for example, cocoa butter and polyethylene glycol.

Another formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art (see, e.g., US Patent No. 5,023,252, issued June 11, 1991, incorporated herein by reference). Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

Controlled release formulations for parenteral administration include liposomal, polymeric microsphere and polymeric gel formulations which are known in the art.

It may be desirable or necessary to introduce the pharmaceutical composition to the patient via a mechanical delivery device. The construction and use of mechanical delivery devices for the delivery of pharmaceutical agents is well known in the art. Direct techniques for, for example, administering a drug directly to the brain usually involve placement of a drug delivery catheter into the patient's ventricular system to bypass the blood-brain barrier. One such implantable delivery system, used for the transport of agents to specific anatomical regions of the body, is described in US Patent No. 5,011,472, issued April 30, 1991.

The compositions of the invention can also contain other conventional pharmaceutically acceptable compounding ingredients, generally referred to as carriers or diluents, as necessary or desired. Conventional procedures for preparing such compositions in appropriate dosage forms can be utilized. Such ingredients and procedures include those described in the following references, each of which is incorporated herein by reference: Powell, M.F. et al, "Compendium of Excipients for Parenteral Formulations" *PDA Journal of Pharmaceutical Science & Technology* **1998**, *52*(5), 238-311; Strickley, R.G "Parenteral Formulations of Small Molecule Therapeutics Marketed in the United States (1999)-Part-1" *PDA Journal of Pharmaceutical Science & Technology* **1999**, *53*(6), 324-349; and Nema, S. et al, "Excipients and Their Use in Injectable Products" *PDA Journal of Pharmaceutical Science & Technology* **1997**, *51*(4), 166-171.

Commonly used pharmaceutical ingredients which can be used as appropriate to formulate the composition for its intended route of administration include:

- acidifying agents (examples include but are not limited to acetic acid, citric acid, fumaric acid, hydrochloric acid, nitric acid);
- alkalinizing agents (examples include but are not limited to ammonia solution, ammonium carbonate, diethanolamine, monoethanolamine, potassium hydroxide, sodium borate, sodium carbonate, sodium hydroxide, triethanolamine, trolamine);
- adsorbents (examples include but are not limited to powdered cellulose and activated charcoal);
- aerosol propellants (examples include but are not limited to carbon dioxide, CCl_2F_2, $F_2ClC\text{-}CClF_2$ and $CClF_3$)
- air displacement agents (examples include but are not limited to nitrogen and argon);
- antifungal preservatives (examples include but are not limited to benzoic acid, butylparaben, ethylparaben, methylparaben, propylparaben, sodium benzoate);
- antimicrobial preservatives (examples include but are not limited to benzalkonium chloride, benzethonium chloride, benzyl alcohol, cetylpyridinium chloride, chlorobutanol, phenol, phenylethyl alcohol, phenylmercuric nitrate and thimerosal);
- antioxidants (examples include but are not limited to ascorbic acid, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorus acid, monothioglycerol, propyl gallate, sodium ascorbate, sodium bisulfite, sodium formaldehyde sulfoxylate, sodium metabisulfite);
- binding materials (examples include but are not limited to block polymers, natural and synthetic rubber, polyacrylates, polyurethanes, silicones, polysiloxanes and styrene-butadiene copolymers);
- buffering agents (examples include but are not limited to potassium metaphosphate, dipotassium phosphate, sodium acetate, sodium citrate anhydrous and sodium citrate dihydrate)
- carrying agents (examples include but are not limited to acacia syrup, aromatic syrup, aromatic elixir, cherry syrup, cocoa syrup, orange syrup, syrup, corn oil, mineral oil, peanut oil, sesame oil, bacteriostatic sodium chloride injection and bacteriostatic water for injection)

- chelating agents (examples include but are not limited to edetate disodium and edetic acid)
- colorants (examples include but are not limited to FD&C Red No. 3, FD&C Red No. 20, FD&C Yellow No. 6, FD&C Blue No. 2, D&C Green No. 5, D&C Orange No. 5, D&C Red No. 8, caramel and ferric oxide red);
- clarifying agents (examples include but are not limited to bentonite);
- emulsifying agents (examples include but are not limited to acacia, cetomacrogol, cetyl alcohol, glyceryl monostearate, lecithin, sorbitan monooleate, polyoxyethylene 50 monostearate);
- encapsulating agents (examples include but are not limited to gelatin and cellulose acetate phthalate)
- flavorants (examples include but are not limited to anise oil, cinnamon oil, cocoa, menthol, orange oil, peppermint oil and vanillin);
- humectants (examples include but are not limited to glycerol, propylene glycol and sorbitol);
- levigating agents (examples include but are not limited to mineral oil and glycerin);
- oils (examples include but are not limited to arachis oil, mineral oil, olive oil, peanut oil, sesame oil and vegetable oil);
- ointment bases (examples include but are not limited to lanolin, hydrophilic ointment, polyethylene glycol ointment, petrolatum, hydrophilic petrolatum, white ointment, yellow ointment, and rose water ointment);
- penetration enhancers (transdermal delivery) (examples include but are not limited to monohydroxy or polyhydroxy alcohols, mono-or polyvalent alcohols, saturated or unsaturated fatty alcohols, saturated or unsaturated fatty esters, saturated or unsaturated dicarboxylic acids, essential oils, phosphatidyl derivatives, cephalin, terpenes, amides, ethers, ketones and ureas)
- plasticizers (examples include but are not limited to diethyl phthalate and glycerol);
- solvents (examples include but are not limited to ethanol, corn oil, cottonseed oil, glycerol, isopropanol, mineral oil, oleic acid, peanut oil, purified water, water for injection, sterile water for injection and sterile water for irrigation);

- stiffening agents (examples include but are not limited to cetyl alcohol, cetyl esters wax, microcrystalline wax, paraffin, stearyl alcohol, white wax and yellow wax);
- suppository bases (examples include but are not limited to cocoa butter and polyethylene glycols (mixtures));
- surfactants (examples include but are not limited to benzalkonium chloride, nonoxynol 10, oxtoxynol 9, polysorbate 80, sodium lauryl sulfate and sorbitan mono-palmitate);
- suspending agents (examples include but are not limited to agar, bentonite, carbomers, carboxymethylcellulose sodium, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, kaolin, methylcellulose, tragacanth and veegum);
- sweetening agents (examples include but are not limited to aspartame, dextrose, glycerol, mannitol, propylene glycol, saccharin sodium, sorbitol and sucrose);
- tablet anti-adherents (examples include but are not limited to magnesium stearate and talc);
- tablet binders (examples include but are not limited to acacia, alginic acid, carboxymethylcellulose sodium, compressible sugar, ethylcellulose, gelatin, liquid glucose, methylcellulose, and pregelatinized starch);
- tablet and capsule diluents (examples include but are not limited to dibasic calcium phosphate, kaolin, lactose, mannitol, microcrystalline cellulose, powdered cellulose, precipitated calcium carbonate, sodium carbonate, sodium phosphate, sorbitol and starch);
- tablet coating agents (examples include but are not limited to liquid glucose, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose, ethylcellulose, cellulose acetate phthalate and shellac);
- tablet direct compression excipients (examples include but are not limited to dibasic calcium phosphate);
- tablet disintegrants (examples include but are not limited to alginic acid, carboxymethylcellulose calcium, microcrystalline cellulose, polacrillin potassium, sodium alginate, sodium starch glycollate and starch);
- tablet glidants (examples include but are not limited to colloidal silica, corn starch and talc);

- tablet lubricants (examples include but are not limited to calcium stearate, magnesium stearate, mineral oil, stearic acid and zinc stearate);
- tablet/capsule opaquants (examples include but are not limited to titanium dioxide);
- tablet polishing agents (examples include but are not limited to carnauba wax and white wax);
- thickening agents (examples include but are not limited to beeswax, cetyl alcohol and paraffin);
- tonicity agents (examples include but are not limited to dextrose and sodium chloride);
- viscosity increasing agents (examples include but are not limited to alginic acid, bentonite, carbomers, carboxymethylcellulose sodium, methylcellulose, sodium alginate and tragacanth); and
- wetting agents (examples include but are not limited to heptadecaethylene oxycetanol, lecithin, sorbitol monooleate, polyoxyethylene sorbitol monooleate, and polyoxyethylene stearate).

Pharmaceutical compositions according to the present invention can be illustrated as follows:

Sterile IV Solution: a 5 mg/mL solution of the desired compound of this invention is made using sterile, injectable water, and the pH is adjusted if necessary. The solution is diluted for administration to 1 – 2 mg/mL with sterile 5% dextrose and is administered as an IV infusion over 60 minutes.

Lyophilized powder for IV administration: A sterile preparation can be prepared with (i) 100 - 1000 mg of the desired compound of this invention as a lypholized powder, (ii) 32- 327 mg/mL sodium citrate, and (iii) 300 – 3000 mg Dextran 40. The formulation is reconstituted with sterile, injectable saline or dextrose 5% to a concentration of 10 to 20 mg/mL, which is further diluted with saline or dextrose 5% to 0.2 – 0.4 mg/mL, and is administered either IV bolus or by IV infusion over 15 – 60 minutes.

Intramuscular suspension: The following solution or suspension can be prepared, for intramuscular injection:

50 mg/mL of the desired, water-insoluble compound of this invention
5 mg/mL sodium carboxymethylcellulose
4 mg/mL Tween 80
9 mg/mL sodium chloride
9 mg/mL benzyl alcohol

Hard Shell Capsules: A large number of unit capsules are prepared by filling standard two-piece hard galantine capsules each with 100 mg of powdered active ingredient, 150 mg of lactose, 50 mg of cellulose and 6 mg of magnesium stearate.

Soft Gelatin Capsules: A mixture of active ingredient in a digestible oil such as soybean oil, cottonseed oil or olive oil is prepared and injected by means of a positive displacement pump into molten gelatin to form soft gelatin capsules containing 100 mg of the active ingredient. The capsules are washed and dried. The active ingredient can be dissolved in a mixture of polyethylene glycol, glycerin and sorbitol to prepare a water miscible medicine mix.

Tablets: A large number of tablets are prepared by conventional procedures so that the dosage unit was 100 mg of active ingredient, 0.2 mg of colloidal silicon dioxide, 5 mg of magnesium stearate, 275 mg of microcrystalline cellulose, 11 mg of starch, and 98.8 mg of lactose. Appropriate aqueous and non-aqueous coatings may be applied to increase palatability, improve elegance and stability or delay absorption.

Immediate Release Tablets/Capsules: These are solid oral dosage forms made by conventional and novel processes. These units are taken orally without water for immediate dissolution and delivery of the medication. The active ingredient is mixed in a liquid containing ingredient such as sugar, gelatin, pectin and sweeteners. These liquids are solidified into solid tablets or caplets by freeze drying and solid state extraction techniques. The drug compounds may be compressed with viscoelastic and thermoelastic sugars and polymers or effervescent components to produce porous matrices intended for immediate release, without the need of water.

Dosage of the pharmaceutical compositions of the present invention

Based upon standard laboratory techniques known to evaluate compounds useful for the treatment of any of the aforementioned disorders, by standard toxicity tests and by standard pharmacological assays for the determination of treatment of the conditions identified above in mammals, and by comparison of these results with the results of known medicaments that are used to treat these conditions, the effective dosage of the compounds of this invention can readily be determined for treatment of each desired indication. The amount of the active ingredient to be administered in the treatment of one of these conditions can vary widely according to such considerations as the particular compound and dosage unit employed, the mode of administration, the period of treatment, the age and sex of the patient treated, and the nature and extent of the condition treated.

The total amount of the active ingredient to be administered can range from about 0.001 mg/kg to about 200 mg/kg, and preferably from about 0.1 mg/kg to about 50 mg/kg body weight per day. A unit dosage may preferably contain from about 5 mg to about 4000 mg of active ingredient, and can be administered one or more times per day. The daily dosage for oral administration will preferably be from 0.1 to 50 mg/kg of total body weight. The daily dosage for administration by injection, including intravenous, intramuscular, subcutaneous and parenteral injections, and use of infusion techniques will preferably be from 0.1 to 10 mg/kg of total body weight. The daily rectal dosage regimen will preferably be from 0.1 to 50 mg/kg of total body weight. The daily vaginal dosage regimen will preferably be from 0.1 to 50 mg/kg of total body weight. The daily topical dosage regimen will preferably be from 0.1 to 10 mg/kg administered between one to four times daily. The transdermal concentration will preferably be that required to maintain a daily dose of from 0.1 to 10 mg/kg. The daily inhalation dosage regimen will preferably be from 0.1 to 10 mg/kg of total body weight. Other dosages and amounts can be selected routinely.

The specific initial and continuing dosage regimen for each patient will vary according to the nature and severity of the condition as determined by the attending diagnostician, the activity of the specific compound employed, the age and general condition of the patient, time of administration, route of administration, rate of excretion of the drug, drug combinations, and the like. The desired mode of treatment and number of doses of a compound of the present invention or a pharmaceutically

acceptable salt or ester or composition thereof can be ascertained by those skilled in the art using conventional treatment tests.

Combination of the compounds and compositions of the present invention with additional active ingredients

Compounds of this invention can be administered as the sole pharmaceutical agent or in combination with one or more other pharmaceutical agents where the combination causes no unacceptable adverse effects. This may be of particular relevance for the treatment of hyper-proliferative diseases such as cancer. In this instance, the compound of this invention can be combined with known cytotoxic agents, signal transduction inhibitors, or with other anti-cancer agents, as well as with admixtures and combinations thereof.

In one embodiment, the compounds of the present invention can be combined with cytotoxic anti-cancer agents. Examples of such agents can be found in the 11th Edition of the *Merck Index* (1996). These agents include, by no way of limitation, asparaginase, bleomycin, carboplatin, carmustine, chlorambucil, cisplatin, colaspase, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin, doxorubicin (adriamycine), epirubicin, etoposide, 5-fluorouracil, hexamethylmelamine, hydroxyurea, ifosfamide, irinotecan, leucovorin, lomustine, mechlorethamine, 6-mercaptopurine, mesna, methotrexate, mitomycin C, mitoxantrone, prednisolone, prednisone, procarbazine, raloxifen, streptozocin, tamoxifen, thioguanine, topotecan, vinblastine, vincristine, and vindesine.

Other cytotoxic drugs suitable for use with the compounds of the invention include, but are not limited to, those compounds acknowledged to be used in the treatment of neoplastic diseases in *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (Ninth Edition, 1996, McGraw-Hill). These agents include, by no way of limitation, aminoglutethimide, L-asparaginase, azathioprine, 5-azacytidine cladribine, busulfan, diethylstilbestrol, 2', 2'-difluorodeoxycytidine, docetaxel, erythrohydroxynonyladenine, ethinyl estradiol, 5-fluorodeoxyuridine, 5-fluorodeoxyuridine monophosphate, fludarabine phosphate, fluoxymesterone, flutamide, hydroxyprogesterone caproate, idarubicin, interferon, medroxyprogesterone acetate, megestrol acetate, melphalan, mitotane, paclitaxel, pentostatin, N-phosphonoacetyl-L-aspartate (PALA), plicamycin, semustine,

teniposide, testosterone propionate, thiotepa, trimethylmelamine, uridine, and vinorelbine.

Other cytotoxic anti-cancer agents suitable for use in combination with the compounds of the invention also include newly discovered cytotoxic principles such as oxaliplatin, gemcitabine, capecitabine, epothilone and its natural or synthetic derivatives, temozolomide (Quinn et al., *J. Clin. Oncology* **2003**, *21*(4), 646-651), tositumomab (Bexxar), trabedectin (Vidal et al., *Proceedings of the American Society for Clinical Oncology* **2004**, *23*, abstract 3181), and the inhibitors of the kinesin spindle protein Eg5 (Wood et al., *Curr. Opin. Pharmacol.* **2001**, *1*, 370-377).

In another embodiment, the compounds of the present invention can be combined with other signal transduction inhibitors. Of particular interest are signal transduction inhibitors which target the EGFR family, such as EGFR, HER-2, and HER-4 (Raymond et al., *Drugs* **2000**, *60* (Suppl.1), 15-23; Harari et al., *Oncogene* **2000**, *19* (53), 6102-6114), and their respective ligands. Examples of such agents include, by no way of limitation, antibody therapies such as Herceptin (trastuzumab), Erbitux (cetuximab), and pertuzumab. Examples of such therapies also include, by no way of limitation, small-molecule kinase inhibitors such as ZD-1839 / Iressa (Baselga et al., *Drugs* **2000**, *60* (Suppl. 1), 33-40), OSI-774 / Tarceva (Pollack et al. *J. Pharm. Exp. Ther.* **1999**, *291*(2), 739-748), CI-1033 (Bridges, *Curr. Med. Chem.* **1999**, *6*, 825-843), GW-2016 (Lackey et al., *92nd AACR Meeting, New Orleans, March 24-28, 2001, abstract 4582*), CP-724,714 (Jani et al., *Proceedings of the American Society for Clinical Oncology* **2004**, 23, abstract 3122), HKI-272 (Rabindran et al., *Cancer Res.* **2004**, *64*, 3958-3965), and EKB-569 (Greenberger et al., *11th NCI-EORTC-AACR Symposium on New Drugs in Cancer Therapy, Amsterdam, November 7-10, 2000, abstract 388).*

In another embodiment, the compounds of the present invention can be combined with other signal transduction inhibitors targeting receptor kinases of the split-kinase domain families (VEGFR, FGFR, PDGFR, flt-3, c-kit, c-fms, and the like), and their respective ligands. These agents include, by no way of limitation, antibodies such as Avastin (bevacizumab). These agents also include, by no way of limitation, small-molecule inhibitors such as STI-571 / Gleevec (Zvelebil, *Curr. Opin. Oncol., Endocr. Metab. Invest. Drugs* **2000**, *2*(1), 74-82), PTK-787 (Wood et al., *Cancer Res.* **2000**, *60*(8), 2178-2189), SU-11248 (Demetri et al., *Proceedings of*

the American Society for Clinical Oncology **2004**, *23*, abstract 3001), ZD-6474 (Hennequin et al., *92nd AACR Meeting, New Orleans, March 24-28, 2001, abstract 3152*), AG-13736 (Herbst et al., *Clin. Cancer Res.* **2003**, *9*, 16 (suppl 1), abstract C253), KRN-951 (Taguchi et al., *95th AACR Meeting, Orlando, FL, 2004, abstract 2575*), CP-547,632 (Beebe et al., *Cancer Res.* **2003**, *63*, 7301-7309), CP-673,451 (Roberts et al., *Proceedings of the American Association of Cancer Research* **2004**, *45*, abstract 3989), CHIR-258 (Lee et al., *Proceedings of the American Association of Cancer Research* **2004**, *45*, abstract 2130), MLN-518 (Shen et al., *Blood* **2003**, *102*, 11, abstract 476), and AZD-2171 (Hennequin et al., *Proceedings of the American Association of Cancer Research* **2004**, *45*, abstract 4539).

In another embodiment, the compounds of the present invention can be combined with inhibitors of the Raf/MEK/ERK transduction pathway (Avruch et al., *Recent Prog. Horm. Res.* **2001**, *56*, 127-155), or the PKB (akt) pathway (Lawlor et al., *J. Cell Sci.* **2001**, *114*, 2903-2910). These include, by no way of limitation, PD-325901 (Sebolt-Leopold et al., *Proceedings of the American Association of Cancer Research* **2004**, *45*, abstract 4003), and ARRY-142886 (Wallace et al., *Proceedings of the American Association of Cancer Research* **2004**, *45*, abstract 3891).

In another embodiment, the compounds of the present invention can be combined with inhibitors of histone deacetylase. Examples of such agents include, by no way of limitation, suberoylanilide hydroxamic acid (SAHA), LAQ-824 (Ottmann et al., *Proceedings of the American Society for Clinical Oncology* **2004**, *23*, abstract 3024), LBH-589 (Beck et al., *Proceedings of the American Society for Clinical Oncology* **2004**, *23*, abstract 3025), MS-275 (Ryan et al., *Proceedings of the American Association of Cancer Research* **2004**, *45*, abstract 2452), and FR-901228 (Piekarz et al., *Proceedings of the American Society for Clinical Oncology* **2004**, *23*, abstract 3028).

In another embodiment, the compounds of the present invention can be combined with other anti-cancer agents such as proteasome inhibitors, and m-TOR inhibitors. These include, by no way of limitation, bortezomib (Mackay et al., *Proceedings of the American Society for Clinical Oncology* **2004**, *23*, Abstract 3109), and CCI-779 (Wu et al., *Proceedings of the American Association of Cancer Research* **2004**, *45*, abstract 3849).

Generally, the use of cytotoxic and/or cytostatic anti-cancer agent in combination with a compound or composition of the present invention for the treatment of cancer will serve to:

(1) yield better efficacy in reducing the growth of a tumor or even eliminate the tumor as compared to administration of either agent alone,

(2) provide for the administration of lesser amounts of the administered chemotherapeutic agents,

(3) provide for a chemotherapeutic treatment that is well tolerated in the patient with fewer deleterious pharmacological complications than observed with single agent chemotherapies and certain other combined therapies,

(4) provide for treating a broader spectrum of different cancer types in mammals, especially humans,

(5) provide for a higher response rate among treated patients,

(6) provide for a longer survival time among treated patients compared to standard chemotherapy treatments,

(7) provide a longer time for tumor progression, and/or

(8) yield efficacy and tolerability results at least as good as those of the agents used alone, compared to known instances where other cancer agent combinations produce antagonistic effects.

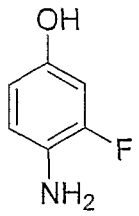
Examples

Abbreviations used in this specification are as follows:

HPLC	high pressure liquid chromatography
MS	mass spectrometry
ES	electrospray
DMSO	dimethylsulfoxide
MP	melting point
NMR	nuclear resonance spectroscopy

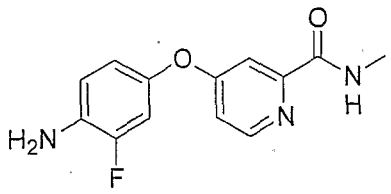
TLC	thin layer chromatography
rt	room temperature

Preparation of 4-amino-3-fluorophenol



To a dry flask purged with Argon was added 10% Pd/C (80 mg) followed by 3-fluoro-4-nitrophenol (1.2 g, 7.64 mmol) as a solution in ethyl acetate (40 mL). The mixture was stirred under an H_2 atmosphere for 4 h. The mixture was filtered through a pad of Celite and the solvent was evaporated under reduced pressure to afford the desired product as a tan solid (940 mg, 7.39 mmol; 97 % yield); ^{1}H-NMR (DMSO-d_6) 4.38 (s, 2H), 6.29-6.35 (m, 1H), 6.41 (dd, J=2.5, 12.7, 1H), 6.52-6.62 (m, 1H), 8.76 (s, 1H).

Preparation of 4-(4-amino-3-fluorophenoxy)pyridine-2-carboxylic acid methylamide

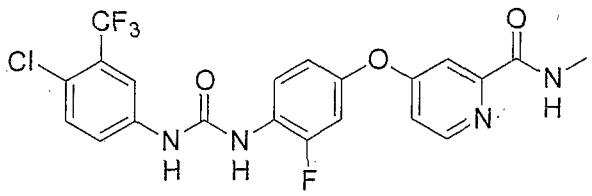


A solution of 4-amino-3-fluorophenol (500 mg, 3.9 mmol) in N,N-dimethylacetamide (6 mL) cooled to 0 °C was treated with potassium tert-butoxide (441 mg, 3.9 mmol), and the brown solution was allowed to stir at 0 °C for 25 min. To the mixture was added 4-chloro-N-methyl-2-pyridinecarboxamide (516 mg, 3.0 mmol) as a solution in dimethylacetamide (4 mL). The reaction was heated at 100 °C for 16 h. The mixture was cooled to room temperature, quenched with H_2O (20 mL), and extracted with ehtylacetate (4 x 40 mL). The combined organics were washed with H_2O (2 x 30 mL),

dried ($MgSO_4$), and evaporated to afford a red-brown oil. ^{1}H-NMR indicated the presence of residual dimethylacetamide, thus the oil was taken up in diethylether (50 mL) and was further washed with brine (5 x 30 mL). The organic layer was dried ($MgSO_4$) and concentrated to give 950 mg of the desired product as a red-brown solid, which was used in the next step without purification.

A method of preparing 4-chloro-N-methyl-2-pyridinecarboxamide is described in Bankston et al., *Org. Proc. Res. Dev.* **2002**, *6*(6), 777-781.

Example 1: Preparation of 4{4-[3-(4-chloro-3-trifluoromethylphenyl)-ureido]-3-fluorophenoxy}-pyridine-2-carboxylic acid methylamide



To a solution of 4-(4-amino-3-fluorophenoxy)pyridine-2-carboxylic acid methylamide (177 mg, 0.68 mmol) in toluene (3 mL) was added 4-chloro-3-(trifluoromethyl)phenyl isocyanate (150 mg, 0.68 mmol). The mixture was stirred at rt for 72 h. The reaction was concentrated under reduced pressure and the residue was triturated with diethylether. The resulting solid was collected by filtration and dried in vacuo for 4 h to afford the title compound (155 mg, 0.32 mmol; 47% yield); ^{1}H-NMR (DMSO-d_6) 2.78 (d, J=4.9, 3H), 7.03-7.08 (m, 1H), 7.16 (dd, J=2.6, 5.6, 1H), 7.32 (dd, J=2.7, 11.6, 1H), 7.39 (d, J=2.5, 1H), 7.60 (s, 2H), 8.07–8.18 (m, 2H), 8.50 (d, J=5.7, 1H), 8.72 (s, 1H), 8.74-8.80 (m, 1H), 9.50 (s, 1H); MS (HPLC/ES) 483.06 m/z = (M + 1).

Example 2: Preparation of 4{4-[3-(4-chloro-3-trifluoromethylphenyl)-ureido]-3-fluorophenoxy}-pyridine-2-carboxylic acid methylamide hydrochloride

The compound of example 1 as a free base (2.0 g) was dissolved in anhydrous tetrahydrofuran (15 mL) and a 4M HCl/dioxane was added (excess). The solution was then concentrated in vacuo to afford 2.32 grams of off-white solids. The crude salt was dissolved in hot ethanol (125 mL), activated carbon was added and the mixture heated at reflux for 15 minutes. The hot suspension was filtered through a pad of Celite 521 and allowed to cool to room temperature. The flask was placed in a freezer overnight. The crystalline solids were collected by suction filtration, washed with ethanol, then hexane and air-dried. The mother liquors were concentrated down and crystallization (in freezer) allowed taking place overnight. A second crop of solids was collected and combined with the first crop. The colorless salt was dried in a vacuum oven at 60 °C over two days. Yield of hydrochloride salt obtained 1.72 g (79%).

Melting point: 215 °C

Elemental analysis:

	Calcd.	Found
C	48.57	48.68
H	3.11	2.76
N	10.79	10.60
Cl	13.65	13.63
F	14.63	14.88

Example 3: Preparation of 4{4-[3-(4-chloro-3-trifluoromethylphenyl)-ureido]-3-fluorophenoxy}-pyridine-2-carboxylic acid methylamide mesylate

The compound of example 1 as a free base (2.25 g) was dissolved in ethanol (100 mL) and a stock solution of methanesulfonic acid (excess) was added. The solution was then concentrated in vacuo to afford a yellow oil. Ethanol was added and concentration repeated, affording 2.41 g of off-white solids. The crude salt was dissolved in hot ethanol (~125 mL) and then cooled slowly to crystallize. After reaching room temperature, the flask was placed in a freezer overnight. The colorless crystalline material was collected by suction filtration; the filter cake was washed with ethanol, then hexane and air-dried, to afford 2.05 g of material, which was dried in a vacuum oven at 60 °C overnight.

Melting point: 231 °C

Elemental analysis:

	Calcd.	Found
C	45.64	45.34
H	3.31	3.08
N	9.68	9.44
Cl	6.12	6.08
F	13.13	13.42
S	5.54	5.59

Example 4: Preparation of 4{4-[3-(4-chloro-3-trifluoromethylphenyl)-ureido]-3-fluorophenoxy}-pyridine-2-carboxylic acid methylamide phenylsulfonate

The compound of example 1 as a free base (2.25 g) was suspended in ethanol (50 mL) and benzensulfonic acid (0.737 g) in ethanol (50 mL) was added. The mixture was heated with vigorous stirring. All solid material dissolved to give a reddish solution. The solution was allowed to cool to room temperature and the flask scratched. Crystal formation was difficult to achieve, some seeds were found, added to solution and placed in freezer overnight. Grayish-tan solids had formed in the flask; the material was broken up & collected by suction filtration. The solids were washed with ethanol, then hexane and air-dried. Weighed product: 2.05 g, 69% yield.

Melting point: 213 °C

Elemental Analysis:

	Calcd.	Found
C	50.59	50.24
H	3.30	3.50
N	8.74	8.54
F	11.86	11.79
Cl	5.53	5.63
S	5.00	5.16

Example 5: c-raf (raf-1) Biochemical Assay

The c-raf biochemical assay was performed with a c-raf enzyme that was activated (phosphorylated) by Lck kinase. Lck-activated c-raf (Lck/c-raf) was

produced in Sf9 insect cells by co-infecting cells with baculoviruses expressing, under the control of the polyhedrin promoter, GST-c-raf (from amino acid 302 to amino acid 648) and Lck (full-length). Both baculoviruses were used at the multiplicity of infection of 2.5 and the cells were harvested 48 h post infection.

MEK-1 protein was produced in Sf9 insect cells by infecting cells with the baculovirus expressing GST-MEK-1 (full-length) fusion protein at the multiplicity of infection of 5 and harvesting the cells 48 hours post infection. Similar purification procedure was used for GST-c-raf 302-648 and GST-MEK-1.

Transfected cells were suspended at 100 mg of wet cell biomass per mL in a buffer containing 10 mM sodium phosphate, 140 mM sodium chloride pH 7.3, 0.5% Triton X-100 and the protease inhibitor cocktail. The cells were disrupted with Polytron homogenizer and centrifuged 30,000g for 30 minutes. The 30,000g supernatant was applied onto GSH-Sepharose. The resin was washed with a buffer containing 50 mM Tris, pH 8.0, 150 mM NaCl and 0.01% Triton X-100. The GST-tagged proteins were eluted with a solution containing 100 mM Glutathione, 50 mM Tris, pH 8.0, 150 mM NaCl and 0.01% Triton X-100. The purified proteins were dialyzed into a buffer containing 20 mM Tris, pH 7.5, 150 mM NaCl and 20% Glycerol.

Test compounds were serially diluted in DMSO using three-fold dilutions to stock concentrations ranging typically from 50 μM to 20 nM (final concentrations in the assay range from 1 μM to 0.4 nM). The c-Raf biochemical assay was performed as a radioactive filtermat assay in 96-well Costar polypropylene plates (Costar 3365). The plates were loaded with 75 μL solution containing 50 mM HEPES pH 7.5, 70 mM NaCl, 80 ng of Lck/c-raf and 1 μg MEK-1. Subsequently, 2 μL of the serially diluted individual compounds were added to the reaction, prior to the addition of ATP. The reaction was initiated with 25 μL ATP solution containing 5μM ATP and 0.3 μCi [33P]-ATP. The plates were sealed and incubated at 32 °C for 1 h. The reaction was quenched with the addition of 50 μL of 4 % Phosphoric Acid and harvested onto P30 filtermats (PerkinElmer) using a Wallac Tomtec Harvester. Filtermats were washed with 1 % Phosphoric Acid first and deinonized H_2O second. The filters were dried in a microwave, soaked in scintillation fluid and read in a Wallac 1205 Betaplate Counter (Wallac Inc., Atlanta, GA, U.S.A.). The results were expressed as percent inhibition.

% Inhibition = $[100-(T_{ib}/T_i)] \times 100$ where

T_{ib} = (counts per minute with inhibitor)-(background)

T_i = (counts per minute without inhibitor)-(background)

The compound of the present invention shows potent inhibition of raf kinase in this assay.

Example 6: p38 kinase *in vitro* assay

Purified and His-tagged p38 α2 (expressed in E. Coli) was activated *in vitro* by MMK-6 to a high specific activity. Using a microtiter format, all reactions were conducted in 100 µL volumes with reagents diluted to yield 0.05 µg/well of activated p38 α2 and 10 µg/well of myelin basic protein in assay buffer (25 mM HEPES 7.4, 20 mM $MgCl_2$, 150 mM NaCl). Test compounds (5 µL of a 10% DMSO solution in water) were prepared and diluted into the assay to cover a final concentration range from 5 nM to 2.5 µM. The kinase assay was initiated by addition of 25 µL of an ATP cocktail to give a final concentration of 10 µM cold ATP and 0.2 µCi [gamma-^{33}P] ATP per well (200–400 dpm/pmol of ATP). The plate was incubated at 32 °C for 35 min., and the reaction quenched with 7 µL of a 1 N aq HCl solution. The samples were harvested onto a P30 Filtermat (Wallac, Inc.) using a TomTec 1295 Harvester (Wallac, Inc.), and counted in a LKB 1205 Betaplate Liquid Scintillation Counter (Wallac, Inc.). Negative controls included substrate plus ATP alone. SW1353 cellular assay: SW1353 cells (human chondro-sarcoma) are seeded (1000 cells/100 µL DMEM 10% FCS/well) into 96-well plates and incubated overnight. After medium replacement, cells are exposed to test compounds for 1 h at 37 °C, at which time human IL-1 (1 ng/mL, Endogen, Woburn, WA) and recombinant human TNFalpha (10 ng/mL) are added. Cultures are incubated for 48 h at 37 °C, then supernatant IL-6 values are determined by ELISA. The compound of this invention shows significant inhibition of p38 kinase.

Example 7: Bio-Plex Phospho-ERK ½ immunoassay.

A 96 well pERK immunoassay, using laser flow cytometry (Bio-Rad) platform has been established to measure inhibition of basal pERK in breast cancer cell line. MDA-MB-231 cells were plated at 50,000 cells per well in 96 well microtitre plates in complete growth media. For effects of test compounds on basal pERK1/2

inhibition, the next day after plating, MDA-MB-231 cells were transferred to DMEM with 0.1% BSA and incubated with test compounds diluted 1:3 to a final concentration of 3 μM to 12 nM in 0.1% DMSO. Cells were incubated with test compounds for 2 h, washed, and lysed in Bio-Plex whole cell lysis buffer A. Samples are diluted with buffer B 1:1 (v/v) and directly transferred to assay plate or frozen at –80 C degrees until processed. 50 μL of diluted MDA-MB-231 cell lysates were incubated with about 2000 of 5 micron Bio-Plex beads conjugated with an anti-ERK1/2 antibody overnight on a shaker at room temperature. The next day, biotinylated phospho-ERK1/2 sandwich immunoassay was performed, beads are washed 3 times during each incubation and then 50 μL of PE-strepavidin was used as a developing reagent. The relative fluorescence units of pERK1/2 were detected by counting 25 beads with Bio-Plex flow cell (probe) at high sensitivity. The IC50 was calculated by taking untreated cells as maximum and no cells (beads only) as background using in an Excel spreadsheet based program.

The compound of this invention shows significant inhibition in this assay.

Example 8: Flk-1 (murine VEGFR-2) Biochemical Assay

This assay was performed in 96-well opaque plates (Costar 3915) in the TR-FRET format. Reaction conditions are as follows: 10 μM ATP , 25 nM poly GT-biotin , 2 nM Eu-labelled phospho-Tyr Ab, 10 nM APC, 7 nM Flk-1 (kinase domain), 1% DMSO, 50 mM HEPES pH 7.5, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.015% BRIJ, 0.1 mg/mL BSA, 0.1% mercapto-ethanol). Reaction is initiated upon addition of enzyme. Final reaction volume in each well is 100 μL. Plates are read at both 615 and 665 nM on a Perkin Elmer Victor V Multilabel counter at about 1.5- 2.0 hours after reaction initiation. Signal is calculated as a ratio: (665 nm / 615 nm) * 10000 for each well.

The compound of this invention shows significant inhibition of VEGFR2 kinase.

Example 9: Murine PDGFR FRET biochemical assay

This assay was formatted in a 96-well black plate (Costar 3915). The following reagents are used: Europium-labeled anti-phosphotyrosine antibody pY20 (Perand streptavidin-APC; poly GT-biotin from, and mouse PDGFR. The reaction conditions are as follows: 1 nM mouse PDGFR is combined with 20 μM ATP, 7 nM poly GT-biotin, 1 nM pY20 antibody, 5 nM streptavidin-APC, and 1% DMSO in assay buffer

(50 mM HEPES pH 7.5, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.015% BRIJ 35, 0.1 mg/mL BSA, 0.1% mercaptoethanol). Reaction is initiated upon addition of enzyme. Final reaction volume in each well is 100 µL. After 90 minutes, the reaction is stopped by addition of 10 µL/well of 5 µM staurosporine. Plates are read at both 615 and 665 nm on a Perkin Elmer VictorV Multilabel counter at about 1 hour after the reaction is stopped. Signal is calculated as a ratio: (665 nm / 615 nm) * 10000 for each well. The compound of this invention shows significant inhibition of PDGFR kinase.

For IC_{50} generation for both PDGFR and Flk-1, compounds were added prior to the enzyme initiation. A 50-fold stock plate was made with compounds serially diluted 1:3 in a 50% DMSO/50% dH2O solution. A 2 µL addition of the stock to the assay gave final compound concentrations ranging from 10 µM – 4.56 nM in 1% DMSO. The data were expressed as percent inhibition: % inhibition = 100-((Signal with inhibitor-background)/(Signal without inhibitor - background)) * 100

Example 10: MDA-MB231 proliferation assay

Human breast carcinoma cells (MDA MB-231, NCI) were cultured in standard growth medium (DMEM) supplemented with 10% heat-inactivated FBS at 37°C in 5% CO_2 (vol/ vol) in a humidified incubator. Cells were plated at a density of 3000 cells per well in 90 µL growth medium in a 96 well culture dish. In order to determine T_{0h} CTG values, 24 hours after plating, 100 µL of CellTiter-Glo Luminescent Reagent (Promega) was added to each well and incubated at room temperature for 30 minutes. Luminescence was recorded on a Wallac Victor II instrument. The CellTiter-Glo reagent results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present, which, in turn is directly proportional to the number of cells present.

Test compounds are dissolved in 100% DMSO to prepare 10 mM stocks. Stocks were further diluted 1:400 in growth medium to yield working stocks of 25 µM test compound in 0.25% DMSO. Test compounds were serially diluted in growth medium containing 0.25% DMSO to maintain constant DMSO concentrations for all wells. 60 µL of diluted test compound were added to each culture well to give a final volume of 180 µL. The cells with and without individual test compounds were incubated for 72 hours at which time ATP dependent luminescence was measured, as described previously, to yield T_{72h} values. Optionally, the IC_{50} values can be

determined with a least squares analysis program using compound concentration versus percent inhibition.

% Inhibition = $[1-(T_{72h\ test}-T_{0h})/(T_{72h\ ctrl}-T_{0h})] \times 100$, where

$T_{72h\ test}$ = ATP dependent luminescence at 72 hours in the presence of test compound

$T_{72h\ ctrl}$ = ATP dependent luminescence at 72 hours in the absence of test compound

T_{0h} = ATP dependent luminescence at Time Zero

The compound of this invention shows significant inhibition of proliferation using this assay.

Example 11: pPDGFR-beta sandwich ELISA in AoSMC cells

100K P3-P6 Aortic SMC were plated in each well of 12-well cluster in 1000 µL volume/ well of SGM-2 using standard cell culture techniques. Next day, cells were rinsed with 1000 µL D-PBS once, then serum starved in 500 µL SBM (smooth muscle cell basal media) with 0.1% BSA overnight. Compounds were diluted at a dose range from (10 µM to 1 nM in 10-fold dilution steps in DMSO. Final DMSO concentration 0.1%). Remove old media by inversion into the sink quickly then add 100 µL of each dilution to corresponding well of cells for 1 h at 37 ^{0}C. Cells were then stimulated with 10 ng/mL PDGF-BB ligand for 7 min at 37 ^{0}C. The media is decanted and 150 µL of isotonic lysis buffer with protease inhibitor tablet (Complete; EDTA-free) and 0.2 mM Na vanadate is added. Cells are lysed for 15 min at 4 ^{0}C on shaker in cold room. Lysates are put in eppendorf tubes to which 15 µL of agarose-conjugated anti-PDGFR-beta antibody is added and incubated at 4 ^{0}C overnight. Next day, beads are rinsed in 50-volumes of PBS three times and boiled in 1x LDS sample buffer for 5 minutes. Samples were run on 3-8% gradient Tris-Acetate gels and transferred onto Nitrocellulose. Membranes were blocked in 1% BSA/TBS-T for 1 hr. before incubation in anti-phospho-PDGFR-b (Tyr-857) antibody in blocking buffer (1:1000 dilution) for 1 h. After three washes in TBS-T, membranes were incubated in Goat anti-rabbit HRP IgG (1:25000 dilution) for 1 hr. Three more washes followed before addition of ECL substrate. Membranes were exposed to Hyperfilm-ECL. Subsequently, membranes were stripped and reprobed with anti-PDGFR-beta antibody for total PDGFR-beta.

Table 1 illustrates the results of *in vitro* kinase biochemical assays for p38 kinase, PDGFR kinase and VEGFR2 kinase. These three kinase targets are all involved in stroma activation and endothelial cell proliferation, leading to angiogenesis, and providing blood supply to the tumor tissue.

Table 1

	mPDGFR IC50, nM	mVEGFR2 IC50, nM	p38 IC50, nM
Example 1	83	5.5	24

Table 2 illustrates the results of two cellular assays for raf kinase activity, which are (i) inhibition of pERK in MDA-MB231 cells, a mechanistic readout of raf kinase activity, and (ii) a proliferation assay of MDA-MB231 cells, a functional assay of raf kinase activity. In addition, Table 2 illustrates the results of PDGFR driven phosphorylation of PDGFR-beta in aortic smooth muscle cells, which is a mechanistic readout of PDGFR kinase inhibition.

Table 2

	pERK in cells (MDA-MB-231) IC50, nM	Proliferation (MDA-MB-231) IC50, nM	pPDGFR (AoSMC) IC50, nM
Example 1	22	600	43.6

Overall, compounds of the present invention provide a unique combination of inhibition of angiogenesis and tumor cell proliferation. They also possess an improved inhibition profile against several key kinase targets such as raf, p38, PDGFR, and VEGFR-2, which are all molecular targets of interest for the treatment of

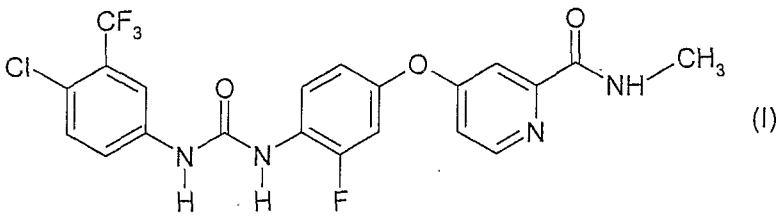
osteoporosis, inflammatory diseases, and hyper-proliferative diseases, including cancer.

It is believed that one skilled in the art, using the preceding information and information available in the art, can utilize the present invention to its fullest extent. It should be apparent to one of ordinary skill in the art that changes and modifications can be made to this invention without departing from the spirit or scope of the invention as it is set forth herein. All publications, applications and patents cited above and below are incorporated herein by reference.

The topic headings set forth above and below are meant as guidance where certain information can be found in the application, but are not intended to be the only source in the application where information on such topic can be found.

Claims

1. A compound of Formula (I) or a salt, or a prodrug or a metabolite or an isolated stereoisomer thereof



(I)

2. A pharmaceutically acceptable salt of a compound of Formula I of claim 1 which is

a) a basic salt of an organic acid or inorganic acid which is hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, methanesulfonic acid, trifluoromethanesulfonic acid, benzenesulfonic acid, p-toluene sulfonic acid (tosylate salt), 1-napthalene sulfonic acid, 2-napthalene sulfonic acid, acetic acid, trifluoroacetic acid, malic acid, tartaric acid, citric acid, lactic acid, oxalic acid, succinic acid, fumaric acid, maleic acid, benzoic acid, salicylic acid, phenylacetic acid, or mandelic acid; or

b) an acid salt of an organic or inorganic base containing an alkali metal cation, an alkaline earth metal cation, an ammonium cation, an aliphatic substituted ammonium cation or an aromatic substituted ammonium cation.

3. A compound which is 4{4-[3-(4-chloro-3-trifluoromethylphenyl)-ureido]-3-fluorophenoxy}-pyridine-2-carboxylic acid methylamide, or a salt thereof.

4. A pharmaceutically acceptable salt of a compound of claim 3 which is a basic salt of an organic acid such as hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, methanesulfonic acid, trifluoromethanesulfonic acid, benzenesulfonic acid, p-toluene sulfonic acid (tosylate salt), 1-napthalene sulfonic

acid, 2-napthalene sulfonic acid, acetic acid, trifluoroacetic acid, malic acid, tartaric acid, citric acid, lactic acid, oxalic acid, succinic acid, fumaric acid, maleic acid, benzoic acid, salicylic acid, phenylacetic acid, or mandelic acid.

5. A compound which is which is a hydrochloride, benzenesulfonate, or methanesulfonate salt of N-(4-chloro-3-(trifluoromethyl)phenyl)-N'-2-fluoro-(4-(2-(N-methylcarbamoyl)-4-pyridyloxy)phenyl) urea.

6. A pharmaceutical composition comprising a compound of claim 1 and a physiologically acceptable carrier.

7. A pharmaceutical composition comprising a compound of claim 3 and a physiologically acceptable carrier.

8. A pharmaceutical composition for the treatment of a disease in a human or other mammal regulated by a protein kinase, associated with an aberration in the protein kinase signal transduction pathway comprising a compound of claim 1 and a physiologically acceptable carrier.

9. A pharmaceutical composition for the treatment of a hyper-proliferative disorder comprising a compound of claim 1 and a physiologically acceptable carrier.

10. A pharmaceutical composition for the treatment of a cancerous cell growth comprising a compound of claim 1 and a physiologically acceptable carrier.

11. A pharmaceutical composition which comprises a pharmaceutically acceptable salt of N-(4-chloro-3-(trifluoromethyl)phenyl)-N'-2-fluoro-(4-(2-(N-methylcarbamoyl)-4-pyridyloxy)phenyl) urea and a physiologically acceptable carrier.

12. A method for regulating tyrosine kinase signal transduction comprising administering to a human or other mammal a compound of claim1.

13. A method for treating or preventing a disease in a human or other mammal which is regulated by tyrosine kinase and associated with an aberration in the tyrosine kinase signal transduction pathway, said method comprising administering to a human or other mammal a compound of claim 1.

14. A method for treating or preventing a disease in a human and/or other mammal which is a VEGFR-2 mediated disorder, said method comprising administering to a human or other mammal a compound of claim 1.

15. A method for treating or preventing a disease in a human and/or other mammal which is a PDGFR mediated disorder, said method comprising administering to a human or other mammal a compound of claim 1.

16. A method for treating or preventing a disease in a human or other mammal which is a raf-mediated disorder, said method comprising administering to a human or other mammal a compound of claim 1.

17. A method for treating or preventing a disease in a human or other mammal which is a p38-mediated disorder, said method comprising administering to a human or other mammal a compound of claim 1.

18. A method for treating or preventing a disease in a human or other mammal which is a VEGF-mediated disorder, said method comprising administering to a human or other mammal a compound of claim 1.

19. A method for treating or preventing a disease in a human or other mammal which is a hyper-proliferative, inflammatory and/or angiogenesis disorder which comprises administering to a human or other mammal a compound of claim 1.

20. A method for treating or preventing a disease in a human or other mammal which is a hyper-proliferative disorder which comprises administering to a human or other mammal a compound of claim 1.

21. A method as in claim 20, wherein the hyper-proliferative disorder is cancer.

22. A method as in claim 21, wherein said method comprises administering to a human or other mammal a compound of claim 1 in combination with one or several additional anti-cancer agents.

23. A method for treating or preventing a disease in a human or other mammal characterized by abnormal angiogenesis or hyperpermiability processes comprising administering to a human or other mammal a compound of claim 1.

24. A method as in claim 23, for treating or preventing a disease in a human or other mammal characterized by abnormal angiogenesis or hyperpermeability processes, comprising administering to a human or other mammal, a compound of claim 1 simultaneously with another anti-angiogenesis agent, either in the same formulation or in separate formulations.

25. A method for treating or preventing one or more of the following conditions in humans and/or other mammals:

tumor growth, retinopathy, ischemic retinal-vein occlusion, retinopathy of prematurity, age related macular degeneration; rheumatoid arthritis, psoriasis, a bullous disorder associated with subepidermal blister formation, including bullous pemphigoid, erythema multiforme, or dermatitis herpetiformis, rheumatoid arthritis, osteoarthritis, septic arthritis, tumor metastasis, periodontal disease, cornal ulceration, proteinuria and coronary thrombosis from atherosclerotic plaque, aneurismal aortic, birth control, dystrophobic epidermolysis bullosa, degenerative cartilage loss following traumatic joint injury, osteopenias mediated by MMP activity, tempero mandibular joint disease or demyelating disease of the nervous system,

said method comprising administering to a human or other mammal, a compound of claim 1.

26. A method for treating or preventing one or more of the following conditions in humans and/or other mammals: tumor growth, retinopathy, ischemic retinal-vein occlusion, retinopathy of prematurity, age related macular degeneration; rheumatoid arthritis, psoriasis, a bullous disorder associated with subepidermal blister formation, including bullous pemphigoid, erythema multiforme, or dermatitis herpetiformis;

in combination with another condition selected from the group consisting of:

rheumatic fever, bone resorption, postmenopausal osteoporosis, sepsis, gram negative sepsis, septic shock, endotoxic shock, toxic shock syndrome, systemic inflammatory response syndrome, inflammatory bowel disease (Krohn's disease and ulcerative colitis), Jarisch-Herxheimer reaction, asthma, adult respiratory distress syndrome, acute pulmonary fibrotic disease, pulmonary sarcoidosis, allergic respiratory disease, silicosis, coal worker's pneumoconiosis, alveolar injury, hepatic failure, liver disease during acute inflammation, severe alcoholic hepatitis, malaria (Plasmodium falciparum malaria and cerebral malaria), non-insulin-dependent diabetes mellitus (NIDDM), congestive heart failure, damage following heart disease, atherosclerosis, Alzheimer's disease, acute encephalitis, brain injury, multiple sclerosis (demyelation and oligiodendrocyte loss in multiple sclerosis), advanced cancer, lymphoid malignancy, pancreatitis, impaired wound healing in infection, inflammation and cancer, myelodysplastic syndromes, systemic lupus erythematosus, biliary cirrhosis, bowel necrosis, radiation injury/ toxicity following administration of monoclonal antibodies, host-versus-graft reaction (ischemia reperfusion injury and allograft rejections of kidney, liver, heart, and skin), lung allograft rejection (obliterative bronchitis) and complications due to total hip replacement,

said method comprising administering to a human or other mammal a compound of claim 1.

27. A method for treating or preventing one or more of the following conditions in humans and/or other mammals: tumor growth, retinopathy, diabetic retinopathy, ischemic retinal-vein occlusion, retinopathy of prematurity, age related macular degeneration; rheumatoid arthritis, psoriasis, bullous disorder associated with subepidermal blister formation, bullous pemphigoid, erythema multiforme, and dermatitis herpetiformis,

in combination with an infectious disease selected from the group consisting of:

tuberculosis, Helicobacter pylori infection during peptic ulcer disease, Chaga's disease resulting from Trypanosoma cruzi infection, effects of Shiga-like toxin resulting from E. coli infection, effects of enterotoxin A resulting from Staphylococcus infection, meningococcal infection, and infections from Borrelia burgdorferi, Treponema pallidum, cytomegalovirus, influenza virus, Theiler's encephalomyelitis virus, and the human immunodeficiency virus (HIV);

said method comprising administering to a human or other mammal a compound of claim 1.

28. A method as in claim 22 wherein the additional anti-cancer agent is selected from the group consisting of asparaginase, bleomycin, carboplatin, carmustine, chlorambucil, cisplatin, colaspase, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin, doxorubicin (adriamycine), epirubicin, etoposide, 5-fluorouracil, hexamethylmelamine, hydroxyurea, ifosfamide, irinotecan, leucovorin, lomustine, mechlorethamine, 6-mercaptopurine, mesna, methotrexate, mitomycin C, mitoxantrone, prednisolone, prednisone, procarbazine, raloxifen, streptozocin, tamoxifen, thioguanine, topotecan, vinblastine, vincristine, vindesine, aminoglutethimide, L-asparaginase, azathioprine, 5-azacytidine cladribine, busulfan, diethylstilbestrol, 2',2'-difluorodeoxycytidine, docetaxel, erythrohydroxynonyl adenine, ethinyl estradiol, 5-fluorodeoxyuridine, 5-fluorodeoxyuridine monophosphate, fludarabine phosphate, fluoxymesterone, flutamide, hydroxyprogesterone caproate, idarubicin, interferon, medroxyprogesterone acetate, megestrol acetate, melphalan, mitotane, paclitaxel, pentostatin, N-phosphonoacetyl-L-aspartate (PALA), plicamycin, semustine, teniposide, testosterone propionate, thiotepa, trimethylmelamine, uridine, and vinorelbine, oxaliplatin, gemcitabine, capecitabine, epothilone and its natural or synthetic derivatives, tositumomab, trabedectin, and temozolomide. trastuzumab, cetuximab, bevacizumab, pertuzumab, ZD-1839 (Iressa), OSI-774 (Tarceva), CI-1033, GW-2016, CP-724,714, HKI-272, EKB-569, STI-571 (Gleevec), PTK-787, SU-11248, ZD-6474, AG-13736, KRN-951, CP-547,632, CP-673,451, CHIR-258, MLN-518, AZD-2171, PD-325901, ARRY-

142886, suberoylanilide hydroxamic acid (SAHA), LAQ-824, LBH-589, MS-275, FR-901228, bortezomib, and CCI-779.

29. A method as in claim 22 wherein the additional anti-cancer agent is a cytotoxic agent selected from the group consisting of DNA topoisomerase I and II inhibitors, DNA intercalators, alkylating agents, anti-metabolites, cell-cycle blockers, microtubule disruptors, and Eg5 inhibitors.

30. A method as in claim 22 wherein the additional anti-cancer agent is selected from the group consisting of inhibitors of growth factor receptor signaling, histone deacetylase inhibitors, inhibitors of the PKB pathway, inhibitors of the Raf/MEK/ERK pathway, inhibitors of the mTOR pathway, and proteasome inhibitors.

31. A method for treating or preventing one or more of the following conditions in humans and/or other mammals:

rheumatic fever, bone resorption, postmenopausal osteoporosis, sepsis, gram negative sepsis, septic shock, endotoxic shock, toxic shock syndrome, systemic inflammatory response syndrome, inflammatory bowel disease (Krohn's disease and ulcerative colitis), Jarisch-Herxheimer reaction, asthma, adult respiratory distress syndrome, acute pulmonary fibrotic disease, pulmonary sarcoidosis, allergic respiratory disease, silicosis, coal worker's pneumoconiosis, alveolar injury, hepatic failure, liver disease during acute inflammation, severe alcoholic hepatitis, malaria (Plasmodium falciparum malaria and cerebral malaria), non-insulin-dependent diabetes mellitus (NIDDM), congestive heart failure, damage following heart disease, atherosclerosis, Alzheimer's disease, acute encephalitis, brain injury, multiple sclerosis (demyelation and oligiodendrocyte loss in multiple sclerosis), advanced cancer, lymphoid malignancy, pancreatitis, impaired wound healing in infection, inflammation and cancer, myelodysplastic syndromes, systemic lupus erythematosus, biliary cirrhosis, bowel necrosis, psoriasis, radiation injury/ toxicity following administration of monoclonal antibodies, host-versus-graft reaction (ischemia reperfusion injury and allograft rejections of kidney, liver, heart, and skin), lung allograft rejection (obliterative bronchitis) or complications due to total hip replacement,

said method comprising administering to a human or other mammal, a compound of claim 1.

32. A method for treating or preventing one or more of the following conditions in humans and/or other mammals:

tuberculosis, Helicobacter pylori infection during peptic ulcer disease, Chaga's disease resulting from Trypanosoma cruzi infection, effects of Shiga-like toxin resulting from E. coli infection, effects of enterotoxin A resulting from Staphylococcus infection, meningococcal infection, and infections from Borrelia burgdorferi, Treponema pallidum, cytomegalovirus, influenza virus, Theiler's encephalomyelitis virus, and the human immunodeficiency virus (HIV) ,

said method comprising administering to a human or other mammal, a compound of claim 1.

33. A method for treating or preventing osteoporosis, inflammation, and angiogenesis disorders, with the exclusion of cancer, in a human and/or other mammal by administering an effective amount of a compound of claim 1 to said mammal.

34. A method for treating or preventing cancer in a human or other mammal which comprises administering to a human or other mammal a single active principle combining inhibition of tumor cell proliferation mediated by the raf / MEK / ERK pathway, and inhibition of angiogenesis mediated by PDGF and VEGF.

35. A method of claim 34 where said inhibition of tumor cell proliferation is caused by inhibition of raf kinase, and said inhibition of angiogenesis is caused by dual inhibition of PDGFR-beta and VEGFR-2 kinases.

36. A method for treating or preventing cancer in a human or other mammal which comprises administering to a human or other mammal a single active principle combining inhibition of tumor cell proliferation mediated by the raf pathway, and inhibition of angiogenesis mediated by PDGF or VEGF.

37. A method of treating and/or preventing a disease and/or condition in a subject in need thereof, comprising administering an effective amount of a compound of claim 1 or 2.

38. A method of claim 37, wherein said method comprises causing tumor regression in a subject or cells therefrom.

39. A method of claim 37, wherein said method comprises inhibiting lymphangiogenesis.

40. A method of claim 37, wherein said method comprises inhibiting angiogenesis.

41. A method of claim 37, wherein said method comprises inhibiting lymphangiogenesis and angiogenesis.

42. A method of claim 37, wherein said method comprises stimulating the proliferation of hematopoietic progenitor cells.

43. A method of claim 37, wherein said method comprises treating a disorder in a mammalian subject mediated by raf, VEGFR-2, VEGFR-3, PDGFR-beta, p38 and/or flt-3.

44. A method of claim 37, wherein said method comprises determining whether a condition can be modulated by said compound, comprising measuring the expression or activity of raf, VEGFR-2, VEGFR-3, PDGFR-beta, p38 and/or flt-3, in a sample comprising cells or a cell extract, wherein said ample is obtained from a subject or cell having said condition, whereby said condition can be modulated by said compound when said expression or activity is different in said condition as compared to a normal control.

45. A method of claim 44, further comprising comparing the expression in said sample to said normal control.

46. A method of claim 37, wherein said method comprises assessing the efficacy of said compound disorder, comprising administering said compound, measuring the expression or activity of raf, VEGFR-2, VEGFR-3, PDGFR-beta, p38, and/or flt-3, and determining the effect of said compound on said expression or activity.

47. A method of claim 37, wherein said method comprises determining the presence of raf, VEGFR-2, VEGFR-3, PDGFR-beta, p38 and/or flt-3 in a sample of a biological material, contacting said sample with said compound, and determining whether said compound binds to said material.

48. A method of claim 37, wherein said method comprises treating a tumor in a subject in need thereof, comprising administering an effective amount of said compound wherein said amount is effective to inhibit tumor cell proliferation and neovascularization.

49. A compound which is a naturally occurring metabolite of the compound of claim 3.

50. A compound of claim 49 where the metabolism site is either one of the two urea nitrogen atoms, or the pyridine nitrogen atom, or the methylamide functionality, or any combination of the above.

51. A compound of claim 49 where either urea nitrogen atom carries a hydroxyl group, and/or the pyridine nitrogen atom is oxidized, and/or the amide functionality is de-methylated.

52. A compound of claim 49 which is selected from:
4{4-[3-(4-chloro-3-trifluoromethylphenyl)-ureido]-3-fluorophenoxy}-pyridine-2-carboxylic acid amide,
4{4-[3-(4-chloro-3-trifluoromethylphenyl)-ureido]-3-fluorophenoxy}-1-hydroxy-pyridine-2-carboxylic acid methylamide, or
4{4-[3-(4-chloro-3-trifluoromethylphenyl)-ureido]-3-fluorophenoxy}-1-hydroxy-pyridine-2-carboxylic acid amide.

53. A method as in claim 19, where the inflammatory disorder is selected from rheumatoid arthritis, COPD, Crohn's disease and proriasis.

54. A method for treating or preventing a disease in a human or other mammal which is a flt-3 mediated disorder, said method comprising administering to a human or other mammal a compound of claim 1.