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(71) Applicant *(for all designated States except US)*: **NORTH SHORE-LONG ISLAND JEWISH HEALTH SYSTEM** [US/US]; 400 Lakeville Road, New Hyed Park, NY 11040 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PILLARISETTI, Sivaram** [IN/US]; 4762 Grand Heron Court, Norcross, GA 30092 (US). **GOLDBERG, Itzhak, D.** [US/US]; 41 Brayton Street, Englewood, NJ 07631 (US).

(74) Agent: **YAMIN, Michael, A.**; Klauber & Jackson, 411 Hackensack Avenue, Hackensack, NJ 07601 (US).

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(54) Title: MODULATORS OF CELLULAR PROLIFERATION AND ANGIOGENESIS, METHODS FOR USE AND IDENTIFICATION THEREOF

(57) Abstract: The invention is directed to small organic molecules and peptides having the ability to mimic or agonize hepatocyte growth factor/ scatter factor (HGF/SF) activity, or inhibit or antagonize HGF/SF activity, the former useful for promoting, for example, vascularization of tissues or organs for promoting wound or tissue healing, or augmenting or restoring blood flow to ischemic tissues such as the heart following myocardial infarction. Inhibition of cellular growth or proliferation is beneficial in the treatment, for example, of inflammatory diseases such as inflammatory joint and skin diseases, and dysproliferative diseases such as cancer.

# MODULATORS OF CELLULAR PROLIFERATION AND ANGIOGENESIS, METHODS FOR USE AND IDENTIFICATION THEREOF

## FIELD OF THE INVENTION

The invention is directed to various therapeutic uses of peptide and small molecule compounds having either hepatocyte growth factor/ scatter factor (HGF/SF), or the property of inhibiting the activity of HGF/SF. Such compounds have the potential for the treatment of conditions and diseases in which modulation of cellular proliferation, among other activities, is desired. Methods for identifying such compounds are also described.

## BACKGROUND OF THE INVENTION

Scatter factor (SF; also known as hepatocyte growth factor [HGF], and hereinafter referred to and abbreviated as HGF/SF) is a pleiotropic growth factor that stimulates cell growth, cell motility, morphogenesis and angiogenesis. HGF/SF is produced as an inactive monomer (~100 kDa) which is proteolytically converted to its active form. Active HGF/SF is a heparin-binding heterodimeric protein composed of a 62 kDa α chain and a 34 kDa β chain. HGF/SF is a potent mitogen for parenchymal liver, epithelial and endothelial cells (Matsumoto, K, and Nakamura, T. , 1997, Hepatocyte growth factor (HGF) as a tissue organizer for organogenesis and regeneration. Biochem. Biophys. Res. Commun. 239, 639-44; Boros, P. and Miller, C.M., 1995, Hepatocyte growth factor: a multifunctional cytokine. Lancet 345, 293-5). It stimulates the growth of endothelial cells and also acts as a survival factor against endothelial cell death (Morishita, R, Nakamura, S, Nakamura, Y, Aoki, M, Moriguchi, A, Kida, I, Yo, Y, Matsumoto, K, Nakamura, T, Higaki, J, Ogihara, T, 1997, Potential role of an endothelium-specific growth factor, hepatocyte growth factor, on endothelial damage in diabetes. Diabetes 46:138-42). HGF/SF synthesized and secreted by vascular smooth muscle cells stimulate endothelial cells to proliferate, migrate and differentiate into capillary-like tubes *in vitro* (Grant, D.S, Kleinman, H.K., Goldberg, I.D., Bhargava, M.M., Nickoloff, B.J., Kinsella, J.L., Polverini, P., Rosen, E.M., 1993, Scatter factor induces blood vessel formation in vivo. Proc. Natl. Acad. Sci. U S A 90:1937-41; Morishita, R., Nakamura, S., Hayashi, S., Taniyama, Y., Moriguchi, A., Nagano, T., Taiji, M., Noguchi, H., Takeshita, S., Matsumoto, K., Nakamura, T., Higaki, J., Ogihara, T., 1999, Therapeutic angiogenesis induced by human recombinant hepatocyte growth factor in rabbit hind limb ischemia model as cytokine supplement therapy. Hypertension 33:1379-84). HGF/SF-containing implants in mouse subcutaneous tissue and rat cornea induce growth of new blood vessels from surrounding tissue. HGF/SF protein is expressed at sites of neovascularization including in tumors (Jeffers, M., Rong, S., Woude, G.F. , 1996, Hepatocyte growth factor/scatter factor-Met signaling in tumorigenicity and invasion/metastasis. J. Mol. Med. 74:505-13; Moriyama, T., Kataoka, H., Koono, M., Wakisaka, S., 1999, Expression of hepatocyte growth factor/scatter factor and its receptor c-met in brain tumors: evidence for a role in progression of astrocytic

tumors Int. J. Mol. Med. 3:531-6). These findings suggest that HGF/SF plays a significant role in the formation and repair of blood vessels under physiologic and pathologic conditions. Further discussion of angiogenic proteins may be found in U.S. Patents 6,011,009 and 5,997,868, both of which are incorporated herein by reference in their entireties.

Modulation of cellular proliferation by exogenously-supplied therapeutic agents has been offered as a new approach for the prophylaxis and/or treatment of various conditions and diseases in which limited cellular proliferation, or, in contrast, excessive proliferation of cells, is responsible for pathology, or at least for the prolongation of rebound from a pathological state to homeostasis. For example, the duration of wound healing, normalization of myocardial perfusion as a consequence of chronic cardiac ischemia or myocardial infarction, development or augmentation of collateral vessel development after vascular occlusion or to ischemic tissues or organs, and vascularization of grafted or transplanted tissues, organs, or wound healing, may be accelerated by promoting cellular proliferation, particularly of vascular cells.

In other cases where abnormal or excessive cellular proliferation is the cause of pathology, such as in dysproliferative diseases including cancer, inflammatory joint and skin diseases such as rheumatoid arthritis, and neovascularization in the eye as a consequence of diabetic retinopathy, suppression of cellular proliferation is a desired goal in the treatment of these and other conditions. In either case, therapy to promote or suppress proliferation may be beneficial locally but not systemically, and for a particular duration, and proliferation modulating therapies must be appropriately applied.

In co-pending application Serial No. 09/606,628, filed June 29, 2000, incorporated herein by reference in its entirety, peptide mimetics with HGF/SF-like, proliferative activity and particularly angiogenic activity, as well as other agents, particularly peptide HGF/SF antagonists which inhibit cellular proliferation and, in particular, angiogenesis, were described. Such peptides have uses, for example, in the treatment of inflammatory diseases, cancer, neovascularization, cardiac ischemia, wound healing, and other conditions in which modulation of cellular proliferation including blood vessel growth is therapeutically beneficial, as described above. Furthermore, co-pending provisional application serial no. 60/276,170, filed March 15, 2001, also incorporated herein by reference in its entirety, describes various small-molecule compounds with HGF/SF-like activity or antagonistic activity, with the same aforementioned uses.

It is toward the identification of peptide and small organic molecules with HGF/SF activity, or those that inhibit HGF/SF activity, as well as methods for identifying and preparing such active molecules, that the present invention is directed.

The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

## SUMMARY OF THE INVENTION

In one broad aspect, the present invention is directed to methods for the modulation of hepatocyte growth factor / scatter factor (HGF/SF) activities in a mammal for the treatment of any of a number of conditions or diseases in which either HGF/SF has a therapeutically useful role, or in which the activity of endogenous HGF/SF is desirably inhibited or abrogated. Such modulation is achieved by the administration to the mammal of a compound of the invention in an amount effective to achieve the desired outcome. In one embodiment, the compounds of the invention modulate the activity of the HGF/SF receptor, c-met. In a further embodiment, the compounds of the invention bind to c-met.

In the instance where HGF/SF activity is desirable, certain compounds of the invention have been found to mimic or agonize the biological activities of HGF/SF, and thus are useful in the treatment, for example, of conditions or diseases in which enhanced cellular or vascular proliferation is desirable, among other desirable activities of HGF/SF. Such conditions or diseases include hepatic disease, renal disease, bone regeneration, hair growth, promoting wound or tissue healing, or augmenting or restoring blood flow to ischemic tissues such as the heart following myocardial infarction. Such compounds may be administered systemically or locally to particular tissues or organs, in order to achieve the desired systemic or local effect. Such desirable activities also include induction of proliferation of endothelial cells, induction of anti-apoptotic activity, induction of scatter activity, or any combination of the foregoing activities. In a preferred embodiment, any one of these activities is reduced or inhibited in the presence of exogenous c-met receptor by a compound of the invention.

In another aspect, the present invention is directed to cellular proliferation promoting agents and in particular peptide agents characterized by the ability to bind to a monoclonal or polyclonal antibody to HGF/SF; and exhibit cellular proliferative activity in one or more in-vitro and/or in-vivo assays. Such agents may further exhibit the property of agonizing c-met, the HGF/SF receptor. In one embodiment, cellular proliferation comprises endothelial cell proliferation and angiogenesis. By way of non-limiting example, the agents may be small-molecule drugs, peptides, or proteins, such as the peptides TMGFTAPRFPHY (SEQ ID No:1) and KVWYHTTSIPSH (SEQ ID No:2), or their conservatively-substituted variants. The angiogenic peptides may further include a heparan sulfate-binding peptide conjugated thereto, such as KVWYHTTSIPSHCRPKAKAKAKAKDQTK (SEQ ID No:7) or conservatively-substituted variants thereof. Non-limiting examples of such conjugates include TMGFTAPRFPHYKVWYHTTSIPSHCRPKAKAKAKAKDQTK (SEQ ID No:9), KVWYHTTSIPSHKVWYHTTSIPSHCRPKAKAKAKAKDQTK (SEQ ID No:10), and conservatively-substituted variants thereof. The invention is further directed to pharmaceutical compositions comprising the foregoing agents as well as polynucleotides comprising sequences encoding the peptide agents.

In a further aspect of the invention, a method is provided for promoting cellular proliferation comprising contacting cells or tissues with an effective proliferation promoting amount of an agent characterized by ability to bind to a monoclonal or polyclonal antibody to HGF/SF; and exhibiting proliferation promoting activity in one or more in-vitro and/or in-vivo assays. Such agents may further exhibit the property of agonizing c-met, the HGF/SF receptor. Such cells or tissues may further exhibit the property of expressing the c-met receptor. In one embodiment, the proliferation promoting activity is angiogenesis. By way of non-limiting example, the agents may be small-molecule drugs, peptides, or proteins, such as the peptides TMGFTAPRFPHY (SEQ ID No:1) and KVWYHTTSIPSH (SEQ ID No:2), or their conservatively-substituted variants. The proliferation-promoting peptides may further include a heparan sulfate-binding peptide conjugated thereto, such as KVWYHTTSIPSHCRPKAKAKAKAKDQTK (SEQ ID No:7) or conservatively-substituted variants thereof. Non-limiting examples of such conjugates include TMGFTAPRFPHYKVWYHTTSIPSHCRPKAKAKAKAKDQTK (SEQ ID No:9), KVWYHTTSIPSHKVWYHTTSIPSHCRPKAKAKAKAKDQTK (SEQ ID No:10); and conservatively-substituted variants thereof. The cells or tissue may be, for example, a transplanted or grafted tissue or organ such as skin, heart, vascular tissue or kidney, an ischemic organ, such as a heart following myocardial infarction or angina, a tissue or organ damaged by wounding, surgical intervention, vascular tissue, neural tissue, a wound, ulcer, etc. The cells may be, by way of non-limiting example, epithelial cells, endothelial cells, and smooth muscle cells, and tissues and organs comprising such cells. Promotion of growth and/or regeneration of neural tissue, teeth, and other tissues comprising the c-met receptor are embraced herein. Methods of treatment include application of an agent of the invention, a pharmaceutical composition comprising an agent of the invention, gene therapy in which endogenous cells are transfected to express a protein comprising an agent of the invention, or implanting cells which secrete an agent of the invention.

In another aspect of the invention, a method is provided for promoting vascularization of a tissue comprising contacting the tissue with an effective angiogenic amount of an agent characterized by ability to bind to a monoclonal or polyclonal antibody to HGF/SF; and exhibit angiogenic activity in one or more in-vitro and/or in-vivo assays. Such agents may further exhibit the property of agonizing c-met, the HGF/SF receptor. By way of non-limiting example, the agents may be small-molecule drugs, peptides, or proteins, such as the peptides T M G F T A P R F P H Y (SEQ ID No:1) and K V W Y H T T S I P S H (SEQ ID No:2), or their conservatively-substituted variants. The angiogenic peptides may further include a heparan sulfate-binding peptide conjugated thereto, such as K V W Y H T T S I P S H C R P K A K A K A K A K D Q T K (SEQ ID No:7) or conservatively-substituted variants thereof. Non-limiting examples of such conjugates include T M G F T A P R F P H Y K V W Y H T T S I P S H C R P K A K A K A K A K D Q T K (SEQ ID No:9), K V W Y H T T S I P S H K V W Y H T T S I P S H C R P K A K A K A K A K D Q T K(SEQ ID No:10); and conservatively-substituted variants thereof. The cells or tissue may be, for example, a transplanted or grafted tissue or organ such as skin, heart, vascular tissue or kidney, an ischemic organ, such as a heart following myocardial infarction or angina, a tissue or organ

damaged by wounding, surgical intervention, a wound, ulcer, etc. Means of delivery of the agent are as described hereinabove.

In a further embodiment, polynucleotides are described which comprise nucleic acids encoding the proliferation promoting peptides of the invention, including vectors encoding the polynucleotides, as well as microorganisms and cells comprising the vectors and expressing the peptides. Therapeutic use of such polynucleotide sequences, including gene therapy with naked DNA, viral vectors, etc., and other means of transfecting cells to express the agents of the invention, for all of the above mentioned purposes, among others, are embraced herein.

In yet another embodiment, the present invention is directed to methods for identifying an proliferation promoting agent, by carrying out the steps of a) providing a candidate agent; b) measuring the ability of the agent to bind to a monoclonal or polyclonal antibody to HGF/SF; and c) measuring the ability of said agent to exhibit cellular proliferation activity in one or more in-vitro and/or in-vivo assays; wherein the candidate agent with the ability to bind to a monoclonal or polyclonal antibody to HGF/SF and ability to exhibit proliferation promoting activity is an proliferation promoting agent. The agent may further exhibit the ability of agonizing the c-met receptor. In one embodiment, the agent is an angiogenic agent. By way of non-limiting example, the agent may be a small-molecule drug, a peptide, or a protein. The ability of the agent to bind to a monoclonal or polyclonal antibody to HGF/SF may be determined by measurement of binding of said agent to the antibody, or by measuring the ability of the agent to compete with the binding of the antibody with HGF/SF or a peptide mimetic thereof. In another embodiment, a method of preparing an proliferation promoting agent may be carried out by a) identifying an proliferation promoting peptide as described above; b) determining the three-dimensional structure of the peptide; and c) modeling a small-molecule drug on the three-dimensional structure of the peptide.

Another broad aspect of the invention is directed to antiproliferative agents characterized by the ability to bind to the extracellular domain of c-met; and inhibit HGF/SF-mediated increase in cellular growth or proliferation. In one embodiment, the inhibition of cellular growth or proliferation is directed towards cells expressing the c-met receptor. In another embodiment, the cells include but are not limited to epithelial cells, endothelial cells, fibroblasts and smooth muscle cells. By way of non-limiting example, the antiproliferative agent may be a small-molecule drug, a peptide or a protein; examples of peptides include but are not limited to A T W S H H L S S A G L (SEQ ID No:3); W P Q L P P R P Y S T L (SEQ ID No:4); S N T S A G T P F T S L (SEQ ID No:5); D S T P K S T P W Y Y I (SEQ ID No:6); and conservatively-substituted variants thereof. Pharmaceutical compositions comprising the aforementioned agents are embraced herein.

In a further aspect, the invention is directed to a method for inhibiting cellular proliferation in a tissue or organ by contacting the tissue or organ with an effective antiproliferative amount of an agent

characterized by the ability to bind to the extracellular domain of c-met; and inhibit HGF/SF-mediated increase in cell growth or proliferation. In one embodiment, the agent inhibits HGF/SF-mediated increase in endothelial, epithelial cell, fibroblast or smooth muscle cell growth or proliferation. In a preferred embodiment, the agent inhibits endothelial cell growth. By way of non-limiting example, the agent may be a small-molecule drug, a peptide or a protein; examples of peptides include but are not limited to A T W S H H L S S A G L (SEQ ID No:3); W P Q L P P R P Y S T L (SEQ ID No:4); S N T S A G T P F T S L (SEQ ID No:5); D S T P K S T P W Y Y I (SEQ ID No:6); and conservatively-substituted variants thereof. The tissue or organ may be a dysproliferative tissue, such as a tumor or metastasis, or psoriasis, a tissue or organ involved in inflammatory diseases such as rheumatoid arthritis, the eye involved in neovascularization such as results from chronic diabetes, an abnormal growth such as keloid formation during wound healing, or an intentional disruption of cellular proliferation such as to prevent the genesis or maturation of a developing organ or tissue. Methods of treatment include application of an agent of the invention to the desired target site(s), a pharmaceutical composition comprising an agent of the invention, gene therapy in which endogenous cells are transfected to express a protein comprising an agent of the invention, or implanting cells which secrete an agent of the invention.

In a further aspect, the invention is directed to a method for inhibiting the vascularization of a tissue or organ by contacting the tissue or organ with an effective angiostatic amount of an agent characterized by the ability to bind to the extracellular domain of c-met; and inhibit HGF/SF-mediated increase in endothelial cell growth or proliferation. By way of non-limiting example, the agent may be a small-molecule drug, a peptide or a protein; examples of peptides include but are not limited to A T W S H H L S S A G L (SEQ ID No:3); W P Q L P P R P Y S T L (SEQ ID No:4); S N T S A G T P F T S L (SEQ ID No:5); D S T P K S T P W Y Y I (SEQ ID No:6); and conservatively-substituted variants thereof. Examples of small-molecule compounds are described below. The tissue or organ may be a dysproliferative tissue, such as a tumor or metastasis, or psoriasis, a tissue or organ involved in inflammatory diseases such as rheumatoid arthritis, the eye involved in neovascularization such as results from chronic diabetes, an abnormal growth such as keloid formation during wound healing, or an intentional disruption of cellular proliferation such as to prevent the genesis or maturation of a developing organ or tissue. Means for application of the agent to the desired site(s) are as described hereinabove.

The present invention is also directed to polynucleotide sequences comprising nucleic acids encoding the aforementioned antiproliferative peptide sequences, including vectors encoding the polynucleotides, as well as microorganisms and cells comprising the vectors and expressing the antiproliferative peptides. Therapeutic use of such polynucleotide sequences, including gene therapy with naked DNA, viral vectors, etc., and other means of transfecting cells to express the agents of the invention are embraced herein. In a preferred embodiment, the antiproliferative peptides and nucleic sequences encoding them are antiangiogenic peptides.

In a further aspect, the invention is directed to a method for identifying an agent capable of inhibiting cellular proliferation comprising the steps of a) providing a candidate agent; b) measuring the ability of the agent to bind to the extracellular domain of C-met; and c) measuring the ability of the agent to inhibit the antiproliferative activity of HGF/SF; wherein the candidate agent with the ability to bind to the extracellular domain of C-met and inhibit the proliferative activity of scatter factor is an antiproliferative agent. By way of non-limiting example, the agent may be a small-molecule drug, a peptide, or a protein.

In yet another aspect, a method of preparing an antiproliferative agent is provided comprising the steps of a) identifying an antiproliferative peptide as described above; b) determining the three-dimensional structure of said peptide; and c) modeling a small-molecule drug on the three-dimensional structure of the peptide.

In a further embodiment, the invention is directed to a method for identifying an agent capable of inhibiting angiogenesis comprising the steps of a) providing a candidate agent; b) measuring the ability of the agent to bind to the extracellular domain of C-met; and c) measuring the ability of the agent to inhibit the angiogenic activity of scatter factor; wherein the candidate agent with the ability to bind to the extracellular domain of C-met and inhibit the angiogenic activity of scatter factor is an angiostatic agent. By way of non-limiting example, the agent may be a small-molecule drug, a peptide, or a protein. In yet another aspect, a method of preparing an angiostatic agent is provided comprising the steps of a) identifying an angiostatic peptide as described above; b) determining the three-dimensional structure of said angiostatic peptide; and c) modeling a small-molecule drug on the three-dimensional structure of the angiostatic peptide.

The invention is also directed to small-molecule compounds that are agonists or antagonists of HGF/SF for all of the aforementioned uses of the agonist and antagonist peptides described above. Such agonist compounds of the invention are useful for mimicking or agonizing HGF/SF activity and are characterized by being non-peptide, non-protein organic molecules with one or more of the activities of promoting proliferation of endothelial cells in vitro or in vivo, promoting angiogenesis in vitro or in vivo, increasing angiogenesis in wounds in vivo, promoting the growth of tumor cells in vitro or in vivo, promoting scatter, promoting anti-apoptotic activity, or inducing gene expression of angiogenic-cascade-related genes such as but not limited to IL-8 and angiopoietin-2. Preferred are compounds in which the aforementioned activity is inhibited or competed in the presence of exogenously-added c-met receptor. The compounds may bind to c-met. The present invention embraces the use of all such molecules for treatment of various conditions or diseases in which increased or enhanced HGF/SF activity is desirable. In one embodiment, a compound of the invention has a molecular weight of under 1,000 Daltons, preferably above about 200 Daltons to about 1,000 Daltons; more preferably between about 300 Daltons and about 750 Daltons, and most preferably between about 300 Daltons and about 500 Daltons.

Thus, a method is provided for increasing hepatocyte growth factor / scatter factor (HGF/SF) activities in a mammal by administration to the mammal an effective amount of a compound having a molecular weight below about 1,000 Daltons, the compound exhibiting HGF/SF-like activity in at least one of the following HGF/SF activity assays:

induction of proliferation of endothelial cells in vitro or in vivo;
induction of angiogenesis in vitro or in vivo;
increasing angiogenesis in wounds in vivo;
promoting tumor growth;
inducing gene expression of angiogenic-cascade-related genes such as but not limited to IL-8 and angiopoietin-2;
inducing anti-apoptotic activity; or
inducing scatter activity.

In a preferred embodiment, the HGF/SF activity of the foregoing compound is inhibited in the presence of c-met. In another preferred embodiment, the compound binds to c-met.

A compound of the invention may exhibit HGF/SF-like activity in at least two of the aforementioned HGF/SF activity assays, or in at least three of the HGF/SF activity assays, or in at least four said HGF/SF activity assays, or in at least five of the HGF/SF activity assays, or in at least six of the HGF/SF activity assays or in all of the HGF/SF activity assays. The compound preferably has a molecular weight between about 200 Daltons and about 750 Daltons, more preferably between about 300 Daltons and about 500 Daltons.

In another embodiment, the invention is directed to a method for the prophylaxis or treatment in a mammal of hepatic disease, renal disease, bone regeneration, hair growth, promoting wound or tissue healing, promoting vascularization of a tissue, promoting vascularization of an ischemic tissue, promoting vascularization of a tissue susceptible to ischemia, or augmenting or restoring blood flow to ischemic tissues such as the heart following myocardial infarction comprising administered systemically or locally to particular tissues or organ in need thereof an effective amount of a compound having a molecular weight between below about 1,000 Daltons, the compound exhibiting HGF/SF-like activity in at least one HGF/SF activity assays:

induction of proliferation of endothelial cells in vitro or in vivo;
induction of angiogenesis in vitro or in vivo;
increasing angiogenesis in wounds in vivo;
promoting tumor growth;
inducing gene expression of angiogenic-cascade-related genes such as but not limited to IL-8 and angiopoietin-2;
inducing anti-apoptotic activity; or

inducing scatter activity.

In a preferred embodiment, the HGF/SF activity of the foregoing compound is inhibited in the presence of c-met.

A compound of the invention may exhibit HGF/SF-like activity in at least two of the aforementioned HGF/SF activity assays, or in at least three of the HGF/SF activity assays, in at least five said HGF/SF assays, in at least six said HGF/SF assays, or in at least four said HGF/SF activity assays, or in all of the HGF/SF activity assays. The compound preferably has a molecular weight between about 200 Daltons and about 750 Daltons, more preferably between about 300 Daltons and about 500 Daltons.

The invention is also directed to a method for inhibiting the activity of hepatocyte growth factor / scatter factor (HGF/SF) in a mammal comprising administering to the mammal an effective amount of a compound having a molecular weight below about 1,000 Daltons, the compound exhibiting HGF/SF inhibitory or antagonistic activity in at least one of the following HGF/SF activity assays:

inhibiting proliferation of endothelial cells in vitro or in vivo;
inhibiting the growth of tumor cells in vitro or in vivo;
inhibiting scatter of normal or tumor cells; or
inhibiting anti-apoptotic activity.

In a preferred embodiment, the HGF/SF inhibitory activity of the foregoing compound occurs in the presence of exogenously added HGF/SF or in cells or tissues in which HGF/SF is expressed or induced.

A compound of the invention may exhibit HGF/SF inhibitory activity in at least two of the aforementioned HGF/SF activity inhibition assays, or in at least three of the HGF/SF activity inhibition assays, or in all of the HGF/SF activity inhibition assays. The compound preferably has a molecular weight between about 200 Daltons and about 750 Daltons, more preferably between about 300 Daltons and about 500 Daltons.

In yet another embodiment, a method is provided for the prophylaxis or treatment in a mammal of a condition of disease selected from the group consisting of excessive cellular proliferation, angiogenesis, a dysproliferative disease, cancer, metastasis, inflammatory disease, diabetic retinopathy, inflammatory joint disease, and inflammatory skin disease comprising administering to a mammal an effective amount of a compound having a molecular weight below about 1,000 Daltons, said compound exhibiting HGF/SF inhibitory or antagonistic activity in at least one of the following HGF/SF activity inhibition assays:

inhibiting proliferation of endothelial cells in vitro or in vivo;
inhibiting the growth of tumor cells in vitro or in vivo;

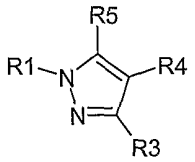
inhibiting scatter of normal or tumor cells; and

inhibiting anti-apoptotic activity.

In a preferred embodiment, the HGF/SF inhibitory activity of the foregoing compound occurs in the presence of exogenously added HGF/SF or in cells or tissues in which HGF/SF is expressed or induced.

A compound of the invention may exhibit HGF/SF inhibitory activity in at least two of the aforementioned HGF/SF activity inhibition assays, or in at least three of the HGF/SF activity inhibition assays, or in all of the HGF/SF activity inhibition assays. The compound preferably has a molecular weight between about 200 Daltons and about 750 Daltons, more preferably between about 300 Daltons and about 500 Daltons.

In another embodiment, the invention is directed to a method for the use for any of the aforementioned purposes of compounds that modulate HGF/SF activity with the general formula I:




Formula I

wherein

R3 and R5 are independently or together a straight-chain or branched C1-C6 alkyl optionally substituted with a cyano or halogen, halogen, trifluoromethyl or difluoromethyl groups;

R1 is hydrogen, methyl, CO-Aryl, $SO_2$-Aryl, CO-heteroaryl, or CO-alkyl; and

R4 is $CH_2$-Aryl, halogen, arylcarbonylvinyl or S-heteroaryl.

Certain of the compounds of Formula I are novel, and the present invention is also directed to all such novel compounds with an activity as described herein.

The invention is also directed to a pharmaceutical composition comprising at least one compound of Formula I and a pharmaceutically-acceptable carrier, for any of the uses described herein.

Non-limiting example of modulators of HGF/SF activity of Formula I include the following compounds, most of which, as will be seen in the examples below, exhibit HGF/SF agonist activity.

3-(5-chloro-1,3-dimethyl-1H-pyrazol-4-yl)-1-(4-chlorophenyl)prop-2-en-1-one

[4-(2,6-dichlorobenzyl)-3,5-dimethyl-1H-pyrazol-1-yl][3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl]methanone

(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(3-(2,6-dichlorophenyl)-5-

methylisoxazol-4-yl)methanone

4-(2-chloro-6-fluorobenzyl)-1-((3,4-dichlorophenyl)sulfonyl)-3,5-dimethyl-1H-pyrazole

4-(2-chloro-6-fluorobenzyl)-1,3,5-trimethyl-1H-pyrazole

4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole

(4-bromo-3,5-dimethyl-1H-pyrazol-1-yl)(3-(2,6-dichlorophenyl)isoxazole-4-carbohydrazide)

3-(4-(2,6-dichlorobenzyl)-3,5-dimethyl-1H-pyrazol-1-yl)propanenitrile

3,5-di(tert-butyl)-4-(2-chloro-6-fluorobenzyl)-1H-pyrazole

(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(2,6-dichlorophenyl)methanone

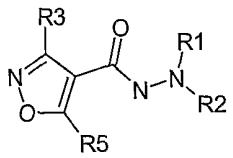
1-(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)2,2-dimethylpropan-1-one

(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(4-chlorophenyl)methanone

(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(2-thienyl)methanone

(4-chlorophenyl)(3,5-dimethyl-4-((1-methyl-1H-imidazol-2-yl)thio)-1H-pyrazol-1-yl)methanone

In another embodiment, the invention is directed to methods for the use for the aforementioned purposes of compounds that modulate HGF/SF activity with the general formula II:




Formula II

wherein

R5 is a C1 to C6 branched or straight-chained alkyl group;

R3 is a substituted or unsubstituted Aryl group;

R1 is hydrogen or a C1 to C4 straight-chained, branched or cycloalkyl group;

R2 is $COCH_2ONCH$-Aryl; heteroaryl, $COCH_2CH_2$Aryl; Aryl; COS-Aryl; CO-Heteroaryl; C1 to C4 straight-chained alkyl, branched alkyl, or cycloalkyl; or wherein R1 and R2 form a cyclic group of 5 or 6 carbon atoms.

Certain of the compounds of Formula II are novel, and the present invention is directed to all such novel compounds with an activity as described herein.

The invention is also directed to a pharmaceutical composition comprising at least one compound of Formula II, in a pharmaceutically-acceptable carrier, for any of the uses described herein.

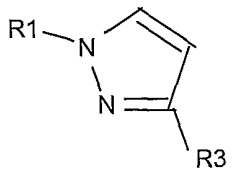
Most of the compounds of Formula II exhibit HGF/SF antagonist or inhibitory activity, as will be seen in the examples below. Non-limiting examples of compounds of Formula II include

N'4,5-dimethyl-N'4-(5-nitro-2-pyridyl)-3-(2,6-dichlorophenyl)isoxazole-4-carbohydrazide

N'4-(2-(((2,4-dichlorobenzylidene)amino)oxy)acetyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-

carbohydrazide
N'4-(3-(3,4,5-trimethoxyphenyl)propanoyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide
2-nitrophenyl 2-((3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)carbonyl)hydrazine-1-carbothioate
N'4-((2-methyl-1,3-thiazol-4-4yl)carbonyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-4carbohydrazide
N1-((2-((3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)carbonyl)hydrazino)(methylthio)methylidene)benzene-1-sulfonamide
N'4-(2,4,6-trichlorophenyl)-3-3(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide
N'4,3-di(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide
N'4-(3,5-dichloro-4-pyridyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide
N'4-phenyl-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide
N'4,N'4,5-trimethyl-3-(2,6-dichlorophenyl)isoxazole-4-carbohydrazide
N4-azepan-1-yl-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxamide
N'4-(6-(trifluoromethyl)-2-pyridyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide
N'4-(3,3-diethoxypropanoyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide

In a further embodiment, the invention is directed to methods for the use for any of the aforementioned purposes of compounds that modulate HGF/SF activity with the general formula III:




Formula III

wherein

R1 is $SO_2$Alkyl, $SO_2$-Aryl, CO-t-Butyl, COAryl, CONHAlkyl; CONHAryl; and

R3 is CHCH-heteroaryl; phenoxyphenyl; heteroaryl; or Aryl substituted heteroaryl.

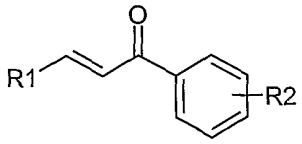
Certain of the compounds of Formula III are novel, and the present invention is directed to all such novel compounds with an activity as described herein.

The invention is also directed to a pharmaceutical composition comprising at least one compound of Formula III, in a pharmaceutically-acceptable carrier, for any of the uses described herein.

These compounds generally exhibit HGF/SF stimulatory or agonist activity. Non-limiting examples of compounds of Formula III include

(4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone;
1-(methylsulfonyl)-3-(2-(2-thienyl)vinyl)-1H-pyrazole;
2,2-dimethyl-1-(3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-yl)propan-1-one
N-methyl-3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-carboxamide
(4-chlorophenyl)(3-(3-phenylisoxazol-5-yl)-1H-pyrazol-1-yl)methanone
(4-chlorophenyl)(3-(3-(4-chlorophenyl)-5-methylisoxazol-4-yl)-1H-pyrazol-1-yl)methanone
(4-chlorophenyl)(3-(5-(2-thienyl)-2-thienyl)-1H-pyrazol-1-yl)methanone
(2,4-dichlorophenyl)(3-(5-(2,4-difluorophenyl)-2-furyl)-1H-pyrazol-1-yl)methanone
N1-phenyl-3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-carboxamide
(4-chlorophenyl)(3-(2-(5-(2-thienyl)-2-thienyl)-4-methyl-1,3-thiazol-5-yl)-1H-pyrazol-1-yl)methanone
(3-benzhydryl-1H-pyrazol-1-yl)(4-chlorophenyl)methanone
N1-(4-chlorophenyl)-3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-carboxamide
(4-chlorophenyl)(3-(2-methylimidazo(1,2-a)pyridin-3-yl)-1H-pyrazol-1-yl)methanone
2-chloro-6-(4-(1-(4-chlorobenzyl)-1H-pyrazol-3-yl)phenoxy)benzonitrile
1-((4-chlorophenyl)sulfonyl)-3-(2-(2-thienyl)vinyl)-1H-pyrazole

In a further embodiment, the invention is directed to methods for the use for any of the aforementioned purposes of compounds that modulate HGF/SF activity with the general formula IV:




Formula IV

Wherein

R1 is Aryl or Heteroaryl; and

R2 is one or more halogen, nitro, C1 to C4 straight-chained alkyl, branched alkyl, or cycloalkyl, or C1 to C4 alkyloxy groups.

Certain of the compounds of Formula IV are novel, and the present invention is directed to all such novel compounds with an activity as described herein.

The invention is also directed to a pharmaceutical composition comprising at least one compound of Formula IV, in a pharmaceutically-acceptable carrier, for any of the uses described herein.

The compounds in this group may be HGF/SF agonists or antagonists. Non-limiting examples of modulators of Formula IV include:

1-(4-chloro-3-methylphenyl)-3-(2,6-dichlorophenyl)-prop-2-en-1-one

1-(4-chloro-3-methylphenyl)-3-(2-chlorophenyl)prop-2-en-1-one

3-(2-chloro-6-fluorophenyl)-1-(4-chloro-3-methylphenyl)prop-2-en-1-one

3-(4-bromo-2-thienyl)-1-(3,4-dichlorophenyl)prop-2-en-1-one

3-(4-bromo-2-thienyl)-1-(4-chloro-3-methylphenyl)prop-2-en-1-one

3-(4-bromo-2-thienyl)-1-(4-fluorophenyl)prop-2-en-1-one

3-(4-bromo-2-thienyl)-1-(4-chlorophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-(2,4-dichlorophenyl)prop-2-en-1-one

3-(1,3-benzodioxol-5-yl)-1-(4-bromophenyl)prop-2-en-1-one

3-(3-phenoxy-2-thienyl)-1-(2-thienyl)prop-2-en-1-one

3-(3-bromo-4-methoxyphenyl)-1-phenylprop-2-en-one

3-(3,4-dichlorophenyl)-1-(2-nitrophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-(3,4-dichlorophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-(3,5-dichloro-2-hydroxyphenyl)prop-2-en-1-one

1-(2-chlorophenyl)-3-(3,5-dichloro-2-hydroxyphenyl)prop-2-en-1-one

3-(4-chlorophenyl)-1-(2,6-dichlorophenyl)prop-2-en-1-one

1-(4-bromophenyl)-3-(4-chlorophenyl)prop-2-en-1-one

1-(2-chlorophenyl)-3-(2,6-dichlorophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-(2,6-dichlorophenyl)prop-2-en-1-one

3-(2,6-dichlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one

3-(4-chloro-1-methyl-1H-pyrazol-3-yl)-1-[4-(trifluoromethyl)phenyl]prop-2-en-1-one

3-(2,4-dichlorophenyl)-1-(2-methylphenyl)prop-2-en-1-one

3-(2,6-dichlorophenyl)-1-(2-methylphenyl)prop-2-en-1-one

3-(3,4-dichlorophenyl)-1-(2-methylphenyl)prop-2-en-1-one

3-(5-bromo-2-hydroxyphenyl)-1-(3-methylphenyl)prop-2-en-1-one

3-(5-bromo-2-hydroxyphenyl)-1-(4-methylphenyl)prop-2-en-1-one

3-(2,4-dichlorophenyl)-1-(3-methylphenyl)prop-2-en-1-one

3-(2,4-dichlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one

1-[4-amino-2-(methylthio)-1,3-thiazol-5-yl]-3-(4-chlorophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-[4-(trifluoromethyl)phenyl]prop-2-en-1-one

1-benzo[b]thiophen-3-yl-3-(4-chlorophenyl)prop-2-en-1-one

1,3-di(5-nitro-3-thienyl)prop-2-en-1-one

1-(4-bromophenyl)-3-(3,5-difluorophenyl)prop-2-en-1-one

3-(3,5-difluorophenyl)-1-(3-nitrophenyl)prop-2-en-1-one

The foregoing compounds also have antagonistic activity to other tyrosine kinase receptor growth factors including VEGF and FGF, and may be used to inhibit such activities for the treatment of various conditions and diseases arising from the activities of these growth factors.

The foregoing general and specific structures of compounds with HGF/SF activity are merely illustrative of compounds of the invention with the desired activities, and are in no way limiting.

In a further embodiment, the invention is directed to pharmaceutical compositions comprising any one or a combination of the foregoing compounds, together with a pharmaceutically-acceptable carrier, for use in any of the aforementioned purposes.

In a further embodiment, the aforementioned compounds with activities of promotion of cellular proliferation or angiogenesis are useful for promoting vascularization of a tissue, particularly of an ischemic tissue or a tissue susceptible to ischemia.

Prophylaxis or treatment may be provided by contacting the tissue with an effective angiogenic amount of an agent of the invention. Contact may be provided by any appropriate means to deliver an effective amount of the agent for a duration to achieve the desired results. By way of non-limiting example, topical application may be applied to the desired target, or by infusion, bathing, or implantation of a sustained delivery device. For systemic administration, oral or parenteral routes may be employed. The target cells or tissue may be, for example, a transplanted or grafted tissue or organ such as skin, heart, vascular tissue or kidney, an ischemic organ, such as a heart following myocardial infarction or angina, a tissue or organ damaged by wounding, surgical intervention, vascular tissue, neural tissue, a wound, ulcer, etc. The cells may be, by way of non-limiting example, epithelial cells, endothelial cells, and smooth muscle cells, and tissues and organs comprising such cells. Promotion of growth and/or regeneration of neural tissue, teeth, and other tissues are embraced herein. Preferred cells, organs and tissues comprise the c-met receptor.

The aforementioned compounds with HGF/SF activity are also desirably useful for the treatment of various hepatic diseases including cirrhosis and liver failure; various renal diseases including renal failure. The compounds are also useful for inducing bone regeneration.

In another broad aspect of the invention, undesirable activities of HGF/SF *in vivo* may be therapeutically inhibited by the administration to a mammal of an effective amount of certain compounds of the invention for the treatment of various conditions and diseases generally involved in cellular proliferation and angiogenesis, among others. Inhibition of HGF/SF is desired, for example, in the treatment of dysproliferative diseases such as cancer and metastases, as well as various inflammatory diseases such as inflammatory joint and skin diseases. Other activities include but are not limited to inhibition of endothelial cell proliferation, inhibition of angiogenesis, angiostasis, tumoricidal activity, and any combination of the foregoing. In a further embodiment, the agents inhibit activity in the presence of exogenously-added or in cells in which activity is present or induced. Abnormal vascular proliferation such as occurs in diabetic retinopathy is also treatable by the methods of the invention.

The compounds of the invention useful for inhibiting HGF/SF activity are characterized by being small organic molecules or peptides with one or more of the activities of inhibiting proliferation of endothelial cells in vitro or in vivo, inhibiting the growth, scatter or metastasis of tumor cells in vitro or in vivo, inhibiting scatter, or inhibiting anti-apoptotic activity. Preferred are compounds in which such activities are exhibitable in the presence of exogenously-added HGF/SF. The present invention embraces the use of all such molecules for treatment of various conditions or diseases in which decreased or inhibited HGF/SF activity is desirable.

In addition, certain compounds of the invention have been found to have either antagonistic (activating) or antagonistic (inhibitory) activities directed not only to HGF/SF but also to VEGF and FGF, as will be seen in the examples below. The invention is also directed to use of these compounds to agonize or antagonize the activities of these growth factors, as well as to other factors which are tyrosine kinase receptors. In particular, the compounds 3,3-dibromo-1-phenyl-1,2,3,4-tetrahydroquinoline-2,4-dione and 4-(4-chlorophenyl)-6-(dimethylamino)-2-phenyl-5-pyrimidinecarbonitrile have VEGF-like activity and these compounds and structurally-related VEGF agonists or mimics are embraced herein for the treatment of various conditions and diseases for which VEGF would be useful for therapy in a mammal, preferably a human, such as but not limited to acceleration of wound healing, and in particular, diabetic wound healing. The compounds are generally useful for promoting proliferation of vascular endothelial cells and promoting vascularization, for such other uses as restenosis for treatment of coronary artery disease, angina and other ischemic diseases, including stroke.

These and other aspects of the present invention will be better appreciated by reference to the following drawings and Detailed Description.

## BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1** depicts the ability of angiogenic peptides of the invention to stimulate endothelial cell proliferation, as compared to HGF/SF, measured by the uptake of radiolabeled thymidine.

**Figure 2** shows the effects of angiogenic peptides and HGF/SF on angiogenesis in a rat aortic ring vascular sprouting assay.

**Figure 3** shows the effects of angiogenic peptides of the invention on angiogenesis in an in-vivo assay using basement membrane matrix implanted subcutaneously in mice.

**Figures 4A-4B** depict the promotion of angiogenic activity of a peptide of the invention when conjugated to a lysine-rich, heparan-sulfate-binding sequence or a control sequence. Figure 4A shows the effect on

endothelial cell proliferation compared to that of the conjugated peptide measured by radiolabeled thymidine incorporation; Figure 4B compared the activity to growth factors HGF/SF and bFGF.

**Figure 5** shows the ability of angiostatic peptides of the invention to inhibit HGF/SF-mediated increase in endothelial cell proliferation, measured by incorporation of radiolabeled thymidine.

**Figures 6A-6B** illustrate the angiostatic activity of peptides of the invention in a human glioblastoma cell line (U87, Figure 6A) and in a human glioma cell line (Hs 683, Figure 6B), the extent of tumor growth measured by incorporation of radiolabeled thymidine.

**Figure 7** depicts the stimulation of endothelial cell proliferation by (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone, a compound of the invention with HGF/SF-like activity, and the inhibition of the observed stimulation by inclusion of c-met.

**Figure 8 A-B** show the induction of scatter of MDCK cells by (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone.

**Figure 9** shows the protection of MDCK cells from adriamycin-induced apoptosis by (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone.

**Figure 10** shows a dose-response curve of the stimulation of endothelial cell proliferation by (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone.

**Figure 11** shows the ability of the compound 4-(2,6-dichlorobenzyl)-3,5-dimethyl-1H-pyrazol-1-yl)-3-(2,6-dichlorophenyl)-5-methanone on HGF/SF-mediated endothelial cell proliferation.

**Figure 12 A-B** shows the results from a Matrigel in-vivo assay using 1-(4-chloro-3-methylphenyl)-3-(2,6-dichlorophenyl)-prop-2-en-1-one and (4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)methanone, respectively.

**Figure 13** shows the results of a clonogenic assay using DU145 cells and (4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)methanone.

**Figure 14** shows the results of a clonogenic assay using DU145 cells and 1-(4-chloro-3-methylphenyl)-3-(2,6-dichlorophenyl)-prop-2-en-1-one.

**Figure 15** shows improved blood flow in mice following removal of the femoral artery after treatment with (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone.

**Figure 16 A-B** shows the effect of the compound 1-(4-chloro-3-methylphenyl)-3-(2,6-dichlorophenyl)-prop-2-en-1-one on inhibition of endothelial cell proliferation induced by the growth factors HGF/SF, VEGF and FGF at 1.5 micromolar (A) and 3.0 micromolar (B).

**Figure 17** shows the VEGF-like activity of two compounds of the invention.

**Figure 18** shows the stimulation of 3H-thymidine incorporation into HUVEC by (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone.

**Figure 19** depicts the phosphorylation of Erk by HGF/SF and (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone.

**Figure 20** demonstrated the efficacy of (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone in a pig wound healing model.

**Figure 21** shows the ability of (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone to increase capillary number in the ischemic mouse hindlimb.

**Figure 22** depicts the dose-dependent phosphorylation of HUVECs and MDCK cells by (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone.

**Figure 23 A-B** shows the increased survival time in mice by intra-tumor injection of 1-(4-chloro-3-methylphenyl)-3-(2,6-dichlorophenyl)-prop-2-en-1-one.

## DETAILED DESCRIPTION OF THE INVENTION

The agents and method of the invention are directed to modulation of cellular proliferation to provide new and effective agents and methods for the prophylaxis and/or treatment of various conditions and diseases in which limited cellular proliferation, or, in contrast, excessive proliferation of cells, is responsible for pathology, or at least for the prolongation of rebound from a pathological state to homeostasis. Using new identification methods, the inventors herein have found surprising and unexpected activity of various peptides as well as of small-molecule compounds, some of which promote, and others inhibit, cellular proliferation. The methods may be used to identify further agents with the aforementioned activities. By way of non-limiting examples, certain of such agents have been found to promote angiogenesis in vitro and in vivo; others inhibit angiogenesis in vitro and in vivo, and inhibit proliferation of dysproliferative tissues as evaluated in two cancer models. Additional activities are also seen. Moreover, and not to be bound by theory, the proliferation promoting and antiproliferative peptides identified herein are believed

to exert their effects by agonizing and antagonizing, respectively, the c-met receptor present on numerous cell types within the body, comprising various tissues and organs, such cells including but not limited to epithelial cells, endothelial cells, fibroblasts, neuronal cells, and smooth muscle cells. Tissues and organs comprising such cell types are targets for the various activities described herein. As the effects of the agents herein on a single cell type comprising a tissue or organ may account for the therapeutic goal described, the agents herein may have profound effects on tissues or organs whose cells expressing c-met comprise only a small fraction. The extent of expression of the target receptor does not detract from the utility of the agents and methods herein. The foregoing activities display agonist or antagonist activities of HGF/SF, and as such the peptides, small-molecule compounds and other compounds embraced herein may be considered to be HGF/SF agonists or antagonists. Furthermore, as will be seen below, the compounds of the invention also act as agonists or antagonists of other tyrosine kinase receptors, including but not limited to VEGF and FGF, and the present invention and identification of small-molecule agonists and antagonists extends to these receptors generally.

For example, the duration of wound healing, vascularization of a damaged and/or ischemic organs, transplants or grafts, normalization of myocardial perfusion as a consequence of chronic cardiac ischemia or myocardial infarction, development or augmentation of collateral vessel development after vascular occlusion or to ischemic tissues or organs, and vascularization of grafted or transplanted tissues, organs, or wound healing, may be accelerated by promoting cellular proliferation, particularly of vascular cells. Further utility is in the promotion of endothelial growth in vascular grafts and transplants.

In other cases where abnormal or excessive cellular proliferation is the cause of pathology, such as in dysproliferative diseases including cancer and psoriasis, various inflammatory diseases characterized by proliferation of cells such as atherosclerosis and rheumatoid arthritis, and neovascularization in the eye as a consequence of diabetic retinopathy, suppression cellular proliferation is a desired goal in the treatment of these and other conditions. As the antiproliferative agents of the invention have been found to possess antiproliferative activity on cells, as well as antiangiogenic activity, both activities may be beneficial in the treatment of, for example, solid tumors, in which both the dysproliferative cells and the enhanced tumor vasculature elicited thereby are targets for inhibition by the agents of the invention. In either case, therapy to promote or suppress proliferation may be beneficial locally but not systemically, and for a particular duration, and proliferation modulating therapies must be appropriately applied. The invention embraces localized delivery of such agents to the affected tissues and organs, to achieve a particular effect.

As noted above, modulating cellular proliferation, either by promoting the growth or new cells and/or formation of new blood vessels, or by inhibiting growth of cells and/or inducing destruction of existing vasculature, is a therapeutically-desirable goal for the prophylaxis or treatment of numerous conditions and diseases, including such major pathologies as myocardial ischemia, cancer, inflammatory joint and

skin diseases, diabetic retinopathy, and wound healing, as well as adjunctive therapy to increase the success rate of, for example, organ transplants and skin grafts. The examples provided herein below are merely illustrative of the range of utilities or proliferation promoting and antiproliferative agents, which include but are not limited to angiogenic and angiostatic agents; such uses are known to the skilled artisan; moreover, various citations referred to herein, and incorporated by reference, offer guides to certain of the uses mentioned here. For example, inhibition of cellular growth is desirable in the treatment of dysproliferative diseases including cancers, and the additional antiangiogenic activity especially of solid tumors is desirable, as such tumors not only require an enhanced blood supply to feed the tumor, but produce factors that stimulate tumor vascularization. Vascularization of the vitreous humor of the eye as a consequence of diabetic retinopathy is a major cause of blindness, and inhibition of such vascularization is desirable. Other conditions in which vascularization is undesirable include certain chronic inflammatory diseases, in particular inflammatory joint and skin disease, but also other inflammatory diseases in which a proliferative response occurs and is responsible for part of all of the pathology. For example, psoriasis is a common inflammatory skin disease characterized by prominent epidermal hyperplasia and neovascularization in the dermal papillae. Proliferation of smooth muscle cells, perhaps as a consequence of growth factors, is a factor in the narrowing and occlusion of the macrovasculature in atherosclerosis, responsible for myocardial ischemia, angina, myocardial infarction, and stroke, to name a few examples. Peripheral vascular disease and arteriosclerosis obliterans comprise an inflammatory component. Numerous diabetic complications such as atherosclerosis, and particularly diabetic nephropathy, characterized by basement membrane thickening and mesangial cell proliferation, are believed to have a component of cellular proliferation attendant to excessive production of growth factors as a consequence of chronic hyperglycemia. These examples of proliferative diseases are given by way of illustration only, and the theoretical basis for their etiology as proliferative processes is not intended to be limiting to the invention, and applicants have no duty to disclose or be bound by such disclosure.

Moreover, localized ablation of tissues or even organs using antiproliferative or antiangiogenic agents may find use in treatment of certain central nervous system diseases or conditions which otherwise may require dangerous invasive procedures; removal of cosmetically undesirable cutaneous lesions are further targets for the antiproliferative agents of the invention. In reproductive biology, such antiproliferative agents may be used as abortifacients or for non-surgical castration, particularly for use in livestock and domesticated animals. These are also merely illustrative of the uses of the instant agents.

On the other hand, poorly perfused tissues and organs, such as the heart as a sequela of myocardial infarction, as well as to promote wound healing, organ transplantation, acceleration of endothelial cell growth and vascularization of vascular grafts in order to promote integration of the graft, prevent graft failure due to reocclusion, and to enhance skin grafting, are desirable targets for increasing vascularization and uses of the angiogenic agents herein. Enhanced vascularization of a chronically ischemic organ is a therapeutically beneficial goal.

The term "angiogenesis," as used herein, refers to the formation of blood vessels. Specifically, angiogenesis is a multistep process in which endothelial cells focally degrade and invade through their own basement membrane, migrate through interstitial stroma toward an angiogenic stimulus, proliferate proximal to the migrating tip, organize into blood vessels, and reattach to newly synthesized basement membrane (see Folkman et al., Adv. Cancer Res., Vol. 43, pp. 175-203 (1985)). These processes are controlled by soluble factors and by the extracellular matrix (see Ingber et al., Cell, Vol. 58, pp. 803-805 (1985)).

Accordingly, an aspect of the present invention extends to methods for identifying agents with cellular proliferation promoting or antiproliferative activity, including but not limited to angiogenic or angiostatic (antiangiogenic) activity. Such agents are not limited to any particular structural or chemical class. As will be seen in the Examples below, the methods described herein were used to identify peptides as well as small-molecule compounds with agonist or antagonist activities, but such methods may be used to identify active agents in other structural classes. Furthermore, once such agents are identified, analysis of the three-dimensional structure may be used to model other small-molecule compounds with similar or increased activities, also as described below. Such methods, which often employ structural determination of the interactions between biomolecules and their ligands, such as by nuclear magnetic resonance spectroscopy or x-ray crystallographic methods, are known to the skilled artisan and may be applied to the identification of the sites of interaction between c-met and the peptides of the invention for the development of further compounds, which may be peptides or small-molecule, organic drugs, which mimic the interactions and activity of the peptides described herein. The present invention embraces methods for developing and screening small-molecule and other mimics of the instant compounds based upon the properties hereindescribed for the peptides of the invention.

The methods for identifying an proliferation promoting agent are based upon the ability of certain active agents, particularly peptides, to 1) bind to a monoclonal or polyclonal antibody to HGF/SF, and 2) exhibit proliferation promoting activity, such as angiogenic activity, in one or more in-vitro and/or in-vivo assays. Using these methods, which will be elaborated upon below, several peptides have been identified with potent proliferative and angiogenic activity.

Furthermore, antiproliferative agents, such as but not limited to angiostatic agents, may be identified by ability to 1) bind to the HGF/SF receptor, c-met, and 2) exhibit antiproliferative activity, such as but not limited to angiostatic activity, in one or more in-vitro or in-vivo assays. Using these methods, which will be elaborated upon below, several peptides have been identified with potent antiproliferative activity.

Before describing the particular aspects of the invention in more detail, the following discussion is applicable to all of the various aspects of the invention described herein, in particular the peptide and small-molecule compounds with agonist or antagonist activity.

The agents of the invention may be administered to the desired site in the body or target tissue or organ by any means that achieves the desired therapeutic effect. By way of non-limiting example, proliferation promoting agents including angiogenic agents may be administered locally, such as by injection or deposition in a target tissue or organ, or by the implantation of a controlled release delivery device or matrix containing the agent, to achieve local effects. Such sites may be accessed surgically, or via transcutaneous catheterization to gain access to a tissue or organ through the major vasculature. For example, enhancing the perfusion of the ischemic heart may be achieved by use of a transcutaneous catheter that may be positioned to release the angiogenic agent of the invention into the coronary vasculature.

For antiproliferative agents including antiangiogenic (angiostatic) agents, local administration of an agent at the desired site of activity, such as a tumor or the vitreous humor, may be carried out, or implantation of a controlled release delivery device containing an agent of the invention in the tumor or eye, may be desirable to achieve local effects. Surgical or transcutaneous methods may also be used. These and other means for contacting the agents of the invention with the desired target cells, tissue or organs will be readily apparent to the skilled artisan.

In yet another aspect of the present invention, provided are pharmaceutical compositions of the above agents. As noted above, the application and duration of application of the compounds of the invention may require particular local placement or delivery, for example, exposure of the antiproliferative compounds to a solid tumor or within the vitreous humor; and avoidance, for example, of systemic exposure. Exposure of proliferation promoting agents such as angiogenic compounds to a transplanted or ischemic tissue or organ is desirable without exposing such agents to other sites in the body. Such considerations, depending on the target cells, tissues or organs, whether the therapy is to promote or suppress proliferation, and the duration of exposure, may be determined readily by the skilled artisan. The formulation of the instant compounds in appropriate vehicles or carriers or drug delivery systems is also determinable by the skilled artisan, and all such methods of delivery are embraced herein. Examples are provided herein by way of illustration only, and are not intended to be limiting whatsoever.

Such pharmaceutical compositions may be for administration to a particular site by injection, catheterization or implantation, but may also be delivered for certain uses by other routes including oral, pulmonary, nasal or other forms of administration. In general, comprehended by the invention are pharmaceutical compositions comprising effective amounts of an agent or agents, or derivative products, of the invention together with pharmaceutically acceptable diluents, preservatives, solubilizers,

emulsifiers, adjuvants and/or carriers. Such compositions may include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (e.g., Tween 80, Polysorbate 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol). The foregoing examples are merely illustrative and non-limiting, as the skilled artisan will be amply aware of suitable excipients and other component of a pharmaceutical composition comprising one or more agents of the invention.

For controlled delivery, incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes may be used, or the use of a controlled release device, such as an implantable osmotic or other type of pump. Another form of a controlled release of this therapeutic is by a method based on the Oros therapeutic system (Alza Corp.), i.e. the drug is enclosed in a semipermeable membrane which allows water to enter and push drug out through a single small opening due to osmotic effects. Likewise, the skilled artisan will be amply aware of suitable delivery methods that may be extended to the agents of the invention to achieve the intended therapeutic goals of the invention. Such local release may be desirably, for example, with antiproliferative agents for treatment of a tumor or abnormal vascularization in the eye; and proliferative agents at the site of grafts or transplants.

In another embodiment of the invention, cells of the body may be transfected with a polynucleotide encoding the various peptides of the invention, or a polynucleotide encoding a protein which comprise a peptide agent of the invention, including degenerate polynucleotides which encode the same peptides or proteins comprising the aforementioned peptides. Furthermore, such degenerate polynucleotides may be optimized for expression in the target mammalian cells. The transfection carried out by any number of means, for example, using a viral vector, wherein the transfected cells express and secrete the peptide with attendant local effects. The DNA, virus, or other conveyance or vector for the polynucleotide can be administered to the target site by catheter or other means. Such vectors include an attenuated or defective DNA virus, such as but not limited to herpes simplex virus (HSV), papillomavirus, Epstein Barr virus (EBV), adenovirus, adeno-associated virus (AAV), lentivirus and the like. Defective viruses, which entirely or almost entirely lack viral genes, are preferred. Defective virus is not infective after introduction into a cell. Use of defective viral vectors allows for administration to cells in a specific, localized area, without concern that the vector can infect other cells. Thus, particular tissues can be specifically targeted. Examples of particular vectors include, but are not limited to, a defective herpes virus 1 (HSV1) vector [Kaplitt et al., Molec. Cell. Neurosci. 2:320-330 (1991)], an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet et al. [J. Clin. Invest. 90:626-630 (1992)], and a defective adeno-associated virus vector [Samulski et al., J. Virol. 61:3096-3101 (1987); Samulski et al., J. Virol. 63:3822-3828 (1989)]. Alternatively, the vector can be introduced in vivo by lipofection. For the past decade, there has been increasing use of liposomes for encapsulation and transfection of

nucleic acids in vitro. Synthetic cationic lipids designed to limit the difficulties and dangers encountered with liposome mediated transfection can be used to prepare liposomes for in-vivo transfection of a gene encoding a marker [Felgner, et. al., Proc. Natl. Acad. Sci. U.S.A. 84:7413-7417 (1987); see Mackey, et al., Proc. Natl. Acad. Sci. U.S.A. 85:8027-8031 (1988)]. The use of cationic lipids may promote encapsulation of negatively charged nucleic acids, and also promote fusion with negatively charged cell membranes [Felgner and Ringold, Science 337:387-388 (1989)]. The use of lipofection to introduce exogenous genes into the specific organs in vivo has certain practical advantages. Molecular targeting of liposomes to specific cells represents one area of benefit. It is clear that directing transfection to particular cell types would be particularly advantageous in a tissue with cellular heterogeneity, such as pancreas, liver, kidney, and the brain. Lipids may be chemically coupled to other molecules for the purpose of targeting [see Mackey, et. al., supra]. Targeted peptides, e.g., hormones or neurotransmitters, and proteins such as antibodies, or non-peptide molecules could be coupled to liposomes chemically.

It is also possible to introduce the vector in vivo as a naked DNA plasmid. Naked DNA vectors for gene therapy can be introduced into the desired host cells by methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun, or use of a DNA vector transporter [see, e.g., Wu et al., J. Biol. Chem. 267:963-967 (1992); Wu and Wu, J. Biol. Chem. 263:14621-14624 (1988); Hartmut et al., Canadian Patent Application No. 2,012,311, filed March 15, 1990].

In a preferred embodiment of the present invention, a gene therapy vector as described above employs a transcription control sequence operably associated with the sequence for the angiogenic or angiostatic peptide inserted in the vector. That is, a specific expression vector of the present invention can be used in gene therapy.

In addition, cells or tissues may be transfected to express a protein of the invention or a protein comprising a peptide of the invention, and then implanted at the desired site. Such cells may be, for example, derived from a patient's own body such that acceptance of the cells will occur. Alternatively, exogenous cells may be used. Such patient-derived or exogenous cells may be prepared such that they may be selectively activated or destroyed, for example, by sensitivity to a particular drug, or expression activated by a particular drug, such that the secretion of the desired agent may be initiated, maintained, or terminated as appropriate for the duration of appropriate therapy. The ability to engineer such cells with the appropriate promoters and sensitivity markers is known in the art.

As noted above, the peptides of the invention may be conservatively substituted, wherein functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a conservative amino acid substitution. Such alterations define the term "a conservatively-substituted variant" as used herein. For example, one or more amino acid residues within the sequence can be substituted by another

amino acid of a similar polarity, which acts as a functional equivalent. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. Amino acids containing aromatic ring structures are phenylalanine, tryptophan, and tyrosine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Such alterations will not be expected to affect apparent molecular weight as determined by polyacrylamide gel electrophoresis, or isoelectric point.

Particularly preferred conservative substitutions are:

- Lys for Arg and vice versa such that a positive charge may be maintained;
- Glu for Asp and vice versa such that a negative charge may be maintained;
- Ser for Thr such that a free -OH can be maintained; and
- Gln for Asn such that a free $NH_2$ can be maintained.

It is noted that all amino acid sequences are written starting with the amino-terminal residue and ending with the carboxy-terminal residue. Moreover, sequences are provided using one-letter or three-letter abbreviations.

The appropriate effective dosage of an agent of the invention, whether a peptide, small-molecule compound, DNA vector, or other active agent, may be readily determinable by following standard methods. Several animal models are described herein which model conditions and diseases encountered in the clinical setting, and as part of a drug development process, efficacious doses in animal studies, in particular, dose-response studies, are translated into appropriate doses for testing in humans, by following guidelines well known to those skilled in the art. Thus, an effective dose in a human may be determined following such industry-standard guidelines.

In another embodiment, a conjugate between the proliferation promoting or antiproliferative agent of the invention, particularly a peptide agent, and another moiety may be provided to enhance particular characteristics of the agent, such as but not limited to targeting, delivery, persistence in the body or at the site of administration, etc. As shown in the examples below, a heparan sulfate-binding polypeptide was added to the carboxy-terminus of an angiogenic peptide (carried out by solid-phase peptide synthesis), and the resultant polypeptide exhibited superior angiogenic activity. Thus, the present invention embraces fusion peptides or other conjugates between the peptides of the invention and another peptide sequence, which may be administered as described hereinabove, or cells in the body may be transfected with a polynucleotide sequence comprising the fusion peptide resulting in expression of the agent within the body. Thus, the agents of the invention need only comprise as a portion thereof an active agent of the invention to be embraced herein.

Identification of Proliferation Promoting Agents

In accordance with the invention, proliferation promoting agents and in particular peptide agents may be identified by having the properties of 1) binding to an antibody to HGF/SF; and 2) having proliferative activity such as but not limited to angiogenic activity in one or more in-vitro or in-vivo assays. Binding to an antibody to HGF/SF refers to agents, including but not limited to peptides and proteins, which comprise an epitope recognized by an monoclonal or polyclonal antibody to HGF/SF. A monoclonal antibody is preferred. Non-limiting examples of such antibodies include a polyclonal rabbit anti-HGF/SF antibody (designated 813), and a monoclonal anti-HGF/SF antibodies (e.g., clone 23C2). Means for assessing the binding includes various immunochemical methods known to one of skill in the art. Binding may be measured by binding of the antibody to the agent, wherein the agent is conjugated to another entity, such as the phage display method described in the examples below. Alternatively, the binding to the anti-HGF/SF antibody may be assessed by measuring competition by the agent in binding of the antibody to HGF/SF, any peptide mimetic of HGF/SF, or any agent identified by the methods herein as an proliferation promoting agent. Thus, small-molecule drugs on the order of the size of a hapten may be identified as interfering with the binding of the antibody to its binding partner.

Moreover, an activity that may be but not necessarily exhibited by the proliferation promoting agents of the invention is the agonism of c-met, the HGF/SF receptor. Thus, an assay for such agonism may be included in the methods for identifying such active agents.

In-vivo and in-vitro assays for proliferation promoting including angiogenic agents are known to the skilled artisan; several non-limiting examples are described in the Examples, below. Appropriate cells for such assays express c-met.

In one non-limiting example of the practice of the invention, randomly-generated 12-amino acid peptides expressed in a phage display system were screened for binding to either a monoclonal antibody or polyclonal antibody to HGF/SF, and the phages expressing a peptide binding to the antibody were amplified, and rescreened, and after three rounds, twenty positive clones were selected. Other methods for generating peptides, as well as small-molecule drugs, are useful as well. The assay for binding to an antibody to HGF/SF may be carried out as above, or in a screen in which direct binding of the candidate agent to the antibody is assessed, or in the case of small molecules or haptens, the assay for binding to an anti-HGF/SF antibody may be carried out by measuring the inhibition by the candidate molecule of the binding of the antibody to either intact HGF/SF, a fragment of the molecule comprising the epitope recognized by the antibody, or inhibition of binding of the antibody to one of the peptides or other small molecules previously identified as an proliferation promoting or angiogenic compound by the methods described herein. As known to one of skill in the art, standard immunoassay procedures, including ELISA techniques, can be used, for example, with a bound antibody and labeled binding partner to the antibody.

After screening of compounds to identify those capable of binding to the anti-HGF/SF antibody or interfering with the binding of the antibody with an angiogenic molecule, the candidate compound is evaluated in one or more functional proliferation and/or angiogenesis assays. Such assays include but are not limited to an endothelial cell proliferation assay, which measured the effect of the compound on the proliferation of endothelial cells in vitro. Other assays include proliferation of cells which express c-met, such as but not limited to endothelial cells, epithelial cells, neuronal cells, and smooth muscle cells. Such methods are known in the art, examples of which are described in the examples below. Another assay is the vascular sprouting or aortic ring assay, in which the outgrowth of endothelial cells from aortic rings embedded in collagen gels is evaluated. In an in-vivo assay, a solid gel comprising basement membrane and the test sample is implanted subcutaneously in mice, and after a period of time, ingrowth of blood vessels into the matrix is determined histologically. As noted in the Examples below, two peptides T M G F T A P R F P H Y (SEQ ID No:1) and K V W Y H T T S I P S H (SEQ ID No:2) were identified as having activity in the angiogenesis assays.

Moreover, once agents have found, three-dimensional structural analysis may be used to model other compounds, including small-molecule drugs, as described herein.

In another embodiment, the proliferation promoting peptides may be modified to enhance activity. One non-limiting means is by conjugation of active peptides to a heparan sulfate binding polypeptide. Such modification includes chemical conjugation, co-expression on the same peptide chain; i.e., linkage through a peptide bond, etc. By way of non-limiting example, the lysine-rich sequence K V W Y H T T S I P S H C R P K A K A K A K A K D Q T K (SEQ ID No:7) was added to the carboxy-terminus of SEQ ID No:2 (forming SEQ ID No:10). As shown in the Examples, the angiogenic activity of the conjugate was about three-fold higher than a control conjugate in which the same angiogenic peptide was conjugated to a non-specific sequence with the same amino acid composition but without heparan sulfate-binding activity (Y H T T S I P S H C Q K A K T R A K A A K P D K K [SEQ ID No:8]), and had comparable or greater than the mitogenic activity of SF and bFGF. These data show that the growth promoting and possibly the angiogenic activity of SEQ ID No:2 can be enhanced by increasing its affinity to heparin.

Use of Proliferation promoting Agents

As described above, the proliferation promoting agents including angiogenic agents of the invention may be used to promote endothelial cell and microvessel growth, with the goal of increasing vascularization and perfusion of tissues and organs in the body. They may also be used to promote growth of other cells types, such as those expressing c-met. Also as noted above, the agents may be locally applied or delivered to the desired site or sites. While the invention embraces any and all uses of angiogenic an generally proliferation promoting agents for humans and other mammals, some examples include

treatment of ischemic tissues and organs, such as after injury, including myocardial damage after a heart attack, promoting vascularization of transplanted, reattached or translocated tissues or organs, such as following organ transplants, traumatic injury, promotion of wound healing, skin and other organ grafting, to name some examples. They are particularly useful for promoting the growth of endothelial cells in vascular grafts and transplants.

The agents of the invention may be formulated into suitable pharmaceutical compositions for administration. As noted above, such compositions and methods of administration include the transfection of cells in the target tissue or organ of the body with a vector, such as a viral vector, or naked DNA, to induce expression of the agents of the invention in situ, including the implantation of cells expressing an agent or agents of the invention to act locally upon a desired target to continuously produce the agent. Such cells may have engineered susceptibility to, for example, a drug such that at the conclusion of desired therapy, the cells expressing the agent may be readily destroyed.

Identification of Antiproliferative Agents

Agents with antiproliferative activity, including angiostatic activity, may be identified by their ability to bind to the HGF/SF receptor c-met. As will be shown in the Examples below, a phage display peptide library as described herein which expresses a combinatorial library of 12-mers fused to the pIII coat protein were screened for binding to the extracellular domain of HGF/SF receptor C-met. Following this procedure, four peptides were identified and synthesized by solid-phase synthesis: A T W S H H L S S A G L (SEQ ID No:3); W P Q L P P R P Y S T L (SEQ ID No:4); S N T S A G T P F T S L (SEQ ID No:5); and D S T P K S T P W Y Y I (SEQ ID No:6). The ability of these peptides to stimulate endothelial proliferation or inhibit HGF/SF-mediated increase in endothelial proliferation was subsequently determined. The peptides did not significantly affect endothelial growth, but completely inhibited the SF-mediated increase in endothelial growth. These data indicate that met peptides can bind HGF/SF receptor C-Met thereby inhibit HGF/SF binding. Thus, these peptides may have potential angiostatic activity. Moreover, inhibition of growth of several tumor cells in vitro in the absence of a tumor vasculature demonstrates that the antiproliferative agents of the invention have general antiproliferative activity, and not necessarily only antiangiogenic activity. Both activities are useful for the treatment of certain conditions, such as a solid tumor with both tumor cellular and vascular targets.

These peptides and conservatively-substituted variants thereof may be provided in pharmaceutical compositions for various uses to inhibit growth and proliferation of cells, tissues and organs, as well as inhibit vascularization of tissues and organs, as described above. Also noted above, the foregoing methods may be used to identify agents other than peptides with antiproliferative including angiostatic activity, by identifying compounds with the property of binding to c-met, and exhibiting antiproliferative, such as but not limited to angiostatic, activity. Moreover, once agents have found, three-dimensional structural analysis may be used to model other compounds, including small-molecule drugs.

The antiproliferative peptides of the invention were evaluated in two tumor models: human glioma and glioblastoma cell lines. These data show that C-met peptides each alone or in combination can block endogenous HGF/SF activity and associated tumor growth.

Use of Antiproliferative Agents

Expression of scatter factor (HGF/SF), and its receptor, c-met, is often associated with malignant progression of human tumors, including gliomas. Overexpression of HGF/SF in experimental gliomas enhances tumorigenicity and tumor-associated angiogenesis (i.e., growth of new blood vessels). More recent studies showed that human glioblastomas are HGF/SF-c-met dependent and that a reduction in endogenous HGF/SF or c-met expression can lead to inhibition of tumor growth and tumorigenicity. Thus, targeting the HGF/SF-c-met signaling pathway may be an important approach in controlling tumor progression.

Examples of cancers, tumors, malignancies, neoplasms, and other dysproliferative diseases that can be treated according to the invention include leukemias such as myeloid and lymphocytic leukemias, lymphomas, myeloproliferative diseases, and solid tumors, such as but not limited to sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

The present invention is also directed to treatment of non-malignant tumors and other disorders involving inappropriate cell or tissue growth by administering a therapeutically effective amount of an agent of the invention. For example, it is contemplated that the invention is useful for the treatment of arteriovenous (AV) malformations, particularly in intracranial sites. The invention may also be used to treat psoriasis, a dermatologic condition that is characterized by inflammation and vascular proliferation; benign prostatic hypertrophy, a condition associated with inflammation and possibly vascular proliferation; and cutaneous fungal infections. Treatment of other hyperproliferative disorders is also contemplated. The agents may also be used topically to remove warts, birthmarks, moles, nevi, skin tags, lipomas, angiomas including hemangiomas, and other cutaneous lesions for cosmetic or other purposes.

As noted above, other uses of the agents herein include intentional ablation or destruction of tissues or organs in a human or animal, for example, in the area of animal husbandry, and in the field of reproductive biology, to reduce the number of developing embryos; as an abortifacient, and as a means to achieve a biochemical castration, particularly for livestock and domesticated animals such as pets.

The agents of the invention may be formulated into suitable pharmaceutical compositions for administration. As noted above, such compositions and methods of administration include vectors and microorganisms including cells expressing the peptide agents of the invention and larger polypeptides comprising the peptides, and extend to the transfection of cells in the target tissue or organ of the body with a vector, such as a viral vector, or naked DNA, to induce expression of the agents of the invention in situ, including the implantation of cells expressing an agent or agents of the invention to act locally upon a desired target to continuously produce the agent. Such cells may have engineered susceptibility to, for example, a drug such that at the conclusion of desired therapy, the cells expressing the angiogenic agent may be readily destroyed.

In addition to the peptide agents of the invention described above, the inventors herein have identified various small organic molecules of molecular weight below about 1,000 Daltons with the ability to either mimic or antagonize the biological activities of various growth factors that bind the tyrosine kinase receptor, such as hepatocyte growth factor / scatter factor (HGF/SF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF). The present invention is directed to methods for the modulation of the various activities exhibited by such growth factors, for example, hepatocyte growth factor / scatter factor (HGF/SF), using small non-protein or non-peptide molecules. The inventors herein have identified for the first time small organic molecules that either mimic or have HGF/SF, VEGF or FGF activities, as well as those that are capable of inhibiting or antagonizing the activities of HGF/SF, VEGF and FGF. Thus, small molecule compounds having the HGF/SF-like or HGF/SF-inhibitory activities are described and their uses for the treatment of various conditions and diseases embraced herein. With the attendant difficulties in administering protein therapeutic agents at a desired level and for a duration effective to achieve acute and particularly chronic therapeutic goals, notwithstanding the cost of manufacture, the discovery by the inventors herein of small molecules with the desirable growth factor modulating activities offers pharmaceutically-desirable means to address a large number of conditions and diseases heretofore poorly or minimally treatable with available therapies.

Small-molecule, non-protein or non-peptide compounds with HGF/SF-like activity are characterized by one or more of the following activities: promoting proliferation of endothelial cells in vitro or in vivo, promoting angiogenesis in vitro or in vivo, promoting angiogenesis in wounds in vivo, promoting the growth of tumor cells in vitro or in vivo, promoting scatter, promoting anti-apoptotic activity, or inducing gene expression of angiogenic-cascade-related genes such as but not limited to IL-8 and angiopoietin-2.

The modulator compounds of the invention, whether exhibiting HGF/SF-like activity, herein referred to interchangeably as HGF/SF agonist activity, or exhibiting HGF/SF inhibitory activity, herein referred to interchangeably as HGF/SF antagonist activity, are by theory acting through the HGF/SF receptor c-met. While Applicants have no duty to disclose the theory by which the compounds of the invention are operating and are not bound thereto, the small-molecule compounds of the invention modulate c-met activity, and bind to c-met. Preferred are compounds in which the aforementioned activity is inhibited or competed in the presence of exogenously-added c-met receptor. The skilled artisan can readily identify such compounds by carrying out the foregoing assays as described in the examples below, and the present invention embraces the use of any and all such compounds for the purposes described herein.

While the discussions herein describe the activities of HGF/SF agonists and antagonists, the skilled artisan will recognize, based on the studies described herein using other growth factors, that similar uses are afforded the agonists and antagonist compounds of the invention for these other growth factors.

Small-molecule, non-protein or non-peptide compounds with HGF/SF-antagonist activity are characterized by one or more of the following activities: inhibiting proliferation of endothelial cells in vitro or in vivo, inhibiting the growth, scatter or metastasis of tumor cells in vitro or in vivo, inhibiting scatter, or inhibiting anti-apoptotic activity. Preferred are compounds in which such activities are exhibitable in the presence of exogenously-added HGF/SF. The skilled artisan can readily identify such compounds by carrying out the foregoing assays as described in the examples below, and the present invention embraces the use of any and all such compounds for the purposes described herein.

The small organic molecules of the invention preferably have a molecular weight below 1,000 Daltons and more preferably of about 200 Daltons to about 1,000 Daltons; most preferably between about 300 Daltons and about 750 Daltons, and even most preferably between about 300 Daltons and about 500 Daltons. Moreover, the compounds preferably are not proteins or peptides, but may fall into any other class of organic molecule.

Compounds with HGF/SF activity are therapeutically useful for the treatment of numerous conditions and diseases in many but not all cases related to enhancement of cellular proliferation or vascular proliferation (angiogenesis). These have been described in detail above. One aspect of the invention embraces the uses of the small molecule compounds described herein for the treatment of these conditions and diseases. These conditions and diseases are related to organ dysfunction and regeneration, reducing duration of wound healing, normalization of myocardial perfusion as a consequence of chronic cardiac ischemia or myocardial infarction, development or augmentation of collateral vessel development after vascular occlusion of or to ischemic tissues or organs, and vascularization of grafted or transplanted tissues, organs, or wound healing. These desired activities may be accelerated by administration of a compound of the invention. For example, promoting cellular proliferation, particularly of vascular cells, may be

applied to the treatment of an ischemic, damaged or transplanted organ. Prophylaxis or treatment may be provided by contacting the tissue with an effective angiogenic amount of a compound of the invention. Contact may be provided by any appropriate means to deliver an effective amount of the agent for a duration to achieve the desired results. By way of non-limiting example, topical application may be applied to the desired target, or by infusion, bathing, or implantation of a sustained delivery device. For systemic administration, oral or parenteral routes may be employed. The target cells or tissue may be, for example, the liver or kidney, a transplanted or grafted tissue or organ such as skin, heart, vascular tissue or kidney, an ischemic organ, such as a heart following myocardial infarction or angina, a tissue or organ damaged by wounding, surgical intervention, vascular tissue, neural tissue, a wound, ulcer, etc. The cells may be, by way of non-limiting example, epithelial cells, endothelial cells, and smooth muscle cells, and tissues and organs comprising such cells. Promotion of growth and/or regeneration of neural tissue, teeth, and other tissues are embraced herein. Preferred cells, organs and tissues comprise the c-met receptor. The aforementioned compounds with HGF/SF activity are also desirably useful for the treatment of various hepatic diseases including cirrhosis and liver failure; various renal diseases including renal failure. The compounds are also useful for inducing bone regeneration.

In one preferred embodiment, treatment of the endothelial cell dysfunction, vasculopathy and wound healing dysfunction that typifies diabetes mellitus is among the uses of the methods and compounds herein.

Thus, one aspect of the invention is directed to methods for the prophylaxis or treatment of a condition or disease in a mammal in which HGF/SF activity is desired or increased activity is desired comprising administering to the mammal an effective amount of a small-molecule compound with HGF/SF activity. The HGF/SF activity of a small-molecule compound of the invention is inhibited or blocked in the presence of or by preincubation with c-met receptor.

In another broad aspect of the invention, compounds which antagonize HGF/SF activity have therapeutically-desirable properties for the treatment of conditions and diseases in which HGF/SF activity is undesirable. The use of any and all such small-molecule compounds is embraced herein. For example, abnormal cellular proliferation such as occurs in dysproliferative diseases such as various cancers and psoriasis, are such amenable conditions. Other conditions include inflammatory diseases, which exhibit a proliferative component, such as the intimal thickening and smooth muscle cell proliferation in atherosclerosis, among other inflammatory conditions.

Thus, another embodiment of the invention is directed to methods for the prophylaxis or treatment of a condition or disease in a mammal in which HGF/SF activity is not desired or decreased activity is desired comprising administering to the mammal an effective amount of a small-molecule compound with HGF/SF antagonist activity. The HGF/SF antagonist activity of a small-molecule HGF/SF antagonist

compound of the invention may occur alone or only in the presence of exogenously-added HGF/SF or in cells or tissues in which HGF/SF is expressed.

Expression of scatter factor (HGF/SF), and its receptor, c-met, is often associated with malignant progression (metastasis) of human tumors, including gliomas. Overexpression of HGF/SF in experimental gliomas enhances tumorigenicity and tumor-associated angiogenesis (i.e., growth of new blood vessels). More recent studies showed that human glioblastomas are HGF/SF-c-met dependent and that a reduction in endogenous HGF/SF or c-met expression can lead to inhibition of tumor growth and tumorigenicity. Thus, targeting the HGF/SF-c-met signaling pathway using a compound as characterized above is an important approach in controlling tumor progression.

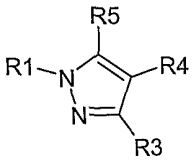
The present invention is also directed to treatment of non-malignant tumors and other disorders involving inappropriate cell or tissue growth by administering a therapeutically effective amount of an agent of the invention. For example, it is contemplated that the invention is useful for the treatment of arteriovenous (AV) malformations, particularly in intracranial sites. The invention may also be used to treat psoriasis, a dermatologic condition that is characterized by inflammation and vascular proliferation; benign prostatic hypertrophy, a condition associated with inflammation and possibly vascular proliferation; and cutaneous fungal infections. Treatment of other hyperproliferative disorders is also contemplated. The agents may also be used topically to remove warts, birthmarks, moles, nevi, skin tags, lipomas, angiomas including hemangiomas, and other cutaneous lesions for cosmetic or other purposes.

The small-molecule HGF/SF activity modulator compounds of the invention fall generally into four groups, as described below. As used herein, the term "alkyl" means straight-chain, branched-chain or cyclo saturated aliphatic hydrocarbon groups preferably containing from one to about 6 carbon atoms. Representative of such straight-chain groups are methyl, ethyl, butyl, pentyl, hexyl and the like. Examples of branched-chain groups include isopropyl, isobutyl and t-butyl. Cycloalkyl includes groups such as but not limited to cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. The term "aryl" refers to, for example, phenyl, biphenyl and naphthyl groups, which are optionally substituted by one or more halogen (F, Cl, Br and I), C1 to C4 alkyl, or C1 to C4 alkyloxy, where alkyloxy refers to an alkyl group as defined above attached to the remainder of the molecule by oxygen. Examples of alkyloxy include methoxy, ethoxy, propoxy, isopropoxy and the like. The term "heteroaryl" refers to heterocyclic groups containing 4-10 ring members and 1-3 heteroatoms selected from the group consisting of oxygen, nitrogen and sulfur. Examples include but are not limited to isoxazolyl, phenylisoxazolyl, furyl, pyrimidinyl, quinolyl, tetrahydroquinolyl, pyridyl, imidazolyl, pyrrolidinyl, 1,2,4-triazoylyl, thiazolyl, thienyl, and the like. The aryl or heteroaryl group may be optionally substituted by one or more halogen (F, Cl, Br and I), C1 to C4 alkyl, C1 to C4 alkyloxy as described above, trifluoromethyl, difluoromethyl, nitro, hydroxy, amine (optionally alkyl substituted), or another aryl or another heteroaryl group as described above.

The organic compounds described herein with HGF/SF-like agonist or antagonist activity are merely illustrative of compounds which modulate one or more of the activities of HGF/SF and the uses of which are embraced herein.

Among the compounds and the formulae described below, certain of such compounds are known, and others are heretofore undescribed. The present invention embraces all such novel compounds with one or more of the activities hereindescribed, as well as pharmaceutical compositions comprising such compounds.

In one embodiment, the invention is directed to the use for any one or more of the aforementioned purposes of compounds that modulate HGF/SF activity with the general formula I:




Formula I

wherein

R3 and R5 are independently or together a straight-chain or branched C1-C6 alkyl optionally substituted with a cyano or halogen, halogen, trifluoromethyl or difluoromethyl groups;

R1 is hydrogen, methyl, CO-Aryl, $SO_2$-Aryl, CO-heteroaryl, or CO-alkyl; and

R4 is $CH_2$-Aryl, halogen, arylcarbonylvinyl or S-heteroaryl.

The definitions of the substituents are as described hereinabove. R3 and R5 preferably may be methyl, t-butyl or chloro groups. The aryl group of substituent R1 is preferably an aromatic group such as phenyl, naphthyl, or biphenyl, substituted with one or more halogen, C1 to C4 alkyl or C1 to C4 alkyloxy groups. The heteroaryl group of substituent R1 preferably is a 3-aryl-substituted isoxazole or 3-aryl-substituted thienyl group. The alkyl group of substituent R1 preferably is t-butyl, or a C1-C6 straight, branched or cycloalkyl group. In a most preferred embodiment, R3 is methyl, R5 is chloro, R1 is methyl, and R4 is 4-chlorophenylcarbonylvinyl group.

Certain of the compounds of Formula I are novel, and the present invention is directed to all such novel compounds. The invention is also directed to a pharmaceutical composition comprising at least one compound of Formula I, in a pharmaceutically-acceptable carrier, for any of the uses described herein.

The invention is also directed to methods for the prophylaxis or treatment of a condition or disease in a mammal wherein the effects of HGF/SF would be beneficial by administering to the mammal an effective amount of a pharmaceutical composition comprising an active HGF/SF agonist of Formula I above. As

mentioned above, such activities include but are not limited to promoting the proliferation of cells, including endothelial cells, vascular cells, hepatic cells, renal cells, among others; promoting angiogenesis; promoting vascularization; promoting wound healing and angiogenesis in wound healing, improving blood flow to ischemic tissues; and other desirable activities attendant to the desirable biological activities of endogenously-present or exogenously-administered HGF/SF. Those compounds of Formula I exhibiting HGF/SF antagonist activity, determinable readily by one of skill in the art, would be useful for the prophylaxis or treatment of a condition or disease in a mammal wherein the effects of HGF/SF would be undesirable, by administering to the mammal an effective amount of a pharmaceutical composition comprising an active HGF/SF antagonist of Formula I above. Such utilities include but are not limited to inhibition of angiogenesis or neovascularization, prevention of tumor growth or metastasis, inhibiting scatter, and inhibiting anti-apoptotic activities.

In a further embodiment, the invention is directed to methods for the prophylaxis or treatment of the aforementioned conditions and diseases using a therapeutically effective amount of a compound of Formula I whose HGF/SF-like activities are inhibited in the presence of, or by preincubation with, the HGF/SF receptor c-met.

Non-limiting example of modulators of HGF/SF activity of Formula I include the following compounds, most of which, as will be seen in the examples below, exhibit HGF/SF agonist activity.

3-(5-chloro-1,3-dimethyl-1H-pyrazol-4-yl)-1-(4-chlorophenyl)prop-2-en-1-one

[4-(2,6-dichlorobenzyl)-3,5-dimethyl-1H-pyrazol-1-yl][3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl]methanone

(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)methanone

4-(2-chloro-6-fluorobenzyl)-1-((3,4-dichlorophenyl)sulfonyl)-3,5-dimethyl-1H-pyrazole

4-(2-chloro-6-fluorobenzyl)-1,3,5-trimethyl-1H-pyrazole

4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole

(4-bromo-3,5-dimethyl-1H-pyrazol-1-yl)(3-(2,6-dichlorophenyl)isoxazole-4-carbohydrazide)

3-(4-(2,6-dichlorobenzyl)-3,5-dimethyl-1H-pyrazol-1-yl)propanenitrile

3,5-di(tert-butyl)-4-(2-chloro-6-fluorobenzyl)-1H-pyrazole

(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(2,6-dichlorophenyl)methanone

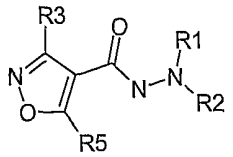
1-(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)2,2-dimethylpropan-1-one

(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(4-chlorophenyl)methanone

(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(2-thienyl)methanone

(4-chlorophenyl)(3,5-dimethyl-4-((1-methyl-1H-imidazol-2-yl)thio)-1H-pyrazol-1-yl)methanone

In yet another embodiment, the invention is directed to the use for any one or more of the aforementioned purposes of compounds that modulate HGF/SF activity with the general formula II:




Formula II

wherein

R5 is a C1 to C6 branched or straight-chained alkyl group;

R3 is a substituted or unsubstituted Aryl group;

R1 is hydrogen or a C1 to C4 straight-chained, branched or cycloalkyl group;

R2 is $COCH_2ONCH$-Aryl; heteroaryl, $COCH_2CH_2$Aryl; Aryl; COS-Aryl; CO-Heteroaryl; C1 to C4 straight-chained alkyl, branched alkyl, or cycloalkyl; or wherein R1 and R2 form a cyclic group of 5 or 6 carbon atoms.

The substituents are as defined hereinabove. Preferably, R5 is methyl. R3 is preferably an alkyl-, halogen- or alkyloxy-substituted phenyl group such as 2,6-dichlorophenyl. R1 is preferably hydrogen or methyl. R2 is preferably a substituted pyridyl group such as 2-(6-trifluoromethyl)pyridyl, a substituted arylthiocarbonyl group such as 2-(nitrophenyl)thiocarbonyl, or a 4-aryl-substituted-5-methylisoxazonecarbonyl group.

Most of the compounds of Formula II exhibit HGF/SF antagonist or inhibitory activity, as will be seen in the examples below. When R2 is $COCH_2ONCH$-Aryl, the compounds may exhibit agonist activity.

Certain of the compounds of Formula II are novel, and the present invention is directed to all such novel compounds. The invention is also directed to a pharmaceutical composition comprising at least one compound of Formula II, in a pharmaceutically-acceptable carrier, for any of the uses described herein.

Thus, this aspect of the invention is directed to method for the prophylaxis or treatment of a condition or disease in a mammal wherein the effects of HGF/SF would be undesirable, by administering to the mammal an effective amount of a pharmaceutical composition comprising an active HGF/SF antagonist of Formula II above. Such utilities include but are not limited to inhibition of angiogenesis or neovascularization, prevention of tumor growth or metastasis, inhibiting scatter, and inhibiting anti-apoptotic activities. As noted above, some compounds in Formula II exhibit HGF/SF-like activity and are likewise useful for the prophylaxis or treatment of a condition or disease in a mammal wherein the effects of HGF/SF would be beneficial by administering to the mammal an effective amount of a pharmaceutical composition comprising an active HGF/SF agonist of Formula II above. As mentioned above, such activities include but are not limited to promoting the proliferation of cells, including endothelial cells, vascular cells, hepatic cells, renal cells, among others; promoting angiogenesis; promoting

vascularization; treatment of wound healing and endothelial cell dysfunction, improving blood flow to ischemic tissues; and other desirable activities attendant to the desirable biological activities of endogenously-present or exogenously-administered HGF/SF. The activity of a compound of Formula II would be readily determinable by one of skill in the art.

In a further embodiment, the invention is directed to methods for the prophylaxis or treatment of the aforementioned conditions and diseases using a therapeutically effective amount of a compound of Formula II whose HGF/SF antagonist activities occur in the presence of exogenously-added or endogenously produced HGF/SF, the latter within the cells or tissues.

Non-limiting examples of compounds of Formula II include:

N'4,5-dimethyl-N'4-(5-nitro-2-pyridyl)-3-(2,6-dichlorophenyl)isoxazole-4-carbohydrazide

N'4-(2-(((2,4-dichlorobenzylidene)amino)oxy)acetyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide

N'4-(3-(3,4,5-trimethoxyphenyl)propanoyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide

2-nitrophenyl 2-((3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)carbonyl)hydrazine-1-carbothioate

N'4-((2-methyl-1,3-thiazol-4-4yl)carbonyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-4carbohydrazide

N1-((2-((3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)carbonyl)hydrazino)(methylthio)methylidene)benzene-1-sulfonamide

N'4-(2,4,6-trichlorophenyl)-3-3(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide

N'4,3-di(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide

N'4-(3,5-dichloro-4-pyridyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide

N'4-phenyl-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide

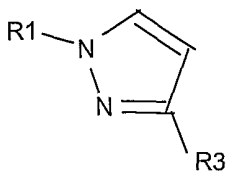
N'4,N'4,5-trimethyl-3-(2,6-dichlorophenyl)isoxazole-4-carbohydrazide

N4-azepan-1-yl-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxamide

N'4-(6-(trifluoromethyl)-2-pyridyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide

N'4-(3,3-diethoxypropanoyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide

In still a further embodiment, the invention is directed to the use for the aforementioned purposes of compounds that modulate HGF/SF activity with the general formula III:




Formula III

wherein

R1 is $SO_2$Alkyl, $SO_2$-Aryl, CO-t-Butyl, COAryl, CONHAlkyl; CONHAryl; and

R3 is CHCH-heteroaryl; phenoxyphenyl; heteroaryl; or Aryl substituted heteroaryl.

Preferably, R1 may be $SO_2$Alkyl, wherein Alkyl is C1 to C4 straight-chained, branched or cyclo, most preferably $SO_2CH_3$; $SO_2$-Aryl, wherein Aryl is halo, C1-4 alkyl or alkyloxy substituted phenyl; COAlkyl, wherein alkyl is C1 to C6 straight-chained alkyl, branched alkyl or cycloalkyl, most preferably CO-t-Butyl ; COAryl wherein Aryl is phenyl substituted with halo, C1-C4 alkyl or alkyloxy; CONHAlkyl wherein alkyl is C1 to C6 straight-chained alkyl, branched alkyl or cycloalkyl, most preferably $CONHCH_3$; or CONHAryl, wherein aryl is phenyl substituted with halo, C1 to C4 alkyl or C1 to C4 alkyloxy. R3 may be CHCH-heteroaryl, where in heteroaryl includes but is not limited to both cis and trans CHCH-3-thienyl, CHCH-2-furyl and CHCH-3-furyl, and substituted CHCH-thienyl and CHCH-furyl, most preferably CHCH-2-thienyl; phenoxyphenyl; heteroaryl; or aryl substituted heteroaryl.

Certain of the compounds of Formula III are novel, and the present invention is directed to all such novel compounds. Moreover, the invention is also directed to a pharmaceutical composition comprising at least one compound of Formula III, in a pharmaceutically-acceptable carrier, for any of the uses described herein.

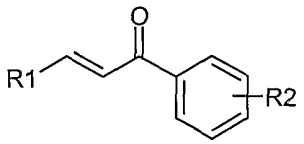
The invention is also directed to methods for the prophylaxis or treatment of a condition or disease in a mammal wherein the effects of HGF/SF would be beneficial by administering to the mammal an effective amount of a pharmaceutical composition comprising an active HGF/SF agonist of Formula III above. As mentioned above, such activities include but are not limited to promoting the proliferation of cells, including endothelial cells, vascular cells, hepatic cells, renal cells, among others; promoting angiogenesis; promoting vascularization; improving or enhancing wound healing; treating endothelial cell dysfunction; improving blood flow to ischemic tissues; and other desirable activities attendant to the desirable biological activities of endogenously-present or exogenously-administered HGF/SF. Those compounds of Formula III exhibiting HGF/SF antagonist activity, determinable readily by one of skill in the art, would be useful for the prophylaxis or treatment of a condition or disease in a mammal wherein the effects of HGF/SF would be undesirable, by administering to the mammal an effective amount of a pharmaceutical composition comprising an active HGF/SF antagonist of Formula III above. Such utilities include but are not limited to inhibition of angiogenesis or neovascularization, prevention of tumor growth or metastasis, inhibiting scatter, and inhibiting anti-apoptotic activities.

In a further embodiment, the invention is directed to methods for the prophylaxis or treatment of the aforementioned conditions and diseases using a therapeutically effective amount of a compound of Formula III whose HGF/SF-like activities are inhibited in the presence of, or by preincubation with, the HGF/SF receptor c-met.

These compounds generally exhibit HGF/SF stimulatory or agonist activity. Non-limiting examples of compounds of Formula III include

(4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone;
1-(methylsulfonyl)-3-(2-(2-thienyl)vinyl)-1H-pyrazole
2,2-dimethyl-1-(3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-yl)propan-1-one
N-methyl-3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-carboxamide
(4-chlorophenyl)(3-(3-phenylisoxazol-5-yl)-1H-pyrazol-1-yl)methanone
(4-chlorophenyl)(3-(3-(4-chlorophenyl)-5-methylisoxazol-4-yl)-1H-pyrazol-1-yl)methanone
(4-chlorophenyl)(3-(5-(2-thienyl)-2-thienyl)-1H-pyrazol-1-yl)methanone
(2,4-dichlorophenyl)(3-(5-(2,4-difluorophenyl)-2-furyl)-1H-pyrazol-1-yl)methanone
N1-phenyl-3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-carboxamide
(4-chlorophenyl)(3-(2-(5-(2-thienyl)-2-thienyl)-4-methyl-1,3-thiazol-5-yl)-1H-pyrazol-1-yl)methanone
(3-benzhydryl-1H-pyrazol-1-yl)(4-chlorophenyl)methanone
N1-(4-chlorophenyl)-3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-carboxamide
(4-chlorophenyl)(3-(2-methylimidazo(1,2-a)pyridin-3-yl)-1H-pyrazol-1-yl)methanone
2-chloro-6-(4-(1-(4-chlorobenzyl)-1H-pyrazol-3-yl)phenoxy)benzonitrile
1-((4-chlorophenyl)sulfonyl)-3-(2-(2-thienyl)vinyl)-1H-pyrazole

In a further embodiment, the invention is directed to the use for any one or more of the aforementioned purposes of compounds that modulate HGF/SF activity with the general formula IV:




Formula IV

Wherein

R1 is Aryl or Heteroaryl; and

R2 is one or more halogen, nitro, C1 to C4 straight-chained alkyl, branched alkyl, or cycloalkyl, or C1 to C4 alkyloxy groups.

The definitions of the aforementioned substituents are described hereinabove. Preferably, R1 is a phenyl group substituted with one or more halogen, C1 to C4 alkyl, or C1 to C4 alkyloxy groups, or a heteroaryl, most preferably 4-bromo-2-thienyl, 4-pyridyl, 2-furyl, 3-thienyl, substituted with halogens and/or C1 to C4 alkyl. R2 preferably is halogen (F, Cl, Br), nitro, or a C1 to C4 straight-chained alkyl, branched alkyl, or cycloalkyl group or a C1 to C4 alkyloxy group; most preferably, R2 is a methyl group and a chloro group.

Certain of the compounds of Formula IV are novel, and the present invention is directed to all such novel compounds. In addition, the invention is also directed to a pharmaceutical composition comprising at

least one compound of Formula IV, in a pharmaceutically-acceptable carrier, for any of the uses described herein.

Certain compounds of Formula IV exhibit HGF/SF agonist activity and others exhibit HGF/SF antagonist activity. The skilled artisan may readily determine the activity of the compounds, and the dose at which the compound exhibits such activity. Thus, the invention is also directed to methods for the prophylaxis or treatment of a condition or disease in a mammal wherein the effects of HGF/SF would be beneficial by administering to the mammal an effective amount of a pharmaceutical composition comprising an active HGF/SF agonist of Formula IV above. As mentioned above, such activities include but are not limited to promoting the proliferation of cells, including endothelial cells, vascular cells, hepatic cells, renal cells, among others; promoting angiogenesis; promoting vascularization; improving wound healing,; improving vascularization of wounds; improving endothelial cell dysfunction; improving blood flow to ischemic tissues; and other desirable activities attendant to the desirable biological activities of endogenously-present or exogenously-administered HGF/SF. Those compounds of Formula IV exhibiting HGF/SF antagonist activity, determinable readily by one of skill in the art, would be useful for the prophylaxis or treatment of a condition or disease in a mammal wherein the effects of HGF/SF would be undesirable, by administering to the mammal an effective amount of a pharmaceutical composition comprising an active HGF/SF antagonist of Formula IV above. Such utilities include but are not limited to inhibition of angiogenesis or neovascularization, prevention of tumor growth or metastasis, inhibiting scatter, and inhibiting anti-apoptotic activities. Moreover, compounds of Formula IV show antagonistic activity to the growth factors VEGF and FGF, and may be used for the treatment of any condition or disease in which inhibition of VEGF or FGF activity is desired.

In a further embodiment, the invention is directed to methods for the prophylaxis or treatment of the aforementioned conditions and diseases using a therapeutically effective amount of a compound of Formula IV whose HGF/SF-like activities are inhibited in the presence of, or by preincubation with, the HGF/SF receptor c-met. The antagonist activity of an HGF/SF antagonist compound of Formula IV may be active alone, or may be active in the presence of either exogenously-administered HGF/SF or in cells or tissues in which HGF/SF is expressed or induced to be expressed.

The compounds in this group may be HGF/SF agonists or antagonists. Non-limiting examples of modulators of Formula IV include:

1-(4-chloro-3-methylphenyl)-3-(2,6-dichlorophenyl)-prop-2-en-1-one

1-(4-chloro-3-methylphenyl)-3-(2-chlorophenyl)prop-2-en-1-one

3-(2-chloro-6-fluorophenyl)-1-(4-chloro-3-methylphenyl)prop-2-en-1-one

3-(4-bromo-2-thienyl)-1-(3,4-dichlorophenyl)prop-2-en-1-one

3-(4-bromo-2-thienyl)-1-(4-chloro-3-methylphenyl)prop-2-en-1-one

3-(4-bromo-2-thienyl)-1-(4-fluorophenyl)prop-2-en-1-one

3-(4-bromo-2-thienyl)-1-(4-chlorophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-(2,4-dichlorophenyl)prop-2-en-1-one

3-(1,3-benzodioxol-5-yl)-1-(4-bromophenyl)prop-2-en-1-one

3-(3-phenoxy-2-thienyl)-1-(2-thienyl)prop-2-en-1-one

3-(3-bromo-4-methoxyphenyl)-1-phenylprop-2-en-one

3-(3,4-dichlorophenyl)-1-(2-nitrophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-(3,4-dichlorophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-(3,5-dichloro-2-hydroxyphenyl)prop-2-en-1-one

1-(2-chlorophenyl)-3-(3,5-dichloro-2-hydroxyphenyl)prop-2-en-1-one

3-(4-chlorophenyl)-1-(2,6-dichlorophenyl)prop-2-en-1-one

1-(4-bromophenyl)-3-(4-chlorophenyl)prop-2-en-1-one

1-(2-chlorophenyl)-3-(2,6-dichlorophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-(2,6-dichlorophenyl)prop-2-en-1-one

3-(2,6-dichlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one

3-(4-chloro-1-methyl-1H-pyrazol-3-yl)-1-[4-(trifluoromethyl)phenyl]prop-2-en-1-one

3-(2,4-dichlorophenyl)-1-(2-methylphenyl)prop-2-en-1-one

3-(2,6-dichlorophenyl)-1-(2-methylphenyl)prop-2-en-1-one

3-(3,4-dichlorophenyl)-1-(2-methylphenyl)prop-2-en-1-one

3-(5-bromo-2-hydroxyphenyl)-1-(3-methylphenyl)prop-2-en-1-one

3-(5-bromo-2-hydroxyphenyl)-1-(4-methylphenyl)prop-2-en-1-one

3-(2,4-dichlorophenyl)-1-(3-methylphenyl)prop-2-en-1-one

3-(2,4-dichlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one

1-[4-amino-2-(methylthio)-1,3-thiazol-5-yl]-3-(4-chlorophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-[4-(trifluoromethyl)phenyl]prop-2-en-1-one

1-benzo[b]thiophen-3-yl-3-(4-chlorophenyl)prop-2-en-1-one

1,3-di(5-nitro-3-thienyl)prop-2-en-1-one

1-(4-bromophenyl)-3-(3,5-difluorophenyl)prop-2-en-1-one

3-(3,5-difluorophenyl)-1-(3-nitrophenyl)prop-2-en-1-one

The compounds of Formulae I-IV described above may be synthesized and isolated following standard methods readily available to one skilled in the art of synthetic organic chemistry. Moreover, the compounds may be readily prepared at a purity acceptable for administration to a mammal, preferably a human, at a dose effective to prophylax or treat any of the conditions and diseases related to the desired or undesired activities of HGF/SF as mentioned above.

The various uses, formulations including pharmaceutical compositions, and dose considerations are as described hereinabove.

While the foregoing discussions have been directed principally to the compounds useful for the promotion or inhibition of HGF/SF activity, and its receptor c-met, they are generally applicable to agonists and antagonists of other growth factors, particularly growth factors whose activities involve binding to a tyrosine kinase receptor. As will be seen in the examples below, compounds of the invention have been shown to inhibit VEGF and FGF activities, and certain other compounds have VEGF-like activities. The present invention embraces these as well as other tyrosine kinase receptor growth factors and small-molecule compounds including but not limited to those described herein, as agents useful for the various agonist and antagonist activities directed to such growth factors generally.

The present invention may be better understood by reference to the following non-limiting Examples, which are provided as exemplary of the invention. The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

**Example 1**

**Methods**

Peptide library. The Ph.D.-12 phage display peptide library (New England BioLabs) was used. The original library contained 1.5 X 109 pfu/µl. This peptide library is based on a combinatorial library of random peptide 12-mers fused to a minor coat protein (pIII) of M13 phage. The displayed 12-mer peptides are expressed at the N-terminus of pIII.

Phage Display. To identify peptide mimics of HGF/SF, peptide libraries were screened twice each with polyclonal HGF/SF antibodies (813, rabbit) and monoclonal HGF/SF antibodies (clone-23C2). Antibodies were diluted in $NaHCO_3$, pH 8 buffer (final concentration ~100 µg/100 µl) and 96 well plates were coated with 100 µl of antibody for 16 h at 4oC. Wells were washed with TBST (Tris buffered saline containing + 0.1% Tween-20). Ten µl of original phage ($4 \times 10^{10}$) was diluted to 10 ml and 100 µl of this solution was incubated with antibody coated plate with gentle rocking for 60 min at room temperature. Non-binding phage was removed and wells were washed 10 times with TBST. Bound phage was eluted with 100 µl of elution buffer. (0.2 M Glycine-HCl, pH 2.2, 1 mg/ml albumin), neutralized with 15 ml 1M Tris-HCl (pH 9.1) and amplified. The titer of the first round eluate is $2x10^{10}$ pfu/µL. A second round of biopanning was carried out with $2 \times 10^{11}$ pfu of the first round amplified phage as above. This procedure was repeated once more.

Characterization of Binding Clones and peptide synthesis. Twenty clones from the third round were picked to detect the consensus binding sequence. Ten clones were from the Monoclonal antibody plate and 10 clones were from the polyclonal antibody plate. Following amplification DNA was extracted and sequenced. Out of 20 clones sequenced, 9 unique sequences were identified: S G W H M R S P F N H M (SEQ ID No:12); H L K P H F W P S S P Y (SEQ ID No:13); T M G F T A P R F P H Y (SEQ ID No:1);

K V W Y H T T S I P S H (SEQ ID No:2); L L A D T T H H R P W T (SEQ ID No:14); N H P H P T P A R G I I (SEQ ID No:15); V S R H Q S W H P H D L (SEQ ID No:16); A L N W S R K L P V P P (SEQ ID No:18); and Q T G H W N A E W H T R (SEQ ID No:19). The peptides were synthesized based on the DNA sequence using solid phase peptide synthesizer at the North Shore University Research Building.

Endothelial cell proliferation. Bovine aortic endothelial cells (BAEC) and human microvascular endothelial cells (MMEC) were used for proliferation assays (19). BAEC were grown in minimal essential medium containing 10% FBS. HMEC were grown in RPMI medium containing 10% FBS and 10% NuSerum. Endothelial cell proliferation was determined as described previously. Subconfluent (50-60%) endothelial cells were incubated in serum free medium containing various concentrations of peptides or growth factor for 16 h-24 h. $^{3}$H-thymidine was then added to medium and incubation was continued under culture conditions for another 4-5 h. The cells were washed and $^{3}$H-thymidine incorporation (increase in DNA synthesis) was determined.

Vascular sprouting (aortic ring) assay. Rat aortic ring assay of angiogenesis was performed as described previously (20). Briefly, one mm-long aortic rings were sectioned from rat aorta and embedded in collagen gels. Following gelation, sections were incubated in medium containing 4% serum in the absence (control) or presence of SEQ ID No: 2 (100 µg/ml) or HGF (100 ng/ml).

Murine angiogenesis assay. Angiogenesis was assayed as growth of blood vessels from subcutaneous tissue into a solid gel of basement membrane containing the test sample. Matrigel in liquid form (0.5 ml) was mixed with SEQ ID No:2 or basic fibroblast growth factor and injected into the abdominal subcutaneous tissue of mice as previously described (21). After 10 days, mice were sacrificed and the Matrigel plugs were removed, fixed, sectioned, stained and examined for ingrowth of blood vessels.

## Example 2

## HGF/SF Antibody-binding Peptides

Two of these aforementioned nine peptides, T M G F T A P R F P H Y and K V W Y H T T S I P S H (designated SEQ ID No:1 and SEQ ID No:2, respectively), showed stimulation of endothelial proliferation and were characterized further.

Incubation of endothelial cells with SEQ ID No:1 and SEQ ID No:2 (100 µg/ml) significantly increased 3H-thymidine incorporation into DNA ($p<0.01$). This increase was comparable with increase showed by HGF/SF (20 ng/ml). As shown in Figure 1, subconfluent endothelial cells were incubated with SEQ ID No:2 (100 µg/ml) or scatter factor (HGF/SF, 20 ng/ml) for 24 h in serum-free medium under culture conditions. 3H-thymidine was then added and DNA synthesis was assessed after 5 h. Values represent Mean ± SD of six measurements. *$p<0.01$.

The peptide depicted in SEQ ID No:2 stimulated growth of microvessels in an in-vitro angiogenesis assay. Figure 2 shows the effects of peptide 4 (SEQ ID No:2) and HGF on angiogenesis in rat aortic ring assay: One mm-long aortic rings were sectioned from rat aorta and embedded in collagen gels. Following gelation, sections were incubated in medium containing 4% serum in the absence (control) or presence of SEQ ID No:2 (100 μg/ml) or HGF (100 ng/ml). Values represent Mean ± SD (n=70-120).

The ability of the peptides to induce blood vessel growth in vivo was evaluated. These experiments were performed at Paragon Bioservices, Baltimore, MD. In this assay SEQ ID No:1 or SEQ ID No:2 or an equal amount of water (control) was mixed with Matrigel, a matrix of reconstituted basement membrane. Samples were injected subcutaneously into mice. After 10 days, mice were sacrificed for histologic and morphometric analysis of Matrigel plugs. Plugs containing SEQ ID No:2 show significantly higher number of endothelial cells (Figure 3, $p<0.0001$, n=56).

## Example 3

### Addition of a heparin-binding sequence enhances growth promoting activity

HGF/SF is a heparin binding protein and recent studies show that HGF/SF binding to cell surface HSPG greatly enhances HGF/SF-induced signal transduction by c-met (11,22). To determine whether the growth promoting activity of SEQ ID No:2 can be enhanced by increasing its heparin-affinity, a lysine-rich heparin-binding sequence (23) was added to the carboxy-terminus of SEQ ID No:2 ("HS-P4"; K V W Y H T T S I P S H C R P K A K A K A K A K D Q T K [SEQ ID No:7]). A non-specific sequence with the same amino acid composition was added and treated as non-specific control ("NS-P4"; K V W Y H T T S I P S H C Q K A K T R A K A A K P D K K [SEQ ID No:8]). The ability of HS-P4 and NS-P4 to stimulate endothelial cell proliferation was compared with SEQ ID No:2 (Figure 4A) and with growth factors (Figure 4B). Endothelial cells were incubated with peptides for 24 h and 3H-thymidine incorporation was determined. HS-P4 but not NS-P4 increased endothelial growth by about three fold (Figure 4A) and is comparable or greater than the mitogenic activity of HGF/SF and bFGF (Figure 4B). These data suggest that the growth promoting and possibly the angiogenic activity of SEQ ID No:2 can be enhanced by increasing its affinity to heparin.

## Example 4

### Identification of peptides that bind scatter factor receptor c-met

The Ph.D.-12 phage display peptide library was used as described above. The extracellular domain of HGF/SF receptor C-met was obtained from R&D Systems. Peptide libraries were screened twice with plates coated with C-met as described above for HGF/SF antibody screening. Eighteen binding clones were sequenced. Of these, 15 clones were found to contain unique sequences. Four peptides (SEQ ID No:3, 4, 5 and 6) were synthesized: A T W S H H L S S A G L (SEQ ID No:3); W P Q L P P R P Y S T L (SEQ ID No:4); S N T S A G T P F T S L (SEQ ID No:5); and D S T P K S T P W Y Y I (SEQ ID No:6). The ability of these peptides to stimulate endothelial proliferation or inhibit HGF/SF-mediated

increase in endothelial proliferation was determined (as assessed by 3H-thymidine incorporation). SEQ ID Nos. 3-6 did not significantly affect endothelial growth (Figure 5). As shown in Figure 5, subconfluent endothelial cells were incubated with each of the peptides (100 μg/ml), scatter factor alone (HGF/SF, 20 ng/ml), or HGF/SF plus each of the peptides for 24 h in serum-free medium under culture conditions. 3H-thymidine was then added and DNA synthesis was assessed after 5 h. Values represent Mean ± SD of four measurements. HGF/SF induced endothelial growth by about 2 fold. However, SEQ ID Nos:3-6 completely inhibited HGF/SF-mediated increase in endothelial growth. These data suggest that these peptides can bind HGF/SF receptor C-Met thereby inhibit HGF/SF binding. Thus, these peptides may have potential angiostatic activity.

**Example 5**

**Antiproliferative Activity In vivo**

A human glioblastoma cell line (U87 MG, ATCC Cat. No. HTB-14) and glioma cell line (Hs 683, ATCC Cat. NO. HTB-138) were obtained from American Type Culture Collection (ATCC), Va. U87 MG cells were maintained in Minimal Essential Medium (MEM) containing 10% fetal bovine serum, 1 mM pyruvate and 0.1 mM non-essential amino acids. Hs 683 cells were maintained in DMEM containing 10% FBS. Cells were plated in a 48 well plate (10, 000 cells per well). Twenty-four h following seeding, medium was replaced with serum free medium. After 8 h cells were incubated with medium with or without peptides (100 μg/ml final concentration) for 20 h. 3H-thymidine was then added and DNA synthesis was determined for 4 h.

Peptides corresponding to SEQ ID Nos:3-6 and A K T Y A G S S Y Q F G (SEQ ID No:11) were evaluated.

The peptide designated as SEQ ID No:5 was most effective in inhibiting the growth of Hs 683 cells ($p<0.001$) (Figure 6A). However peptides designated as SEQ ID No:4, 5 and 6 showed significant inhibition on U87 MG cell growth (Figure 6B). These data show that C-met peptides each alone or in combination could block endogenous HGF/SF activity and associated tumor growth.

**Example 6**

**Assay Methods for Small-molecule Compounds**

The following assays were used to evaluate the activity of the various compounds discussed herein. Certain of the compounds and groups thereof express HGF/SF-like activity, i.e., they induce scatter, cell proliferation, inhibit apoptosis, among other activities, and if acting through the c-met receptor, agonize or stimulate c-met receptor activity. The specificity for such compounds working through the c-met receptor may be identified by performing the stimulation assay in the presence of free c-met receptor. Reduction of a proliferative response in the presence of c-met indicates such specificity.

To evaluate inhibitors of activity, compounds may be evaluated directly for anti-proliferative activities, such as the inhibition of cellular proliferation, inhibition of tumor growth, inhibition of scatter, and inhibition of gene expression, and may also be evaluated on their ability to inhibit activity when exposed to cells together with HGF/SF. In such instances, the scatter and/or proliferative activities induced by added will be inhibited by the attendant presence of an inhibitory compound of the invention. Thus, certain inhibitors may be inhibitory in the absence of exogenously-added HGF/SF; these and/or other compounds may exhibit inhibitory activity only in the presence of HGF/SF.

It is noted that Applicants have no duty to disclose the theory or mechanism by which or through the compounds of the invention operate, nor are they in any way bound by such disclosure.

Cell proliferation assays Endothelial cells (HUVECs) were seeded in 48 well plates at a density of 10,000 to 20,000 cells per well in the normal growth medium (EGM-2- Clonetics) containing 2% fetal bovine serum, FGF, VEGF, IGF, ascorbic acid, EGF, GA, heparin and hydrocortisone. The cells were grown normally in the growth medium for 24 hr at 37° C and 5% $CO_2$. The cells were then rinsed with RPMI-1% BSA and starved for 1-2 hrs. The stock solutions of all the compounds were made at a concentration of 10 mg/ml in DMSO and diluted in RPMI-1% BSA at a final concentrations of 1 to 12 microgram/ml. The cells were then washed and treated with the compounds and incubated for another 24 hr at 37°C. Then $^3H$ thymidine (0.5 microgram/ml in RPMI-BSA) was added to the cells and incubated at 37° C for 4 to 5 hours. The unincorporated thymidine was removed by washing the cells four times with 1x PBS. Then the cells were lysed with 0.5M NaOH for 30 min and the radioactivity counted in the beta counter.

In other experiments, human iliac artery endothelial cells were used under similar conditions as those described above.

Effect on growth of tumor cells. The activity of the compounds herein to promote or inhibit the growth of tumor cells was evaluated using human endometrial cancer cells.

Scatter Assay. A standard assay for scatter of MDCK cells was performed. Results were evaluated by microscopic examination.

Anti-apoptotic Activity Assay. The ability of compounds of the present invention to protect cells from apoptosis was performed using a MTT viability assay with MDCK cells exposed to adriamycin (15 micromolar) to induce apoptosis. HGF/SF was evaluated as a positive control.

Effect of the compounds on gene expression patterns. Additional data on the cellular effects of the compounds of the invention was obtained from GeneChip studies. In particular, effects on induction of angiogenic-cascade-related genes including interleukin-8 and angiopoietin-2 were evaluated.

**Example 7**

**Compounds**

The following compounds were evaluated herein.

5

(4-chlorophenyl)[5-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone (C16H11CIN2OS/315)

1-(methylsulfonyl)-5-(2-(2-thienyl)vinyl)-1H-pyrazole

2,2-dimethyl-1-(3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-yl)propan-1-one

(4-chlorophenyl)(3,5-di(tert-butyl)-1H-pyrazol-1-yl)methanone

N-methy-3-(2-(2thienyl)vinyl)-1H-pyrazole-1-carboxamide

1-(4-chlorobenzoyl)-5-cyclopropyl-1H-pyrazole-4-carbonitrile

(4-chlorophenyl)(3-(3-phenylisoxazol-5-yl)-1H-pyrazol-1-yl)methanone

5-(2-(2-thienyl)vinyl)-1H-pyrazole

ethyl 1-(4-chlorobenzoyl)-3-methyl-1H-pyrazol-5-carboxylate

(4-chlorophenyl)(3,5-dimethyl-4-(pyrimidin-2-ylthio)-1H-pyrazol-1-yl)methanone

(4-chlorophenyl)(3-(3-(4-chlorophenyl)-5-methylisoxazol-4-yl)-1H-pyrazol-1-yl)methanone

(4-chlorophenyl)(3-(5-(2-thienyl)-2-thienyl)-1H-pyrazol-1-yl)methanone

(2,4-dichlorophenyl)(3-(5-(2,4-difluorophenyl)-2-furyl)-1H-pyrazol-1-yl)methanone

(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazol-1-yl)(4-chlorophenyl)methanone

methyl 4-(1-(4-chlorobenzoyl)-1H-pyrazol-5-yl)-5-methylisoxazole-3-carboxylate

(4-chlorophenyl)(5-(methylthio)-3-(4-phenoxyphenyl)-1H-pyrazol-1-yl)methanone

(4-chlorophenyl)(3,5-dimethyl-4-((1-methyl-1H-imidazol-2-yl)thio)-1H-pyrazol-1-yl)methanone

N1-phenyl-3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-carboxamide

(4-chlorophenyl)(3-(2-(5-(2-thienyl)-2-thienyl)-4-methyl-1,3-thiazol-5-yl)-1H-pyrazol-1-yl)methanone

(4-chlorophenyl)(3,5-dimethyl-4-phenoxy-1H-pyrazol-1-yl)methanone

(3-benzhydryl-1H-pyrazol-1-yl)(4-chlorophenyl)methanone

(4-chlorophenyl)(3,5-dimethyl-4-((5-(trifluoromethyl)-2-pyridyl)thio)-1H-pyrazol-1-yl)methanone

N1-(4-chlorophenyl)-3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-carboxamide

methyl 1-(4-chlorobenzoyl)-5-(dimethoxymethyl)-1H-pyrazole-4-carboxylate

(4-chlorophenyl)(3-(2-methylimidazo(1,2-a)pyridin-3-yl)-1H-pyrazol-1-yl)methanone

(4-chlorophenyl)(3,5-dimethyl-4-)((1-phenyl-1H-1,2,3,4-tetraazol-5-yl)thio)-1H-pyrazol-1-yl)methanone

methyl 1-(4-chlorobenzoyl)-5-isoxazol-5-yl-3-methyl-1H-pyrazole-4-carboxylate

(3-(tert-butyl)5-(methylthio)-1H-pyrazol-1-yl)(4-chlorophenyl)methanone

2-chloro-6-(4-(1-(4-chlorobenzyl)-1H-pyrazol-5-yl)phenoxy)benzonitrile

(4-chlorophenyl)(5-(5-methyl-3-phenylisoxazol-4-yl)-1H-pyrazol-1-yl)methanone

1-((4-chlorophenyl)sulfonyl)-3-(2-(2-thienyl)vinyl)-1H-pyrazole

(4-chlorophenyl)(3,5-dimethyl-4-((4-methyl-5-(trifluoromethyl)-4H-1,2,4-triazol-3-yl)thio)-1H-pyrazol-

1-yl)methanone
methyl 1-(4-chlorobenzoyl)-3-methyl-5-(4-methyl-1,2,3-thiadiazol-5-yl)-1H-pyrazole-4-carboxylate

[4-(2,6-dichlorobenzyl)-3,5-dimethyl-1H-pyrazol-1-yl][3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl]methanone (C23H17Cl4N3O2/509)
4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1-(phenylsulfonyl)-1H-pyrazole
(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)methanone
4-(2-chloro-6-fluorobenzyl)-1-((3,4-dichlorophenyl)sulfonyl)-3,5-dimethyl-1H-pyrazole
4-(2-chloro-6-fluorobenzyl)-1,3,5-trimethyl-1H-pyrazole
4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole
(4-bromo-3,5-dimethyl-1H-pyrazol-1-yl)(3-(2,6-dichlorophenyl)isoxazole-4-carbohydrazide)
N'4,5-dimethyl-N'4-(5-nitro-2-pyridyl)-3-(2,6-dichlorophenyl)isoxazole-4-carbohydrazide
N'4-(2-(((2,4-dichlorobenzylidene)amino)oxy)acetyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide
3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide
N'4-(3-(3,4,5-trimethoxyphenyl)propanoyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide
2-nitrophenyl 2-((3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)carbonyl)hydrazine-1-carbothioate
4-(2,6-dichlorobenzyl)-1-((3,5pdi(trifluoromethyl)phenyl)sulfonyl)-3,5-dimethyl-1H-pyrazole
1-((4-chlorophenyl)sulfonyl)-4-(2,6-dichlorobenzyl)-3,5-dimethyl-1H-pyrazol
(4-(2,6-dichlorobenzyl)-3,5-dimethyl-1H-pyrazol-1-yl)(2,6-dichlorophenyl)methanone
3-(4-(2,6-dichlorobenzyl)-3,5-dimethyl-1H-pyrazol-1-yl)propanenitrile
N'4-((2-methyl-1,3-thiazol-4-4yl)carbonyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-4carbohydrazide
N1-((2-((3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)carbonyl)hydrazino)(methylthio)methylidene)benzene-1-sulfonamide
N'4-(2,4,6-trichlorophenyl)-3-3(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide
N'4,3-di(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide
3,5-di(tert-butyl)-4-(2-chloro-6-fluorobenzyl)-1H-pyrazole
N'4-(3,5-dichloro-4-pyridyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide
N'4-phenyl-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide
(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(2,6-dichlorophenyl)methanone
1-(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)2,2-dimethylpropan-1-one
N'4,N'4,5-trimethyl-3-(2,6-dichlorophenyl)isoxazole-4-carbohydrazide
N4-azepan-1-yl-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxamide
N'4-(6-(trifluoromethyl)-2-pyridyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide
(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(4-chlorophenyl)methanone

N4Piperidino-3-(2,6-dichlorophenyl)-5methylisoxazole-4-carboxamide

N'4-(3,3-diethoxypropanoyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide

(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(2-thienyl)methanone

N'4-(2,5-dichlorophenyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide

1-(4-chloro-3-methylphenyl)-3-(2,6-dichlorophenyl)-prop-2-en-1-one

1-(4-chloro-3-methylphenyl)-3-(2-chlorophenyl)prop-2-en-1-one

3-(2-chloro-6-fluorophenyl)-1-(4-chloro-3-methylphenyl)prop-2-en-1-one

3-(4-bromo-2-thienyl)-1-(3,4-dichlorophenyl)prop-2-en-1-one

3-(4-bromo-2-thienyl)-1-(4-chloro-3-methylphenyl)prop-2-en-1-one

3-(4-bromo-2-thienyl)-1-(4-fluorophenyl)prop-2-en-1-one

3-(4-bromo-2-thienyl)-1-(4-chlorophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-(2,4-dichlorophenyl)prop-2-en-1-one

3-(1,3-benzodioxol-5-yl)-1-(4-bromophenyl)prop-2-en-1-one

3-(3-phenoxy-2-thienyl)-1-(2-thienyl)prop-2-en-1-one

3-(3-bromo-4-methoxyphenyl)-1-phenylprop-2-en-one

3-(3,4-dichlorophenyl)-1-(2-nitrophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-(3,4-dichlorophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-(3,5-dichloro-2-hydroxyphenyl)prop-2-en-1-one

1-(2-chlorophenyl)-3-(3,5-dichloro-2-hydroxyphenyl)prop-2-en-1-one

3-(4-chlorophenyl)-1-(2,6-dichlorophenyl)prop-2-en-1-one

1-(4-bromophenyl)-3-(4-chlorophenyl)prop-2-en-1-one

1-(2-chlorophenyl)-3-(2,6-dichlorophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-(2,6-dichlorophenyl)prop-2-en-1-one

3-(2,6-dichlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one

3-(4-chloro-1-methyl-1H-pyrazol-3-yl)-1-[4-(trifluoromethyl)phenyl]prop-2-en-1-one

3-(2,4-dichlorophenyl)-1-(2-methylphenyl)prop-2-en-1-one

3-(2,6-dichlorophenyl)-1-(2-methylphenyl)prop-2-en-1-one

3-(3,4-dichlorophenyl)-1-(2-methylphenyl)prop-2-en-1-one

3-(2,6-dichlorophenyl)-1-(3-methylphenyl)prop-2-en-1-one

3-(5-bromo-2-hydroxyphenyl)-1-(3-methylphenyl)prop-2-en-1-one

3-(5-bromo-2-hydroxyphenyl)-1-(4-methylphenyl)prop-2-en-1-one

3-(2,4-dichlorophenyl)-1-(3-methylphenyl)prop-2-en-1-one

3-(2,4-dichlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one

1-[4-amino-2-(methylthio)-1,3-thiazol-5-yl]-3-(4-chlorophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-[4-(trifluoromethyl)phenyl]prop-2-en-1-one

1-benzo[b]thiophen-3-yl-3-(4-chlorophenyl)prop-2-en-1-one

1,3-di(5-nitro-3-thienyl)prop-2-en-1-one

1-(4-methyl-2-(3-thienyl)-1,3-thiazol-5-yl]-3-(2-thienyl)prop-2-en-1-one

1-(4-bromophenyl)-3-(3,5-difluorophenyl)prop-2-en-1-one

3-(3,5-difluorophenyl)-1-(3-nitrophenyl)prop-2-en-1-one

**Example 8**

**HGF/SF-like cellular proliferative activity of a compound of the invention**

Using the endothelial cell proliferation assay described above, the compound (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone was shown to increase HUVEC proliferation by two to five fold. The specificity of the stimulation of endothelial cell growth by the compound as measured by $^3$H-thymidine incorporation was tested by pre-incubation of cells with the HGF/SF receptor c-met. In **Figure 7**, the first bar represents control cells; the second bar (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone at 6 microgram/ml; and the third bar: (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone at 6 microgram/ml plus c-met receptor, 100 microgram/ml. (4-Chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone by itself stimulated $^3$H-thymidine incorporation by 84%. Thus, (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone is as effective as HGF/SF in stimulating HUVEC proliferation. In the presence of c-met, the (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone stimulation of $^3$H-thymidine incorporation was inhibited by 75%. Although Applicants are not bound by theory, this study also demonstrates that (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone promotes proliferation of HUVECs via the c-met receptor. In another related experiment, (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone (12 microgram/ml) was incubated with the initial target molecule C-met receptor (5 microgram/ml) for 30 min and then added to the cells. Compound-induced EC proliferation was blocked by 40% in the presence of C-met receptor.

**Example 9**

**Scatter of MDCK cells**

(4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone was further tested for HGF/SF activity in a standard scatter assay which is specific for HGF/SF. The ability to scatter was demonstrated for the first time using a non-peptide candidate compound. Scatter of MDCK cells by (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone further demonstrates that its actions are mediated through stimulation of the c-met receptor. As shown in **Figure 8**, the compound caused scattering of MDCK cells similar to that seen with HGF/SF. **Figure 8A**: Control cells; **Figure 8B**: (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone, 6 microgram/ml.

**Example 10**

**Anti-apoptotic activity of (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-**

**1H-pyrazol-1-yl]methanone**

HGF/SF has significant anti-apoptotic activity in a number of cultured cell lines. Using the MTT cell viability assay the ability of (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone to protect cells from adriamycin-induced apoptosis was evaluated. Like HGF/SF, (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone was able to significantly block adriamycin-induced apoptosis in MDCK cells (**Figure 9**). Cell viability was unchanged by either HGF/SF alone (column 2), (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone alone (column 5) or HGF/SF and (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl] methanone combined (column 7). Adriamycin (15 mM) decreased cell viability to 56% of control (column 3). Treatment with either HGF/SF (column 4) or (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone (column 6) effected nearly complete (94%) protection from adriamycin-induced apoptosis.

In another cell line, 90% protection was afforded by (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone.

**Example 11**

**Effect of (4-chlorophenyl)(3-2-(2-thienyl)vinyl)-1H-pyrazol-1-yl-methanone on HUVEC proliferation**

**Figure 10** shows a dose-response relationship between the level of level (4-chlorophenyl)(3-2-(2-thienyl)vinyl)-1H-pyrazol-1-yl-methanone and HUVEC proliferation.

**Example 12**

**Gene Expression**

Preliminary GeneChip studies using the compounds of the invention demonstrate similar gene stimulation profiles including stimulation of interleukin-8 and angiopoetin-2, both of which have important roles in the angiogenic cascade.

**Example 13**

**Effect of [4-(2,6-dichlorobenzyl)-3,5-dimethyl-1H-pyrazol-1-yl][3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl]methanone on HGF/SF-mediated HUVEC proliferation**

**Figure 11** shows the results of a HUVEC growth experiment in the presence of HGF/SF and [4-(2,6-dichlorobenzyl)-3,5-dimethyl-1H-pyrazol-1-yl][3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl]methanone. While the addition of HGF/SF increases the proliferation of HUVEC (second bar), and the compound alone has no effect on baseline proliferation, the combination of both HGF/SF and the compound (fourth bar) results in significant suppression of HGF/SF-mediated stimulation.

**Example 14**

**In vivo blood vessel ingrowth assay**

Angiogenesis was assayed as growth of blood vessels from subcutaneous tissue into a solid gel of basement membrane containing the test compound. Matrigel in liquid form (0.5 ml) was mixed with a compound of the invention, or basic fibroblast growth factor as a control, and injected into the abdominal subcutaneous tissue of mice as previously described (Kibbey, M. C., Grant, D. S. Auerbach, R. and Kleinman, H. K. [1992] Role of the SIKVAV site of laminin in promotion of angiogenesis and tumor growth: an in vivo Matrigel model. J. Natl. Can. Inst. 84, 1633-38). After 10 days, mice were sacrificed and the Matrigel plugs were removed, fixed, sectioned, stained and examined for ingrowth of blood vessels. In **Figure 12A**, the effect of 1-(4-chloro-3-methylphenyl)-3-(2,6-dichlorophenyl)-prop-2-en-1-one was seen as a nearly complete inhibition of blood vessel ingrowth, less than control. In contrast, in **Figure 12B**, (4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)methanone showed significant stimulation of blood vessel ingrowth.

## Example 15

### Clonogenic assays

The effect of (4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)methanone (**Figure 13**) and 1-(4-chloro-3-methylphenyl)-3-(2,6-dichlorophenyl)-prop-2-en-1-one (**Figure 14**) were evaluated in a clonogenic assay over 7 days. Both Figures 13 and 14 show dose responsive inhibition of DU145 cell growth during the experiment.

## Example 16

### In vivo blood flow improvement assay

**Figure 15** shows the results of an in-vivo experiment in which (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone was administered to mice for seven days following removal of the femoral artery. The results show significant improvement in blood flow with the compound.

## Example 17

### Other compounds with activities

In a similar manner as described above, the following compounds related were evaluated for stimulation of endothelial cell proliferation. Three different rounds of testing were performed.

| Compound | Stimulation/Inhibition at 5 micrograms/ml | Stimulation/Inhibition at 10 micrograms/ml |
|---|---|---|
| 1-(methylsulfonyl)-3-(2-(2-thienyl)vinyl)-1H-pyrazole | 74% stimulation | Not significant |
| 2,2-dimethyl-1-(3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-yl)propan-1-one | No effect | 55% stimulation |
| N-methy-3-(2-(2thienyl)vinyl)-1H-pyrazole-1-carboxamide | 70% stimulation | 42% stimulation |
| (4-chlorophenyl)(3-(3-phenylisoxazol-5-yl)-1H-pyrazol-1-yl)methanone | 54% stimulation | 60% stimulation |
| (4-chlorophenyl)(3-(5-(2-thienyl)-2-thienyl)-1H-pyrazol-1-yl)methanone | 40% stimulation | 40% stimulation |

| Compound | Stimulation/Inhibition at 5 micrograms/ml | Stimulation/Inhibition at 10 micrograms/ml |
|---|---|---|
| (4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazol-1-yl)(4-chlorophenyl)methanone | 41% stimulation | 35% stimulation |
| (4-chlorophenyl)(5-(methylthio)-3-(4-phenoxyphenyl)-1H-pyrazol-1-yl)methanone | No effect | 40 % inhibition |
| (4-chlorophenyl)(3,5-dimethyl-4-((1-methyl-1H-imidazol-2-yl)thio)-1H-pyrazol-1-yl)methanone | 52% stimulation | 50% stimulation |
| N1-phenyl-3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-carboxamide | 40% stimulation | Not significant |
| (4-chlorophenyl)(3-(2-(5-(2-thienyl)-2-thienyl)-4-methyl-1,3-thiazol-5-yl)-1H-pyrazol-1-yl)methanone | 20% stimulation | 33% stimulation |
| (3-benzhydryl-1H-pyrazol-1-yl)(4-chlorophenyl)methanone | No effect | 25% stimulation |
| N1-(4-chlorophenyl)-3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-carboxamide | No effect | 55% stimulation |
| methyl 1-(4-chlorobenzoyl)-5-isoxazol-5-yl-3-methyl-1H-pyrazole-4-carboxylate | Not significant | 33% stimulation |
| 2-chloro-6-(4-(1-(4-chlorobenzyl)-1H-pyrazol-5-yl)phenoxy)benzonitrile | 60% stimulation | 90% stimulation |

The following compounds were re-evaluated.

| Compound | Stimulation/Inhibition at 5 micrograms/ml | Stimulation/Inhibition at 10 micrograms/ml |
|---|---|---|
| 4(5-chlorobenzo(b)thiophen-3-yl)-1-(2chlorophenyl)sulfonyl)-3,5dimethyl-1-H-pyrazole | 1.8 fold stimulation | Not significant |
| 4-(2,6-dichlorobenzyl)-3-methyl-1-phenyl-1H-pyrazol-5-ol | 2.3 fold stimulation | two fold stimulation |
| 3-methyl-4-(2-methylallyl)-1-(phenylsulfonyl)-1H-pyrazol-5-ol | two fold stimulation | Not significant |
| [3-(2,6-difluorophenyl)-4-ethyl-1H-pyrazol-1-yl](2-thienyl)methanone | 2.5 fold stimulation | two fold stimulation |
| 4-[(5-chloro-1-benzothiophen-3-yl)methyl]-N,3,5-trimethyl-1H-pyrazole-1-carboxamide | two fold stimulation | two fold stimulation |
| 3-(2,6-difluorophenyl)-4-ethyl-1H-pyrazole | two fold stimulation | 86% stimulation |
| N1-(3-chlorophenyl)-4-[(5-chlorobenzo[b]thiophen-3-yl)methyl]-3,5-dimethyl-1H-pyrazole-1-carboxamide | 86% stimulation | 40% stimulation |
| {4-[(5-chlorobenzo[b]thiophen-3-yl)methyl]-3,5-dimethyl-1H-pyrazol-1-yl}(4-nitrophenyl)methanone | 74% stimulation | 2.5 fold stimulation |
| N1-phenyl-4-[(5-chlorobenzo[b]thiophen-3-yl)methyl]-3,5-dimethyl-1H-pyrazole-1-carboxamide | two fold stimulation | 45% stimulation |
| 4-[(5-chloro-1-benzothiophen-3-yl)methyl]-N-(2,4-dichlorophenyl)-3,5-dimethyl-1H-pyrazole-1-carboxamide | 75% stimulation | 35% stimulation |
| 1-[3-(2,6-difluorophenyl)-4-ethyl-1H-pyrazol-1-yl]-2,2-dimethylpropan-1-one | 58% stimulation | not significant |
| 4-(2-chloro-6-fluorobenzyl)-1-{[3,5-di(trifluoromethyl)phenyl]sulfonyl}-3,5-dimethyl-1H-pyrarole | 3.1 fold stimulation | 2.4 fold stimulation |

**Example 18**

**Inhibitors of Cellular Proliferation**

[4-(2,6-dichlorobenzyl)-3,5-dimethyl-1H-pyrazol-1-yl][3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl]methanone was evaluated for the ability to block HGF/SF induced proliferation of HUVEC as described above. It demonstrated 40-60% blockage at 12 micrograms/ml.

**Example 19**

**Inhibition of Tumor Growth**

[4-(2,6-Dichlorobenzyl)-3,5-dimethyl-1H-pyrazol-1-yl][3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl]methanone was evaluated for inhibition of human endometrial cancer tumor growth. Growth was inhibited by 40-50% at 40 micrograms/ml.

**Example 20**

**Anti-proliferative activity of compounds of the invention**

Using the above-described assays, the following compounds were found to block proliferation of HUVEC in the absence or presence of exogenously-added HGF/SF.

| Compound | Activity at 2 microgram/ml | | Activity at 6 microgram/ml | |
|---|---|---|---|---|
| | Without HGF/SF | With HGF/SF | Without HGF/SF | With HGF/SF |
| (4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)methanone | No effect | 40-90% inhibition | not significant | 50-100% inhibition |
| 4-(2-chloro-6-fluorobenzyl)-1-((3,4-dichlorophenyl)sulfonyl)-3,5-dimethyl-1H-pyrazole | 73% stimulation | not significant | not significant | not significant |
| 4-(2-chloro-6-fluorobenzyl)-1,3,5-trimethyl-1H-pyrazole | 50% stimulation | No effect | No effect | blocked SF by 30% |
| 4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole | 54% stimulation | No effect | No effect | No effect |
| (4-bromo-3,5-dimethyl-1H-pyrazol-1-yl)(3-(2,6-dichlorophenyl)isoxazole-4-carbohydrazide) | 74% stimulation | No effect | Not significant | No effect |
| N'4,5-dimethyl-N'4-(5-nitro-2-pyridyl)-3-(2,6-dichlorophenyl)isoxazole-4-carbohydrazide | No effect | No effect | No effect | blocked SF by 43% |
| N'4-(2-(((2,4-dichlorobenzylidene)amino)oxy)acetyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide | 32% stimulation | 30% stimulation | No effect | No effect |
| 3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide | not significant | not significant | not significant | not significant |
| N'4-(3-(3,4,5-trimethoxyphenyl)propanoyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide | 50% inhibition | Blocked SF by 50% | Cytotoxic | |
| 2-nitrophenyl 2-((3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)carbonyl)hydrazine-1-carbothioate | No effect | No effect | 80% inhibition | 80% inhibition |
| 1-((4-chlorophenyl)sulfonyl)-4-(2,6-dichlorobenzyl)-3,5-dimethyl-1H-pyrazol | No effect | No effect | No effect | 50 % inhibition |
| 3-(4-(2,6-dichlorobenzyl)-3,5-dimethyl-1H-pyrazol-1-yl)propanenitrile | No effect | 36% inhibition | 34% inhibition | 65% inhibition |

| Compound | Activity at 2 microgram/ml | | Activity at 6 microgram/ml | |
|---|---|---|---|---|
| | Without HGF/SF | With HGF/SF | Without HGF/SF | With HGF/SF |
| N'4-((2-methyl-1,3-thiazol-4-4yl)carbonyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-4carbohydrazide | No effect | 50% inhibition | 52% stimulation | 50% inhibition |
| N1-((2-((3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)carbonyl)hydrazino)(methylthio)methylidene)benzene-1-sulfonamide | No effect | No effect | No effect | 25 % inhibition |
| N'4-(2,4,6-trichlorophenyl)-3-3(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide | No effect | No effect | No effect | 60% inhibition |
| N'4,3-di(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide | No effect | not significant | 30% inhibition | 60 % inhibition |
| 3,5-di(tert-butyl)-4-(2-chloro-6-fluorobenzyl)-1H-pyrazole | 51% stimulation | 25% inhibition | No effect | 50% inhibition |
| N'4-(3,5-dichloro-4-pyridyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide | No effect | 25% inhibition | No effect | 25% inhibition |
| N'4-phenyl-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide | No effect | 40% inhibition | No effect | 80% inhibition |
| (4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(2,6-dichlorophenyl)methanone | not significant | 23% inhibition | not significant | 90% inhibition |
| 1-(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)2,2-dimethylpropan-1-one | No effect | 25 % inhibition | No effect | 25 % inhibition |
| N'4,N'4,5-trimethyl-3-(2,6-dichlorophenyl)isoxazole-4-carbohydrazide | No effect | No effect | No effect | 40% inhibition |
| N4-azepan-1-yl-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxamide | No effect | No effect | 50% inhibition | 50% inhibition |
| N'4-(6-(trifluoromethyl)-2-pyridyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide | 40% inhibition | 50% inhibition | 50% inhibition | 80%inhibition |
| (4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(4-chlorophenyl)methanone | 40% stimulation | blocked SF by 30% | 50% inhibition | 90% inhibition |

| Compound | Activity at 2 microgram/ml | | Activity at 6 microgram/ml | |
|---|---|---|---|---|
| | Without HGF/SF | With HGF/SF | Without HGF/SF | With HGF/SF |
| N'4-(3,3-diethoxypropanoyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide | No effect | No effect | No effect | 35%inhibition |
| (4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(2-thienyl)methanone | No effect | No effect | No effect | 61% inhibition |

**Example 21**

**Activities of other compounds**

The following compounds, structurally unrelated to those described in the foregoing examples, were also evaluated for effects on endothelial cell proliferation in the absence and presence of exogenously-added HGF/SF. Several compounds with potential antiproliferative activity showed activity, including those which showed activity only in the presence of exogenous HGF/SF. Moreover, several compounds demonstrated stimulatory activity, and have potential growth promoting activities useful as described hereinabove.

| Compound | Stimulation/Inhibition at 6-12 micrograms/ml | |
|---|---|---|
| | Without HGF/SF | With |
| tetraphenylthiophene | 50% inhibition | No effect |
| pentaphenylbenzene | two to three fold stimulation | 50% inhibition |
| 1,3,5-triphenylbenzene | 40-60% stimulation | two fold stimulation |
| (3- Biphenyl) Trimethyl silane | 40% stimulation | No effect |
| 16 methyl-16 Dehydropregnenolone | No effect | 30% stimulation |
| 9-biphenyl-4-ylmethylene-9H-tri-benzo(A,C,E)-cycloheptene | 30-40% stimulation | 20-30% stimulation |
| 1,1,3-triphenylinedene | 50-100% stimulation | 30-40% stimulation |
| 9,9-Biphenanthrene | No effect | 20-40% inhibition |
| N-(furfurylidene)-2,4-xylidine | 30-40% inhibition | 50-60% inhibition |
| 1-(4-Chloro-3 Methyl Phenyl)3-2(2,6-dichlorophenyl)Prop-2-ene-1-one | No effect at 1 ug/ml | 40-80% inhibition |
| 3-(4-Bromophenyl)-1-phenylprop-2-en-1-one | 40-80% stimulation at 1 ug/ml | some additive effect |

| | Activity at 1ug/ml concentration | |
|---|---|---|
| | Without HGF/SF | With HGF/SF |
| 8-Benzyledene -2,4 Diphenyl-5,6,7,8 Tetrahydrophosphinoline | no significant effect | 40% inhibition |
| 6-(3,5 -Dimethylphenyl) Thio)-3-Phenyl (1,2,4-Triazolo(4,3-b)pyridazine | no significant effect | 30% inhibition |

**Example 22**

**Inhibition of HGF/SF, VEGF and FGF Activities**

In endothelial cell proliferation assays as described above, the effect of the compound 1-(4-chloro-3-methylphenyl)-3-(2,6-dichlorophenyl)-prop-2-en-1-one was evaluated in the presence of HGF/SF, VEGF or FGF for its effects on antagonizing the growth factor-induced stimulation of endothelial cell proliferation. As shown in **Figure 16A**, at 1.5 micromolar, the compound suppressed the HGF/SF-, VEFG- and FGF-mediated increase in endothelial cell proliferation. Similar results were seen with 3 micromolar 1-(4-chloro-3-methylphenyl)-3-(2,6-dichlorophenyl)-prop-2-en-1-one, **Figure 16B**.

**Example 23**

**Compounds with VEGF-like Activity**

Compounds were screened for VEGF-like activity in a standard assay using 2 microgram/ml compound. As shown in **Figure 17**, 3,3-dibromo-1-phenyl-1,2,3,4-tetrahydroquinoline-2,4-dione (VC8) and 4-(4-chlorophenyl)-6-(dimethylamino)-2-phenyl-5-pyrimidinecarbonitrile (VC14) showed positive activity, both of which were better than VEGF (1259 ± 104 cpm). The present invention is also directed to methods of use of these and structurally-related VEGF agonists or mimics for the treatment of various conditions and diseases for which VEGF would be useful for therapy in a mammal, preferably a human, such as but not limited to acceleration of wound healing, and in particular, diabetic wound healing. The compounds are generally useful for promoting proliferation of vascular endothelial cells and promoting vascularization, for such other uses as restenosis for treatment of coronary artery disease, angina and other ischemic diseases, including stroke.

**Example 24**

**Further HGF/SF-like activity of**

**(4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone**

One of the compounds identified with HGF/SF-like activity, (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone, was able to stimulate endothelial cell proliferation in vitro (**Figure 18:** first bar: control cells; second bar (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone, 38 mM; third bar: HGF/SF, 20 ng/ml; fourth bar: (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone + HGF/SF). The specificity of the stimulation by this compound of growth of HUVECs by $^{3}$H-thymidine incorporation was tested by pre-incubation of cells with the HGF/SF receptor c-met. (4-Chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone by itself stimulated $^{3}$H-thymidine

incorporation by more than 5 fold (Figure 18, bar 2). (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone is as effective as HGF/SF in stimulating HUVEC proliferation. In the presence of c-met the (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone stimulation of $^{3}$H-thymidine incorporation was inhibited by 75%. Scattering of MDCK cells in culture is a known specific effect of scatter factor and (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone also has this ability, the first demonstration of this activity in a non-peptide compound. (4-Chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone, like HGF/SF, did not stimulate the growth of fibroblast cell lines and both showed similar inhibitory effects in HepG2 hepatoma cell lines.

**Example 25**

**(4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone causes phosphorylation of c-met and Erk**

Using immunoprecipitation and Western blotting we were able show that like HGF/SF, (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone causes phosphorylation of the signaling protein Erk (**Figure 19**). Both HGF/SF (lane 4) and (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone (lane 2) showed significant amounts of phosphorylated Erk compared to unstimulated control cells (lane 1). A small molecule antagonist, (4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)methanone had no effect on phosphorylation of Erk (Lane 3). Total Erk is shown on the bottom.

**Example 26**

**Wound healing studies**

The angiogenic properties of (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone were further tested in a pig model of wound healing. Full thickness 8-mm skin wounds were produced in pigs and five days later the wounds were excised, stained with H&E and blood vessels counted in five areas from each section under high power. Wounds treated with (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone (500 micrograms) demonstrated a 33% greater density of blood vessels compared to vehicle-treated (DMS) controls (**Figure 20**).

**Example 27**

**Increase in capillary numbers by (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone.**

Mice were subjected to unilateral hindlimb ischemia and treated with either the HGF/SF agonist, (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone (25 micrograms/day) or vehicle for either two or three weeks prior to sacrifice. Hindlimb muscles were frozen in liquid nitrogen and capillaries stained by the alkaline phosphatase technique and the number of capillaries per muscle fiber counted in 6 to 12 random areas of the muscle by a blinded observer.

Recovery in mice with hindlimb ischemia by increasing the number of capillaries in the ischemic muscle was observer (**Figure 21**). At 2 weeks there was a 42% greater number of capillaries per muscle fiber in (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone-treated mice compared to vehicle treated controls. This increased number of capillaries persisted at 3 weeks, the last time point for which samples were analyzed.

**Example 28**

**(4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone produces a dose-dependent phosphorylation of c-met**

Studies of c-met phosphorylation have been extended to demonstrate that the phosphorylation is dose-related and occurs in both HUVECs as well as MDCK cells (**Figure 22**). HUVECs (left set) or MDCK cells (right set) were treated with either HGF/SF or (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone, solubilized lysates were prepared from cells and immunoprecipitation of phosphorylated c-met and total c-met using specific antibodies was performed using standard techniques. Immunoprecipitates were separated on SDS-polyacrylamide gels and proteins were transferred to nitrocellulose membranes and detection of phosphorylated (top) and total c-met (bottom) was performed using an ECL chemiluminescence system (Amersham). Both HGF/SF and (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone showed significant amounts of phosphorylated c-met compared to unstimulated control cells. Total c-met is shown on the bottom.

This result further substantiates the findings that, like HGF/SF, (4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone produces its effects through activation of the c-met receptor.

**Example 29**

**Tumor and angiogenesis inhibition by c-met antagonists.**

Experiments were performed to determine the ability of HGF/SF antagonists to inhibit the growth of tumors and improve survival in in vivo experiments. DU145 tumor cells ($5 \times 10^6$) were injected subcutaneously into the lower right flank of male nude mice. On day 14, mice with established tumors (tumor size, 25-30 $mm^2$) were treated with a single intra-tumor injection of 1-(4-chloro-3-methylphenyl)-3-(2,6-dichlorophenyl)-prop-2-en-1-one (500 ng/50 ml in 50% EtOH/50% cremophor). 1-(4-Chloro-3-methylphenyl)-3-(2,6-dichlorophenyl)-prop-2-en-1-one caused a significant inhibition of the growth of tumors (**Figure 23A**). In addition, survival was significantly prolonged (**Figure 23B**).
Survival of mice recorded as the percentage of surviving animals on a given day. For all animal experiments, the tumor size was measured biweekly using a caliper and expressed as the product of the maximal perpendicular diameters ($mm^2$). Animals were sacrificed when the tumor diameter exceeded 150 mm, which is the end point for the experiment.

The present invention is not to be limited in scope by the specific embodiments describe herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

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WHAT IS CLAIMED IS:

1. A cellular proliferation promoting agent characterized by the ability to
   a) bind to a monoclonal or polyclonal antibody to HGF/SF; and
   b) exhibit proliferative activity in one or more in-vitro or in-vivo assays.

2. The cellular proliferation promoting agent of claim 1 wherein said proliferative activity is angiogenic activity.

3. The cellular proliferation promoting agent of claim 1 of 2 wherein said antibody is a monoclonal antibody.

4. The cellular proliferation promoting agent of any one of claims 1-3 which is capable of agonizing c-met.

5. The cellular proliferation promoting agent of any one of claims 1-4 which is an angiogenic agent.

6. The cellular proliferation promoting agent of any one of claims 1-5 selected from the group consisting of a small-molecule drug, a protein and a peptide.

7. The cellular proliferation promoting agent of any one of claims 1-6 which is a peptide.

8. The peptide of claim 7 selected from the group consisting of
   T M G F T A P R F P H Y (SEQ ID No:1);
   K V W Y H T T S I P S H (SEQ ID No:2);
   and conservatively-substituted variants thereof.

9. The peptide of any one of claims 7-8 further comprising a heparan-sulfate-binding peptide conjugated thereto.

10. The peptide of claim 9 wherein said heparan-sulfate-binding peptide is K V W Y H T T S I P S H C R P K A K A K A K A K D Q T K (SEQ ID No:7) or a conservatively-substituted variant thereof.

11. The peptide of any one of claims 9-10 selected from the group consisting of
   T M G F T A P R F P H Y K V W Y H T T S I P S H C R P K A K A K A K A K D Q T K (SEQ ID No:9);
   K V W Y H T T S I P S H K V W Y H T T S I P S H C R P K A K A

K A K A K D Q T K(SEQ ID No:10); and
a conservatively-substituted variant thereof.

12. A pharmaceutical composition comprising a cellular proliferation promoting agent of any one of claims 1-11 and a pharmaceutically-acceptable carrier.

13. A cellular proliferation promoting agent selected from the group consisting of
T M G F T A P R F P H Y (SEQ ID No:1);
K V W Y H T T S I P S H (SEQ ID No:2);
T M G F T A P R F P H Y K V W Y H T T S I P S H C R P K A K A K A K A K D Q T K (SEQ ID No:9); and
K V W Y H T T S I P S H K V W Y H T T S I P S H C R P K A K A
K A K A K D Q T K (SEQ ID No:10).

14. A polynucleotide sequence comprising a nucleic acid encoding the peptide of any one of claims 7-11 or 13 or a degenerate variant thereof.

15. A polynucleotide sequence comprising a nucleic acid encoding the proliferation promoting agent of claim 9, and degenerate variants thereof.

16. A cloning or expression vector comprising the polynucleotide of claim 14 or 15.

17. A host cell transformed with the expression vector of claim 16.

18. A method for promoting proliferation of a cell, tissue or organ comprising contacting said cell, tissue or organ with an effective proliferation promoting amount of an agent of any one of claims 1-13.

19. The method of claim 18 wherein said peptide is administered by means of gene therapy.

20. The method of any one of claims 18-19 wherein said peptide is administered by secretion from a cell expressing said peptide.

21. The method of any one of claims 18-20 wherein said cell, tissue or organ expresses c-met.

22. The method of any one of claims 18-21 wherein said cell is selected from the group consisting of an epithelial cell, an endothelial cell, a neuronal cell, a fibroblast, and a smooth muscle cell.

23. The method of any one of claims 18-22 wherein said cell, tissue or organ is a grafted cell, tissue or organ; a transplanted cell, tissue or organ; an ischemic cell, tissue or organ; skin; heart; kidney; or vascular tissue.

24. The method of any one of claims 18-23 wherein said promoting proliferation of a cell, tissue or organ promotes vascularization of said tissue or organ.

25. A method for treating an disease or condition characterized by inadequate perfusion of a cell, tissue or organ comprising contacting said cell, tissue or organ effective angiogenic amount of an agent of any one of claims 1-13.

26. The method of claim 25 wherein said agent is administered by means of gene therapy.

27. The method of claim 25 or 26 wherein said agent is administered by secretion from a cell expressing said peptide.

28. The method of any one of claims 25-27 wherein said cell, tissue or organ expresses c-met.

29. The method of any one of claims 25-28 wherein said cell is selected from the group consisting of an epithelial cell, an endothelial cell, a neuronal cell, a fibroblast, and a smooth muscle cell.

30. The method of any one of claims 25-28 wherein said tissue or organ is a grafted tissue or organ; a transplanted tissue or organ; an ischemic tissue or organ; skin; heart; kidney; or vascular tissue.

31. A method for identifying a proliferation promoting agent comprising the steps of:

a) providing a candidate agent;

b) measuring the ability of said agent to bind to a monoclonal or polyclonal antibody to HGF/SF; and

c) measuring the ability of said agent to exhibit proliferative activity in one or more in-vitro or in-vivo assays;

wherein said candidate agent with the ability to bind to a monoclonal or polyclonal antibody to HGF/SF and ability to exhibit proliferative activity is a proliferation promoting agent.

32. The method of claim 31 wherein said antibody is a monoclonal antibody.

33. The method of any one of claims 31-32 wherein said proliferative activity is angiogenic activity.

34. The method of any one of claims 31-33 wherein said agent is a small-molecule drug, a peptide, or a protein.

35. The method of any one of claims 31-34 wherein said ability of said agent to bind to a monoclonal or polyclonal antibody to HGF/SF is determined by measurement of binding of said agent to said antibody, or by measuring the ability of said agent to compete with the binding of said antibody with HGF/SF or a peptide mimetic thereof.

36. The method of any one of claims 31-35 wherein said agent additionally agonizes c-met.

37. A method of preparing a proliferation promoting agent comprising the steps of
    a) identifying an angiogenic peptide in accordance with any one of claims 31-36;
    b) determining the three-dimensional structure of said proliferation promoting peptide; and
    c) modeling a small-molecule drug on said three-dimensional structure of said proliferation promoting peptide.

38. An antiproliferative agent characterized by the ability to
    i) bind to the extracellular domain of c-met; and
    ii) inhibit HGF/SF-mediated increase in cellular growth or proliferation.

39. The antiproliferative agent of claim 38 which is capable of antagonizing c-met.

40. The antiproliferative agent of any one of claims 38-39 wherein said cellular growth or proliferation is cellular growth or proliferation of a cell that expresses c-met.

41. The antiproliferative agent of any one of claims 38-40 wherein said cell that expresses c-met is a tumor cell, an epithelial cell, a neuronal cell, a fibroblast, or an endothelial cell.

42. The antiproliferative agent of any one of claims 38-41 wherein said tumor cell is a glioma or a *glioblastoma*.

43. The antiproliferative agent of any one of claims 38-42 which is an antiangiogenic agent.

44. The antiproliferative agent of any one of claims 38-43 selected from the group consisting of a small-molecule drug, a protein or a peptide.

45. The antiproliferative agent of any one of claims 38-44 which is a peptide.

46. The peptide of claim 45 selected from the group consisting of
A T W S H H L S S A G L (SEQ ID No:3);
W P Q L P P R P Y S T L (SEQ ID No:4);
S N T S A G T P F T S L (SEQ ID No:5);
D S T P K S T P W Y Y I (SEQ ID No:6); and
conservatively-substituted variants thereof.

47. A pharmaceutical composition comprising an antiproliferative agent of any one of claims 38-46 and a pharmaceutically-acceptable carrier.

48. An antiproliferative peptide selected from the group consisting of
A T W S H H L S S A G L (SEQ ID No:3);
W P Q L P P R P Y S T L (SEQ ID No:4);
S N T S A G T P F T S L (SEQ ID No:5); and
D S T P K S T P W Y Y I (SEQ ID No:6).

49. A polynucleotide sequence comprising a nucleic acid encoding the peptide of claim 45 or 48, and degenerate variants thereof.

50. A cloning or expression vector comprising the polynucleotide of claim 49.

51. A host cell transformed with the expression vector of claim 50.

52. A method for inhibiting the proliferation of a cell, tissue or organ comprising contacting said cell, tissue or organ with an effective antiproliferative amount of an agent of any one of claims 38-48.

53. The method of claim 52 wherein said cell, tissue or organ expresses c-met.

54. The method of claim 52 or 53 wherein said antiproliferative peptide is administered by means of gene therapy.

55. The method of any one of claims 52-54 wherein said antiproliferative peptide is administered by secretion from a cell expressing said peptide.

56. The method of any one of claims 52-55 wherein said cell, tissue or organ is a dysproliferative tissue, a tumor, a metastasis, a psoriatic lesion; an eye, a dermal lesion, a reproductive organ, or a keloid.

57. A method for treating a disease or condition characterized by the abnormal proliferation of a cell, tissue or organ comprising contacting said cell, tissue or organ with an effective antiproliferative amount of an agent of any one of claims 38-48.

58. The method of claim 57 wherein said disease is cancer, diabetic retinopathy, an inflammatory disease, an inflammatory joint disease, an inflammatory skin disease, psoriasis, peripheral vascular disease, arteriosclerosis obliterans or diabetic nephropathy.

59. The method of claim 57 or 58 wherein said antiproliferative peptide is administered by means of gene therapy.

60. The method of any one of claims 57-59 wherein said antiproliferative peptide is administered by secretion from a cell expressing said peptide.

61. A method for inhibiting the vascularization of a tissue or organ comprising contacting said tissue or organ with an effective antiangiogenic amount of an agent of any one of claims 38-48.

62. The method of claim 61 wherein said antiproliferative peptide is administered by means of gene therapy.

63. The method of claim 61 or 62 wherein said antiproliferative peptide is administered by secretion from a cell expressing said peptide.

64. The method of any one of claims 61-63 wherein said tissue or organ is a dysproliferative tissue, a tumor, a metastasis, a psoriatic lesion; an eye, a dermal lesion, a reproductive organ, or a keloid.

65. A method for identifying an agent capable of inhibiting cellular proliferation comprising the steps of

a) providing a candidate agent;

b) measuring the ability of said agent to bind to the extracellular domain of C-met; and

c) measuring the ability of said agent to inhibit the proliferative activity of HGF/SF;

wherein said candidate agent with the ability to bind to the extracellular domain of C-met and inhibit the proliferative activity of HGF/SF is an antiproliferative agent.

66. The method of claim 65 wherein said agent is a small-molecule drug, a peptide, or a protein.

67. The method of claim 65 or 66 wherein said agent inhibits angiogenesis.

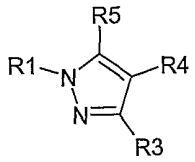
68. A method of preparing an antiproliferative agent comprising the steps of

a) identifying an antiproliferative peptide in accordance with any one of claims 65-67;

b) determining the three-dimensional structure of said antiproliferative peptide; and

c) modeling a small-molecule drug on said antiproliferative peptide.

69. A method for modulating HGF/SF activity in a mammal comprising administering to said mammal an effective HGF/SF activity modulating amount of a compound with the general formula I:




Formula I

wherein

R3 and R5 are independently or together methyl, t-butyl or chloro groups;

R1 is CO-Aryl, $SO_2$-Aryl, CO-heteroaryl, or CO-alkyl, wherein said Aryl is phenyl, naphthyl or diphenyl, said Aryl substituted with one or more halogen, C1 to C4 alkyl, or C1 to C4 alkyloxy groups, or said heteroaryl is a 3-aryl-substituted isoxazole or a 3-aryl substituted thienyl group, or said alkyl group is a t-butyl; and

R4 is $CH_2$-Aryl, halogen, arylcarbonylvinyl or S-heteroaryl.

70. A method for modulating HGF/SF activity in a mammal comprising administering to said mammal an effective HGF/SF activity modulating amount of a compound selected from the group consisting of

3-(5-chloro-1,3-dimethyl-1H-pyrazol-4-yl)-1-(4-chlorophenyl)prop-2-en-1-one;

[4-(2,6-dichlorobenzyl)-3,5-dimethyl-1H-pyrazol-1-yl][3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl]methanone;

(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)methanone;

4-(2-chloro-6-fluorobenzyl)-1-((3,4-dichlorophenyl)sulfonyl)-3,5-dimethyl-1H-pyrazole;

4-(2-chloro-6-fluorobenzyl)-1,3,5-trimethyl-1H-pyrazole;

4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole;

(4-bromo-3,5-dimethyl-1H-pyrazol-1-yl)(3-(2,6-dichlorophenyl)isoxazole-4-carbohydrazide);

3-(4-(2,6-dichlorobenzyl)-3,5-dimethyl-1H-pyrazol-1-yl)propanenitrile;

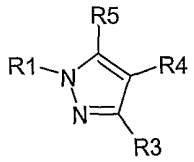
3,5-di(tert-butyl)-4-(2-chloro-6-fluorobenzyl)-1H-pyrazole;

(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(2,6-dichlorophenyl)methanone;

1-(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)2,2-dimethylpropan-1-one;
(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(4-chlorophenyl)methanone;
(4-(2-chloro-6-fluorobenzyl)-3,5-dimethyl-1H-pyrazole-1-yl)(2-thienyl)methanone; and
(4-chlorophenyl)(3,5-dimethyl-4-((1-methyl-1H-imidazol-2-yl)thio)-1H-pyrazol-1-yl)methanone.

71. The method of claim 69 or 70 where said compound is an HGF/SF agonist or an HGF/SF antagonist.

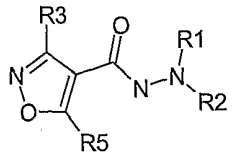
72. A pharmaceutical composition comprising a compound with the general formula I:




Formula I

wherein
R3 and R5 are independently or together methyl, t-butyl or chloro groups;
R1 is CO-Aryl, $SO_2$-Aryl, CO-heteroaryl, or CO-alkyl, wherein said Aryl is phenyl, naphthyl or diphenyl, said Aryl substituted with one or more halogen, C1 to C4 alkyl, or C1 to C4 alkyloxy groups, or said heteroaryl is a 3-aryl-substituted isoxazole or a 3-aryl substituted thienyl group, or said alkyl group is a t-butyl; and
R4 is $CH_2$-Aryl, halogen, arylcarbonylvinyl or S-heteroaryl;
and a pharmaceutically-acceptable carrier.

73. A method for modulating HGF/SF activity in a mammal comprising administering to said mammal an effective HGF/SF activity modulating amount of a compound with the general formula II:




Formula II

wherein
R5 is methyl;
R3 is an alkyl-, halogen- or alkyloxy-substituted phenyl group;
R1 is hydrogen or methyl; and
R2 is a substituted pyridyl or arylthiocarbonyl group or a 4-aryl-substituted 5-methylisoxazonecarbonyl group.

74. The method of claim 73 wherein said pyridyl group is a 2-(6-trifluoromethyl)pyridyl group or said arylthiocarbonyl group is a 2-(nitrophenyl)thiocarbonyl group.

75. A method for modulating HGF/SF activity in a mammal comprising administering to said mammal an effective HGF/SF activity modulating amount of a compound selected from the group consisting of

N'4,5-dimethyl-N'4-(5-nitro-2-pyridyl)-3-(2,6-dichlorophenyl)isoxazole-4-carbohydrazide;

N'4-(2-(((2,4-dichlorobenzylidene)amino)oxy)acetyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide;

N'4-(3-(3,4,5-trimethoxyphenyl)propanoyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide;

2-nitrophenyl 2-((3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)carbonyl)hydrazine-1-carbothioate;

N'4-((2-methyl-1,3-thiazol-4-4yl)carbonyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-4carbohydrazide;

N1-((2-((3-(2,6-dichlorophenyl)-5-methylisoxazol-4-yl)carbonyl)hydrazino)(methylthio)methylidene)benzene-1-sulfonamide;

N'4-(2,4,6-trichlorophenyl)-3-3(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide;

N'4,3-di(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide;

N'4-(3,5-dichloro-4-pyridyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide;

N'4-phenyl-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide;

N'4,N'4,5-trimethyl-3-(2,6-dichlorophenyl)isoxazole-4-carbohydrazide;

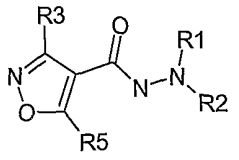
N4-azepan-1-yl-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carboxamide;

N'4-(6-(trifluoromethyl)-2-pyridyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide; and

N'4-(3,3-diethoxypropanoyl)-3-(2,6-dichlorophenyl)-5-methylisoxazole-4-carbohydrazide.

76. The method of claim 73 or 75 where said compound is an HGF/SF agonist or an HGF/SF antagonist.

77. A pharmaceutical composition comprising a compound with the general formula II:

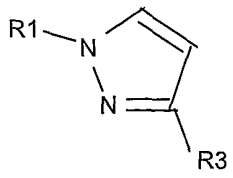



Formula II

wherein

R5 is methyl;
R3 is an alkyl-, halogen- or alkyloxy-substituted phenyl group;
R1 is hydrogen or methyl; and
R2 is a substituted pyridyl or arylthiocarbonyl group or a 4-aryl-substituted 5-methylisoxazonecarbonyl group;
and a pharmaceutically-acceptable carrier.

78. A method for modulating HGF/SF activity in a mammal comprising administering to said mammal an effective HGF/SF activity modulating amount of a compound with the general formula III:




Formula III

wherein

R1 is $SO_2$Alkyl, wherein alkyl is a C1 to C4 straight-chain, branched or cycloalkyl group; $SO_2$-Aryl, wherein aryl is halo, C1 to C4 alkyl- or alkyloxy-substituted phenyl; COAlkyl, wherein alkyl is C1 to C6 straight-chained alkyl, branched alkyl or cycloalkyl; COAryl, wherein Aryl is phenyl substituted with halo, C1-C4 alkyl or alkyloxy; CONHAlkyl wherein alkyl is C1 to C6 straight-chained alkyl, branched alkyl or cycloalkyl; or CONHAryl, wherein aryl is phenyl substituted with halo, C1 to C4 alkyl or C1 to C4 alkyloxy; and

R3 is CHCH-heteroaryl, where in heteroaryl is cis or trans CHCH-3-thienyl, CHCH-2-furyl, CHCH-3-furyl, substituted CHCH-thienyl, or CHCH-furyl; phenoxyphenyl; heteroaryl; or aryl substituted heteroaryl.

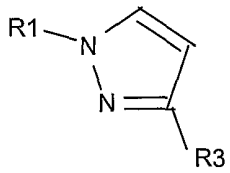
79. The method of claim 78 wherein R1 is $S0_2CH_3$; CO-t-Butyl, or $CONHCH_3$ and R3 is CHCH-2-thienyl.

80. A method for modulating HGF/SF activity in a mammal comprising administering to said mammal an effective HGF/SF activity modulating amount of a compound selected from the group consisting of

(4-chlorophenyl)[3-(2-(2-thienyl)vinyl)-1H-pyrazol-1-yl]methanone;
1-(methylsulfonyl)-3-(2-(2-thienyl)vinyl)-1H-pyrazole;
2,2-dimethyl-1-(3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-yl)propan-1-one;
N-methyl-3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-carboxamide;
(4-chlorophenyl)(3-(3-phenylisoxazol-5-yl)-1H-pyrazol-1-yl)methanone;
(4-chlorophenyl)(3-(3-(4-chlorophenyl)-5-methylisoxazol-4-yl)-1H-pyrazol-1-yl)methanone;
(4-chlorophenyl)(3-(5-(2-thienyl)-2-thienyl)-1H-pyrazol-1-yl)methanone;
(2,4-dichlorophenyl)(3-(5-(2,4-difluorophenyl)-2-furyl)-1H-pyrazol-1-yl)methanone;
N1-phenyl-3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-carboxamide;
(4-chlorophenyl)(3-(2-(5-(2-thienyl)-2-thienyl)-4-methyl-1,3-thiazol-5-yl)-1H-pyrazol-1-yl)methanone;
(3-benzhydryl-1H-pyrazol-1-yl)(4-chlorophenyl)methanone;
N1-(4-chlorophenyl)-3-(2-(2-thienyl)vinyl)-1H-pyrazole-1-carboxamide;
(4-chlorophenyl)(3-(2-methylimidazo(1,2-a)pyridin-3-yl)-1H-pyrazol-1-yl)methanone;
2-chloro-6-(4-(1-(4-chlorobenzyl)-1H-pyrazol-3-yl)phenoxy)benzonitrile; and
1-((4-chlorophenyl)sulfonyl)-3-(2-(2-thienyl)vinyl)-1H-pyrazole.

81. The method of any one of claims 76-80 where said compound is an HGF/SF agonist or an HGF/SF antagonist.

82. A pharmaceutical composition comprising a compound with the formula III:




Formula III

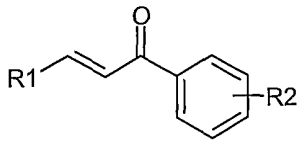
wherein

R1 is $SO_2$Alkyl, wherein alkyl is a C1 to C4 straight-chain, branched or cycloalkyl group; $SO_2$-Aryl, wherein aryl is halo, C1 to C4 alkyl- or alkyloxy-substituted phenyl; COAlkyl, wherein alkyl is C1 to C6 straight-chained alkyl, branched alkyl or cycloalkyl; COAryl, wherein Aryl is phenyl substituted with halo, C1-C4 alkyl or alkyloxy; CONHAlkyl wherein alkyl is C1 to C6 straight-chained alkyl, branched alkyl or cycloalkyl; or CONHAryl, wherein aryl is phenyl substituted with halo, C1 to C4 alkyl or C1 to C4 alkyloxy;

R3 is CHCH-heteroaryl, where in heteroaryl is cis or trans CHCH-3-thienyl, CHCH-2-furyl, CHCH-3-furyl, substituted CHCH-thienyl, or CHCH-furyl; phenoxyphenyl; heteroaryl; or aryl substituted heteroaryl;

and a pharmaceutically-acceptable carrier.

83. A method for modulating HGF/SF activity in a mammal comprising administering to said mammal an effective HGF/SF activity modulating amount of a compound with the general formula IV:




Formula IV

wherein

R1 is a phenyl group substituted with hydrogen, a halogen, C1 to C4 alkyl, or C1 to C4 alkyloxy, or is a heteroaryl group selected from the group consisting of 4-bromo-2-thienyl, 4-pyridyl, 2-furyl, 3-thienyl, optionally substituted with halogens and/or C1 to C4 alkyl; and

R2 is one or more hydrogen, C1 to C4 alkyl, halogen, or C1 to C4 alkyloxy groups.

84. A method for modulating HGF/SF activity in a mammal comprising administering to said mammal an effective HGF/SF activity modulating amount of a compound selected from the group consisting of

1-(4-chloro-3-methylphenyl)-3-(2,6-dichlorophenyl)-prop-2-en-1-one

1-(4-chloro-3-methylphenyl)-3-(2-chlorophenyl)prop-2-en-1-one

3-(2-chloro-6-fluorophenyl)-1-(4-chloro-3-methylphenyl)prop-2-en-1-one

3-(4-bromo-2-thienyl)-1-(3,4-dichlorophenyl)prop-2-en-1-one

3-(4-bromo-2-thienyl)-1-(4-chloro-3-methylphenyl)prop-2-en-1-one

3-(4-bromo-2-thienyl)-1-(4-fluorophenyl)prop-2-en-1-one

3-(4-bromo-2-thienyl)-1-(4-chlorophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-(2,4-dichlorophenyl)prop-2-en-1-one

3-(1,3-benzodioxol-5-yl)-1-(4-bromophenyl)prop-2-en-1-one

3-(3-phenoxy-2-thienyl)-1-(2-thienyl)prop-2-en-1-one

3-(3-bromo-4-methoxyphenyl)-1-phenylprop-2-en-one

3-(3,4-dichlorophenyl)-1-(2-nitrophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-(3,4-dichlorophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-(3,5-dichloro-2-hydroxyphenyl)prop-2-en-1-one

1-(2-chlorophenyl)-3-(3,5-dichloro-2-hydroxyphenyl)prop-2-en-1-one

3-(4-chlorophenyl)-1-(2,6-dichlorophenyl)prop-2-en-1-one

1-(4-bromophenyl)-3-(4-chlorophenyl)prop-2-en-1-one

1-(2-chlorophenyl)-3-(2,6-dichlorophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-(2,6-dichlorophenyl)prop-2-en-1-one

3-(2,6-dichlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one

3-(4-chloro-1-methyl-1H-pyrazol-3-yl)-1-[4-(trifluoromethyl)phenyl]prop-2-en-1-one

3-(2,4-dichlorophenyl)-1-(2-methylphenyl)prop-2-en-1-one

3-(2,6-dichlorophenyl)-1-(2-methylphenyl)prop-2-en-1-one

3-(3,4-dichlorophenyl)-1-(2-methylphenyl)prop-2-en-1-one

3-(5-bromo-2-hydroxyphenyl)-1-(3-methylphenyl)prop-2-en-1-one

3-(5-bromo-2-hydroxyphenyl)-1-(4-methylphenyl)prop-2-en-1-one

3-(2,4-dichlorophenyl)-1-(3-methylphenyl)prop-2-en-1-one

3-(2,4-dichlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-one

1-[4-amino-2-(methylthio)-1,3-thiazol-5-yl]-3-(4-chlorophenyl)prop-2-en-1-one

1-(4-chlorophenyl)-3-[4-(trifluoromethyl)phenyl]prop-2-en-1-one

1-benzo[b]thiophen-3-yl-3-(4-chlorophenyl)prop-2-en-1-one

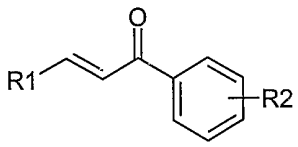
1,3-di(5-nitro-3-thienyl)prop-2-en-1-one

1-(4-bromophenyl)-3-(3,5-difluorophenyl)prop-2-en-1-one

3-(3,5-difluorophenyl)-1-(3-nitrophenyl)prop-2-en-1-one

85. The method of claim 83 or 84 where said compound is an HGF/SF agonist or an HGF/SF antagonist.

86. A pharmaceutical composition comprising a compound with the general formula IV:




Formula IV

wherein

R1 is a phenyl group substituted with hydrogen, a halogen, C1 to C4 alkyl, or C1 to C4 alkyloxy, or is a heteroaryl group selected from the group consisting of 4-bromo-2-thienyl, 4-pyridyl, 2-furyl, 3-thienyl, optionally substituted with halogens and/or C1 to C4 alkyl; and

R2 is one or more hydrogen, C1 to C4 alkyl, halogen, or C1 to C4 alkyloxy groups;

and a pharmaceutically-acceptable carrier.

87. A method for increasing hepatocyte growth factor / scatter factor (HGF/SF) activities in a mammal comprising administration to said mammal an effective amount of a compound having a

induction of proliferation of endothelial cells in vitro or in vivo;
induction of angiogenesis in vitro or in vivo;
increasing angiogenesis in wounds in vivo;
promoting tumor growth;
inducing gene expression of angiogenic-cascade-related genes such as but not limited to IL-8 and angiopoietin-2;
inducing anti-apoptotic activity; and
inducing scatter activity.

95. The method of claim 94 wherein said HGF/SF activity of said compound is inhibited in the presence of c-met.

96. The method of claim 95 or 96 wherein said compounds binds c-met.

97. The method of any one of claims 94-96 wherein said compound exhibits HGF/SF-like activity in at least two said HGF/SF activity assays, at least three said HGF/SF activity assays, at least four said HGF/SF activity assays, at least five said HGF/SF activity assays, at least six said HGF/SF activity assays, or in all said HGF/SF activity assays.

98. The method of claim 94-97 wherein said compound has a molecular weight between about 200 and about 1,000 Daltons.

99. The method of any one of claims 94-98 wherein said compound has a molecular weight between 300 Daltons and about 750 Daltons.

100. The method of any one of claims 94-99 wherein said compound has a molecular weight between about 300 Daltons and about 500 Daltons.

101. A method for inhibiting the activity of hepatocyte growth factor / scatter factor (HGF/SF) in a mammal comprising administering to said mammal an effective amount of a compound having a molecular weight below about 1,000 Daltons, said compound exhibiting HGF/SF inhibitory or antagonistic activity in at least one HGF/SF activity assay selected from the group consisting of
inhibiting proliferation of endothelial cells in vitro or in vivo;
inhibiting the growth of tumor cells in vitro or in vivo;
inhibiting scatter of normal or tumor cells in vitro or in vivo; and
inhibiting anti-apoptotic activity.

102. The method of claim 101 wherein said activities are exhibitable in the presence of exogenously-added HGF/SF.

103. The method of claim 101 or 102 wherein said compound exhibits HGF/SF inhibitory activity in at least two said HGF/SF activity assays, in at least three said HGF/SF activity assays, or in all said HGF/SF activity assays.

104. The method of any one of claims 101-103 wherein said compound has a molecular weight between about 200 Daltons and about 1,000 Daltons.

105. The method of any one of claims 101-104 wherein said compound has a molecular weight between about 300 Daltons and about 750 Daltons.

106. The method of any one of claims 101-105 wherein said compound has a molecular weight between about 300 Daltons and about 500 Daltons.

107. A method for the prophylaxis or treatment in a mammal of a condition of disease selected from the group consisting of excessive cellular proliferation, angiogenesis, a dysproliferative disease, cancer, metastasis, inflammatory disease, diabetic retinopathy, inflammatory joint disease, and inflammatory skin disease comprising administering to said mammal an effective amount of a compound having a molecular weight below about 1,000 Daltons, said compound exhibiting HGF/SF inhibitory or antagonistic activity in at least one HGF/SF activity assay selected from the group consisting of

inhibiting proliferation of endothelial cells in vitro or in vivo;
inhibiting the growth of tumor cells in vitro or in vivo;
inhibiting scatter of normal or tumor cells in vitro or in vivo; and
inhibiting anti-apoptotic activity.

108. The method of claim 107 wherein said activities are exhibitable in the presence of exogenously-added HGF/SF.

109. The method of claim 107 or 108 wherein said compound exhibits HGF/SF inhibitory activity in at least two said HGF/SF activity assays, in at least three said HGF/SF activity assays, or in all said HGF/SF activity assays.

110. The method of any one of claims 107-109 wherein said compound has a molecular weight between about 200 Daltons and about 1,000 Daltons.

111. The method of any one of claims 107-110 wherein said compound has a molecular weight between about 300 Daltons and about 750 Daltons.

112. The method of any one of claims 107-111 wherein said compound has a molecular weight between about 300 Daltons and about 500 Daltons.

113. A compound having a molecular weight of less than about 1,000 Daltons and having tyrosine kinase receptor agonist or stimulatory activity.

114. The compound of claim 113 wherein said tyrosine kinase receptor agonist or stimulatory activity is HGF/SF, VEGF or FGF agonist or stimulatory activity.

115. A pharmaceutical composition comprising a compound of claim 113 or 114 and a pharmaceutically acceptable carrier.

116. A method for treating an individual for a condition or disease benefited from agonizing or stimulating a tyrosine kinase receptor comprising administering to said individual a therapeutically effective amount of a pharmaceutical composition of claim 115.

117. A compound having a molecular weight of less than about 1,000 Daltons and having tyrosine kinase receptor antagonist or inhibitory activity.

118. The compound of claim 115 wherein said tyrosine kinase receptor antagonist or inhibitory activity is HGF/SF, VEGF or FGF antagonist or inhibitory activity.

119. A pharmaceutical composition comprising a compound of claim 117 or 118 and a pharmaceutically acceptable carrier.

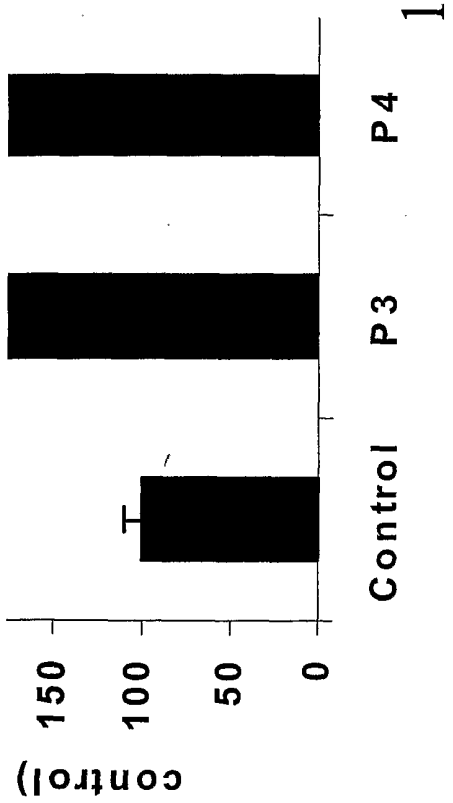
120. A method for treating an individual for a condition or disease benefited from agonizing or stimulating a tyrosine kinase receptor comprising administering to said individual a therapeutically effective amount of a pharmaceutical composition of claim 119.

121. A pharmaceutical composition comprising a tyrosine kinase receptor growth factor agonist selected from the group consisting of 3,3-dibromo-1-phenyl-1,2,3,4-tetrahydroquinoline-2,4-dione, 4-(4-chlorophenyl)-6-(dimethylamino)-2-phenyl-5-pyrimidinecarbonitrile, an analog thereof, and a pharmaceutically-acceptable carrier.

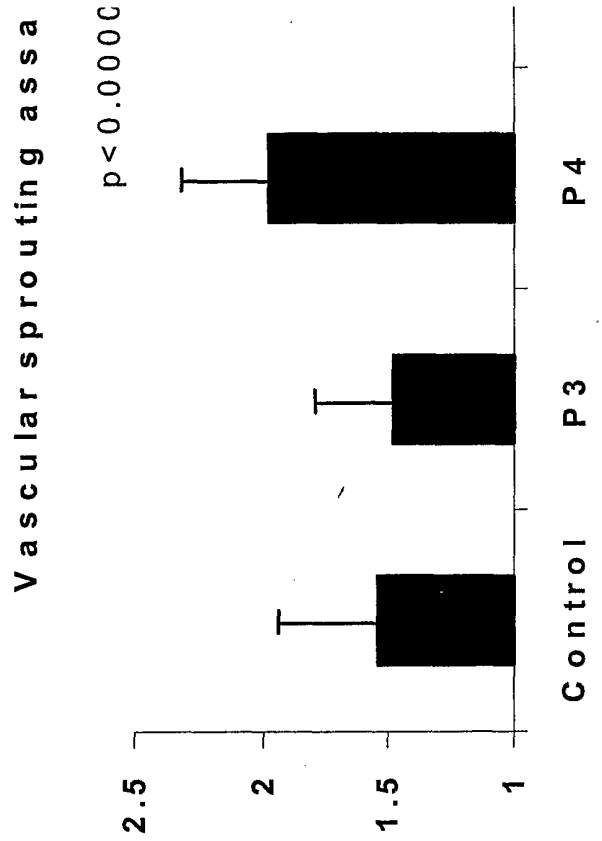
122. A method of treating an individual for a condition or disease in which the activity of VEGF is desired comprising administering to said individual a pharmaceutical composition comprising an effective amount of 3,3-dibromo-1-phenyl-1,2,3,4-tetrahydroquinoline-2,4-dione, 4-(4-

chlorophenyl)-6-(dimethylamino)-2-phenyl-5-pyrimidinecarbonitrile, or an analog thereof, and a pharmaceutically-acceptable carrier.

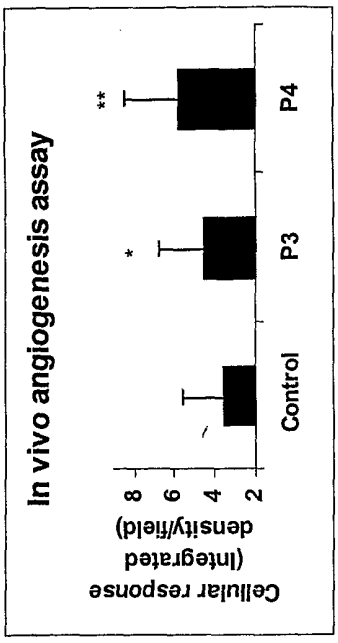
5



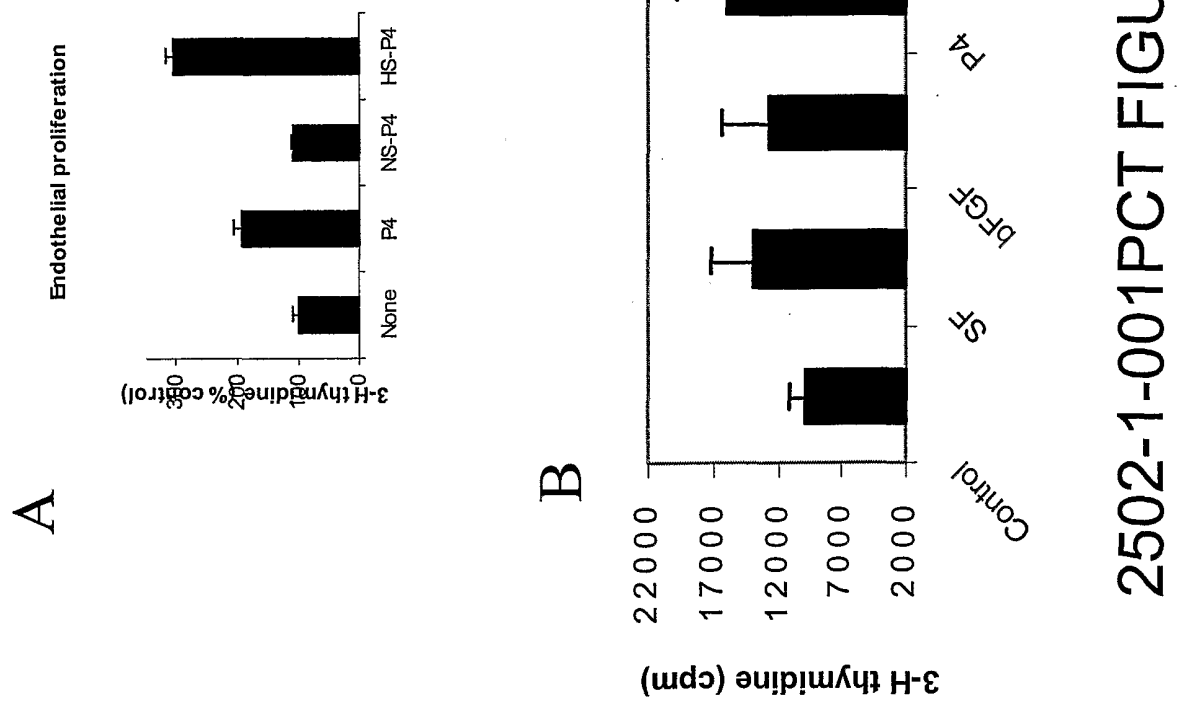

2502-1-001PCT FIGURE 1



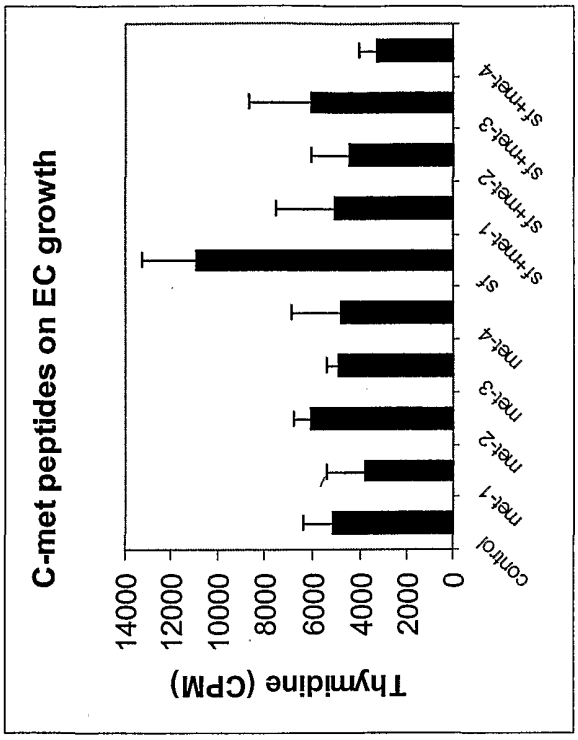

2502-1-001PCT FIGURE 2



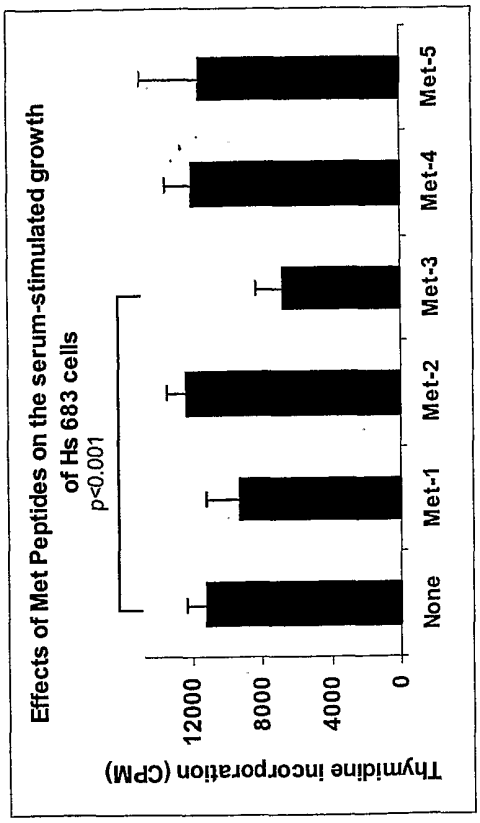

2502-1-001PCT FIGURE 3

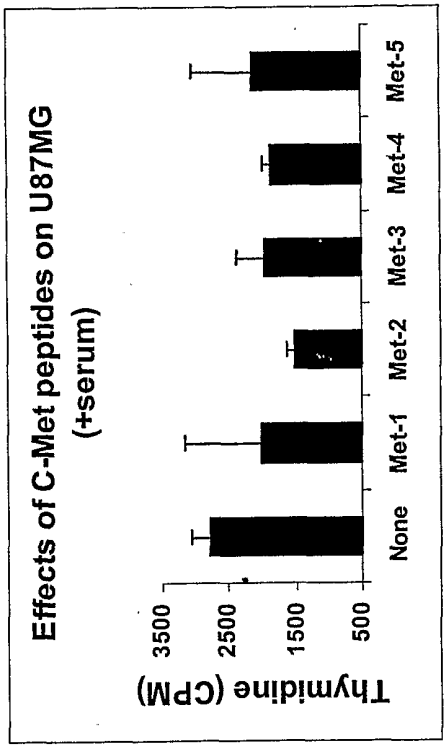



2502-1-001PCT FIGURES 4A and 4B

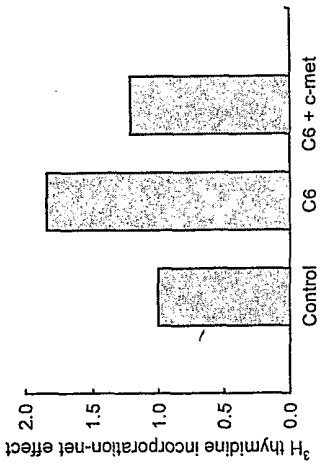



2502-1-001PCT FIGURE 5




2502-1-001PCT FIGURE 6A and 6B




2502-1-001PCT FIGURE 7

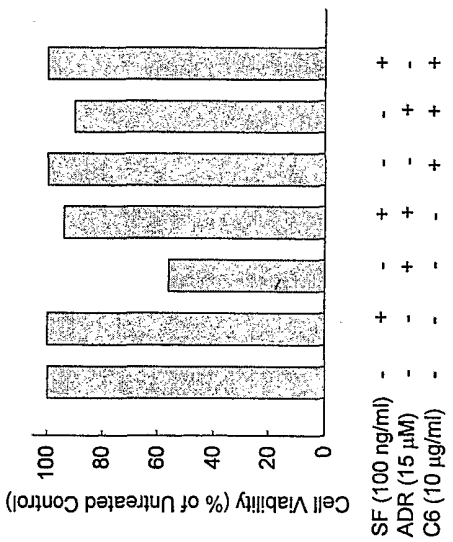
B



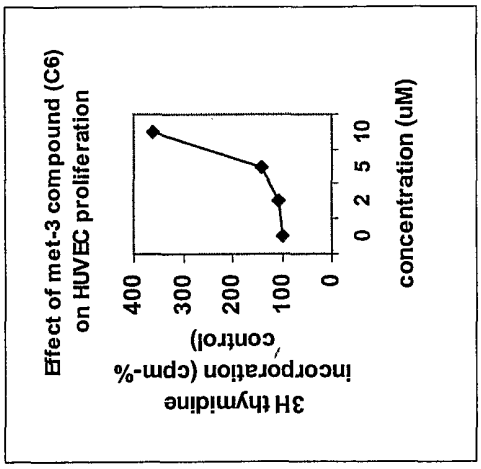
A



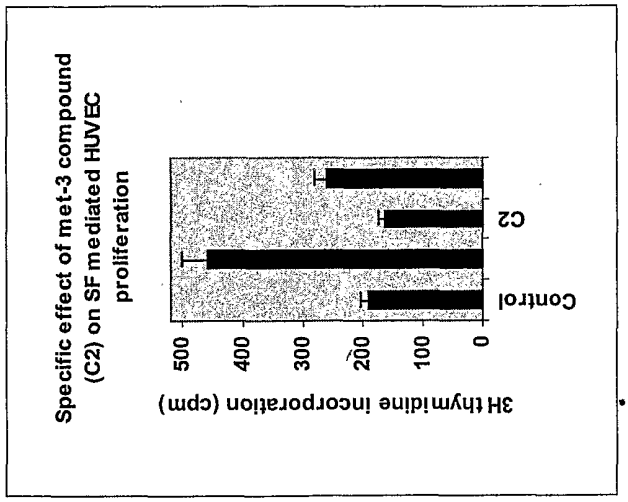
2502-1-001PCT FIGURES 8A AND 8B



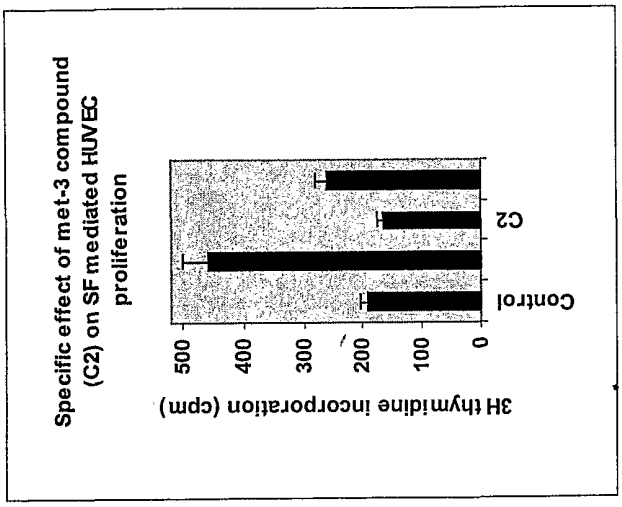

2502-1-001PCT FIGURE 9



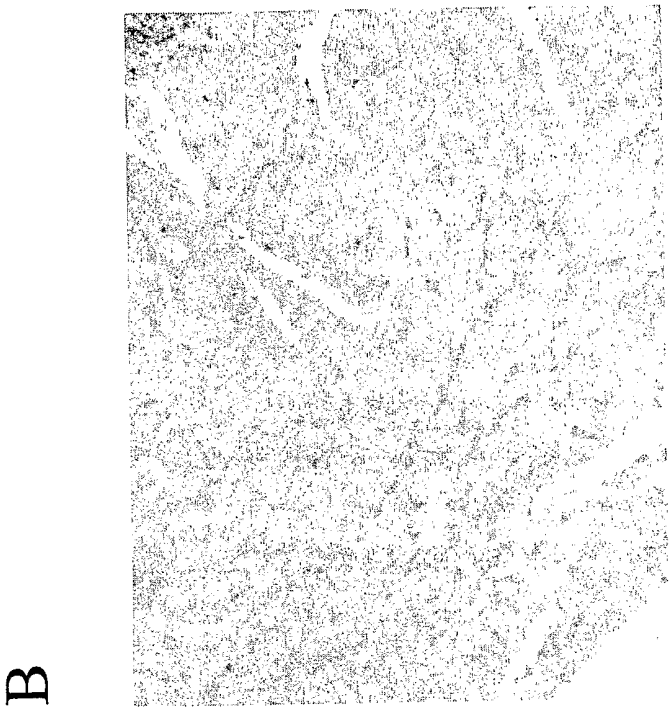

2502-1-001PCT FIGURE 10



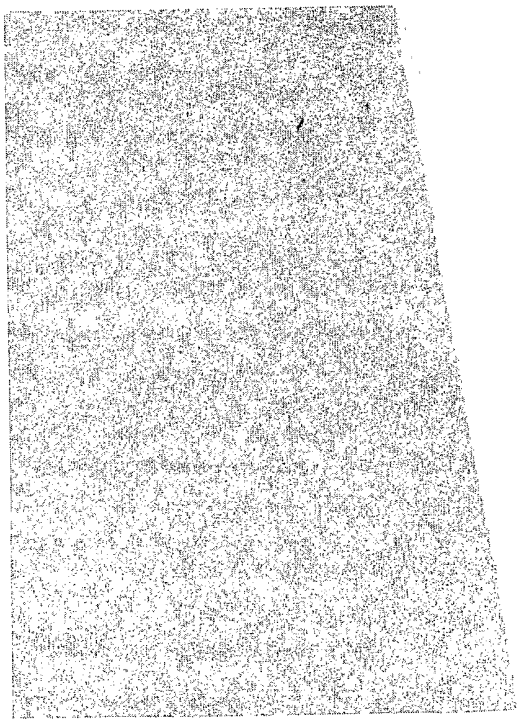

2502-1-001PCT FIGURE 11




2502-1-001PCT FIGURE 11

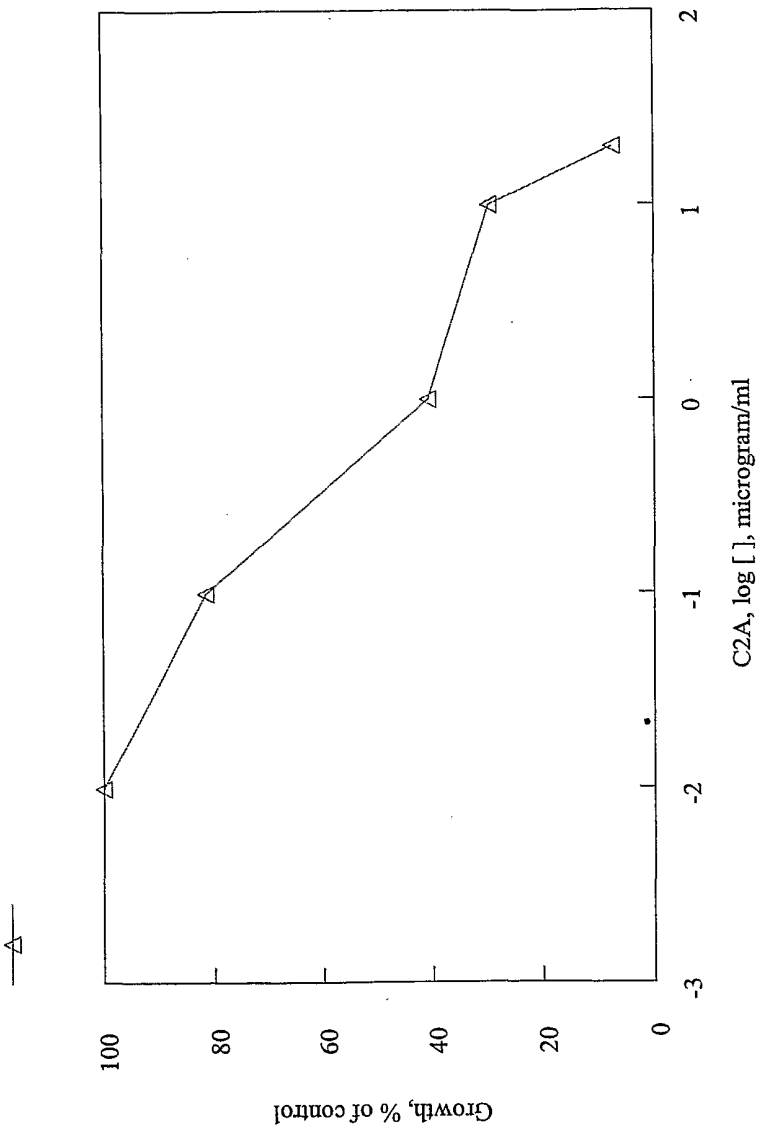


B

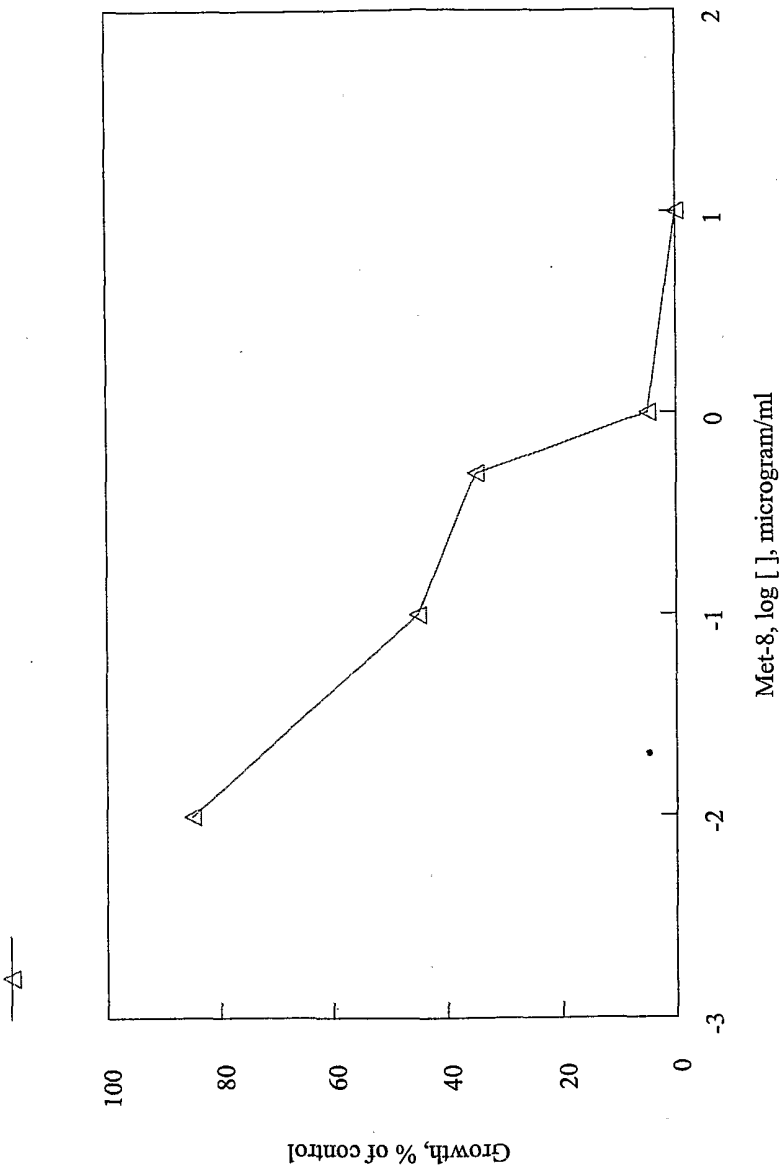


A

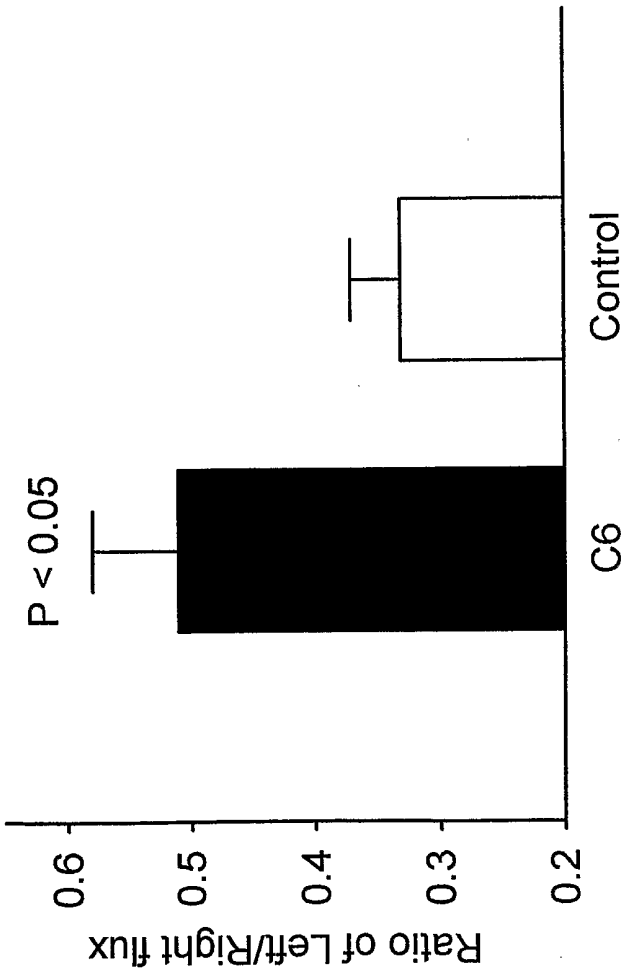
2502-1-001PCT FIGURE 12A and 12B



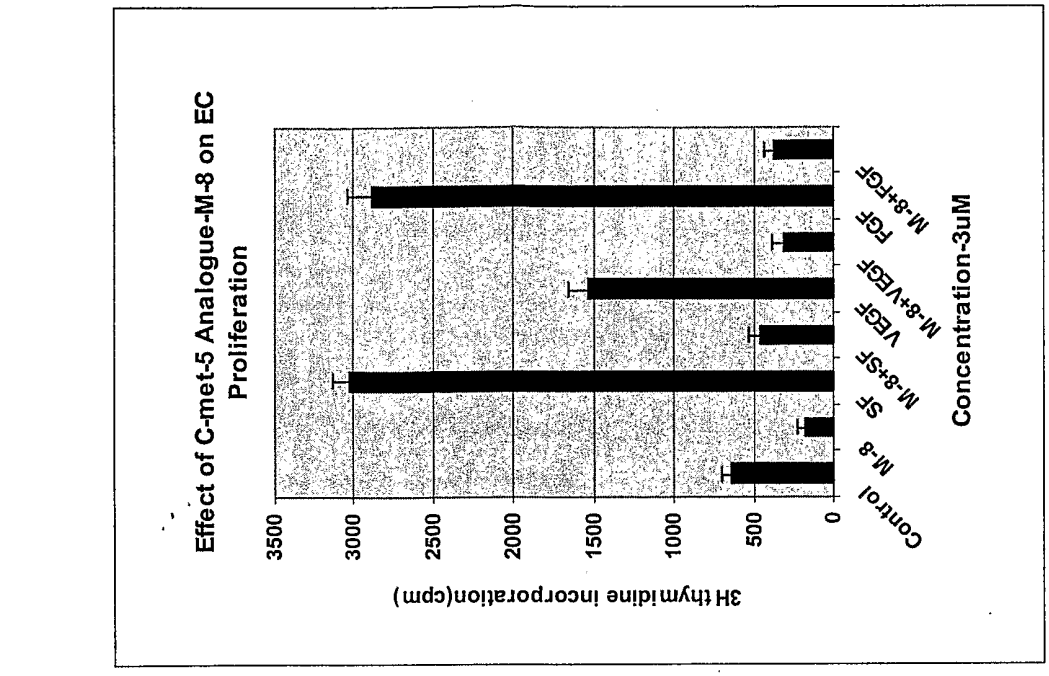

2502-1-001PCT FIGURE 13

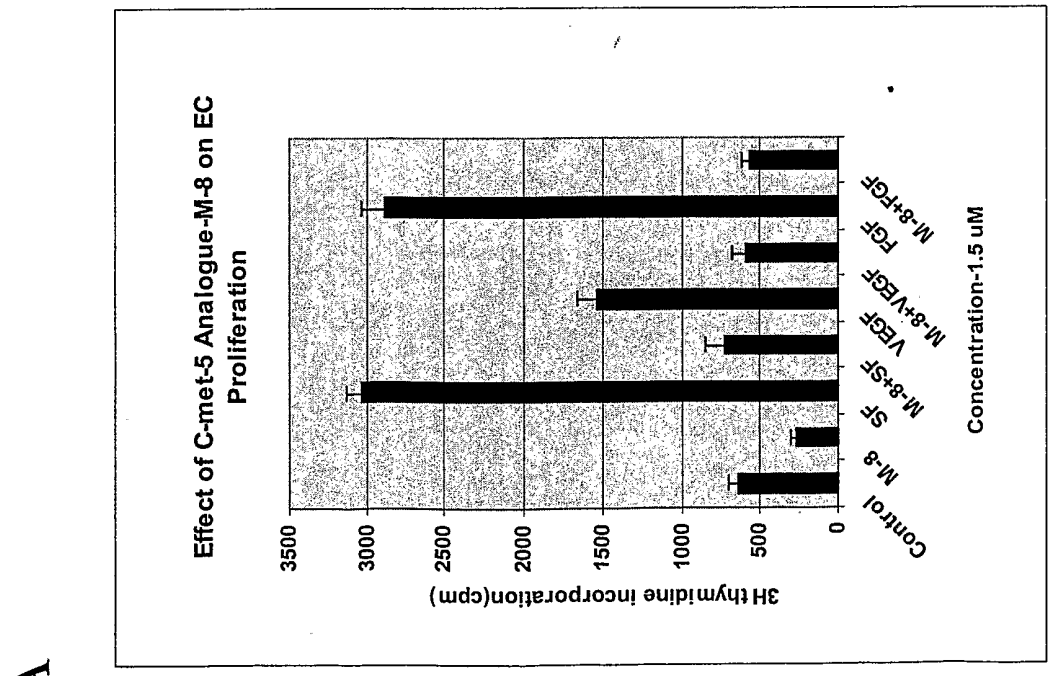



2502-1-001PCT FIGURE 14

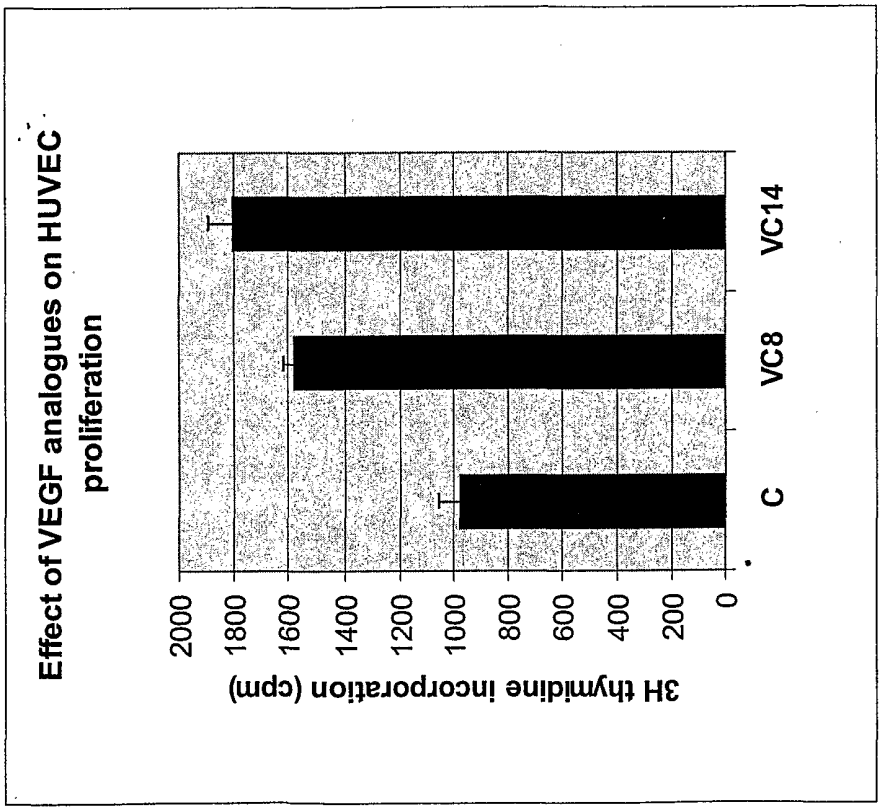



2502-1-001PCT FIGURE 15

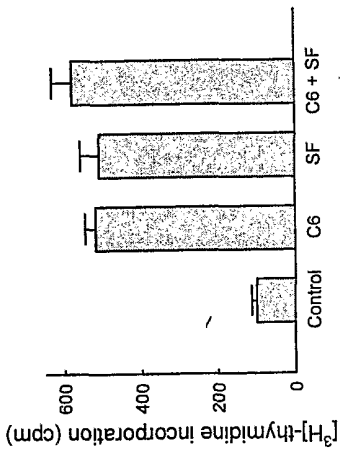


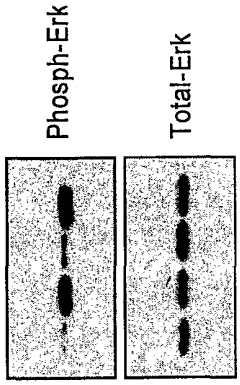

2502-1-001PCT FIGURE 16A and 16B



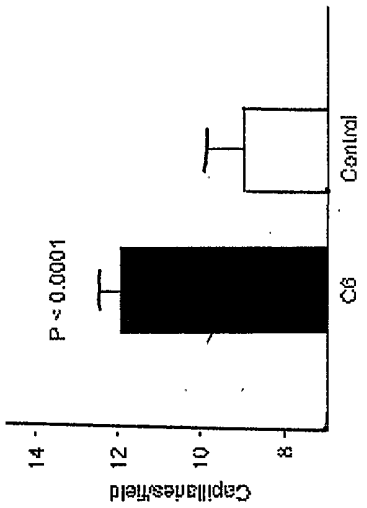

2502-1-001PCT FIGURE 17



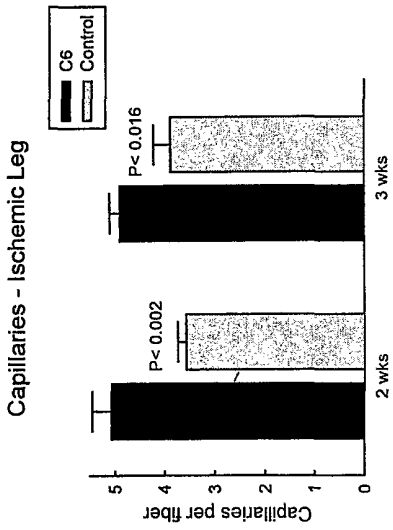

2502-1-001PCT FIGURE 18



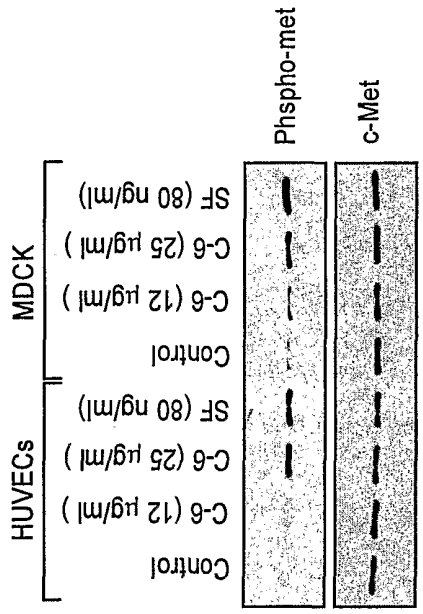

2502-1-001PCT FIGURE 19



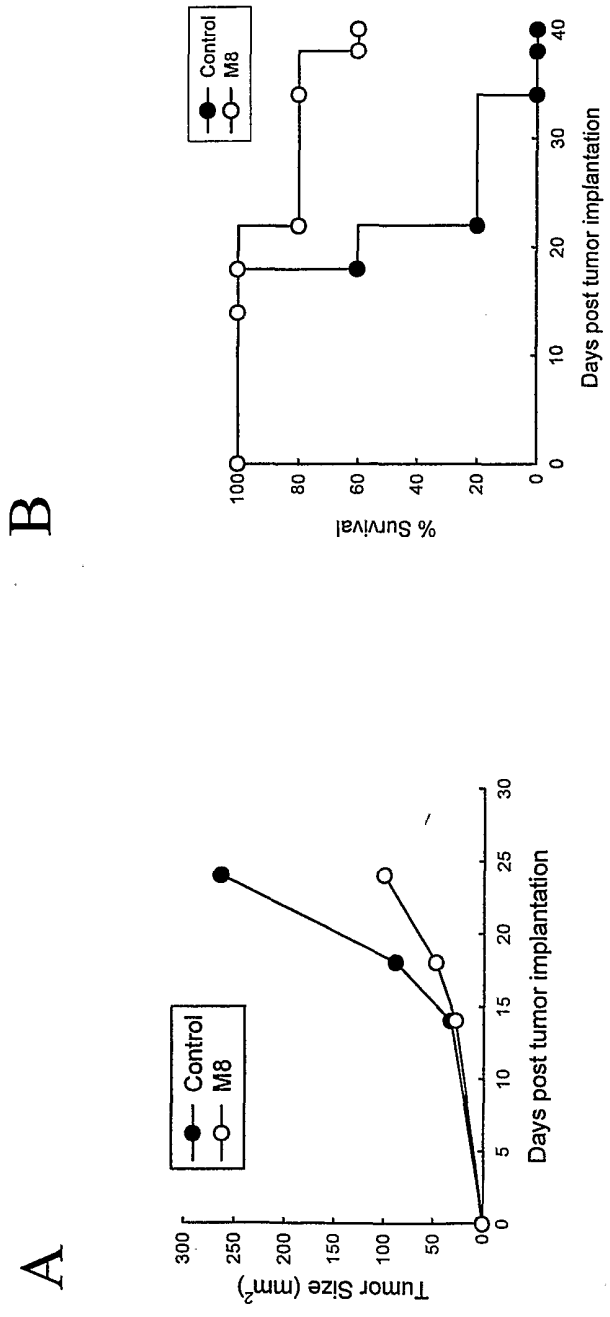

2502-1-001PCT FIGURE 20




2502-1-001PCT FIGURE 21




2502-1-001PCT FIGURE 22




2502-1-001PCT FIGURE 23A and 23B