# INHIBITION OF THE FORMATION OF VASCULAR HYPERPERMEABILITY

## BACKGROUND OF THE INVENTION

Edema can be described as an increase in the interstitial fluid volume. This is usually an abnormal condition for which relief is typically sought. This condition quite often arises because fluid leaves the blood vasculature due to an increase in endothelial permeability, often associated with macromolecular extravasation, and finds a new residence in the interstitial spaces.

There are a variety of physiological and biochemical mechanisms that underlie edema and the formation of the edematous state in an individual. An important mediator in one or more of these mechanisms is "vascular endothelial cell growth This factor upregulates transport in vascular endothelial cells, and causes an increase in the permeability of numerous vascular beds including the skin, subcutaneous tissues, peritoneal wall, mesentery, diaphragm, trachea, bronchi, duodenum and uterus. Significant diapedesis, alterations in exchange across the endothelium, extravasation and deposition of macromolecules at these sites and prolonged hypotension may accompany these increased permeability effects. These processes are thought to be a facilitating prelude to neovascularization. VEGF is expressed by inflammatory T-cells, macrophages, neutrophils and eosinophils, etc. at sites of inflammation. This factor is upregulated by hypoxia, certain vasopressor hormones, growth factors, reproductive hormones and numerous inflammatory cytokines. VEGF-mediated vascular permeability has been implicated in such disorders as tumor ascites, endometriosis, adult respiratory distress syndrome (ARDS), post cardiopulmonary bypass-related hypotension and hyperpermeability blistering, edematous responses to burns and trauma, endothelial dysfunction in diabetes, ovarian hyperstimulation syndrome complications, and ocular edema.

Thus, it is apparent that the inhibition of VEGF production or activity would be beneficial, especially to block the manifestation of the above-listed disorders. In particular, agents that are capable of blocking VEGF mediated hyperpermeability and edema and associated syndromes would be useful for alleviating these disorders.

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*Protein Tyrosine Kinases.* Protein tyrosine kinases (PTKs) comprise a large and diverse class of proteins having enzymatic activity. The PTKs play an important role in the control of cell growth and differentiation (for review, *see* Schlessinger & Ullrich, 1992, *Neuron 9*:383-391).

Aberrant stimulation, expression or mutations in the PTKs have been shown to lead to either uncontrolled cell proliferation (*e.g.*, malignant tumor growth) or to defects in key developmental, regulatory or reparative processes. Consequently, the biomedical community has expended significant resources to discover the specific biological role of members of the PTK family, their function in differentiation processes, their involvement in tumorigenesis and in other diseases, the biochemical mechanisms underlying their signal transduction pathways activated upon ligand stimulation and the development of novel drugs.

Tyrosine kinases can be of the receptor-type (having extracellular, transmembrane and intracellular domains) or the non-receptor type (being wholly intracellular).

*Receptor Tyrosine Kinases (RTKs).* The RTKs comprise a large family of transmembrane receptors with diverse biological activities. At present, at least nineteen (19) distinct RTK subfamilies have been identified. The receptor tyrosine kinase (RTK) family includes receptors that are crucial for the growth and differentiation of a variety of cell types (Yarden and Ullrich, *Ann. Rev. Biochem.* 57:433-478, 1988; Ullrich and Schlessinger, *Cell* 61:243-254, 1990). The intrinsic function of RTKs is activated upon ligand binding, which results in phosphorylation of the receptor and multiple cellular substrates, and subsequently in a variety of cellular responses (Ullrich & Schlessinger, 1990, *Cell 61*:203-212). Thus, receptor tyrosine kinase mediated signal transduction is initiated by extracellular interaction with a specific growth factor (ligand), typically followed by receptor dimerization, stimulation of the intrinsic protein tyrosine kinase activity and receptor trans-phosphorylation. Binding sites are thereby created for intracellular signal transduction molecules and lead to the formation of complexes with a spectrum of cytoplasmic signaling molecules that facilitate the appropriate cellular response. *(e.g.*, cell division, differentiation, metabolic effects, changes in the extracellular microenvironment) *see* Schlessinger and Ullrich, 1992, *Neuron 9*:1-20.

Proteins with SH2 (*src* homology –2) or phosphotyrosine binding (PTB) domains bind activated tyrosine kinase receptors and their substrates with high affinity to propagate signals into cell. Both of the domains recognize phosphotyrosine. (SH2:Fantle *et al.*, 1992, *Cell 69*:413-423; Songyant *et al.*, 1994,*Mol. Cell. Biol. 14*;2777-2785; Songyang *et al.*, 1993, *Cell 72*:767-778; and Koch *et al.*, 1991, *Science 252*:668-678; Schoelson, Curr. Opin. Chem. Biol. (1997), 1(2), 227-234; Cowburn, Curr. Opin. Struct. Biol. (1997), 7(6), 835-838). Several intercellular substrate proteins that associate with receptor tyrosine kinases (RTKs) have been identified. They may be divided into two principal groups: (1) substrates which have a catalytic domain; and (2) substrates which lack such a domain but serve as adapters and associate with catalytically active molecules (Songyang *et al.*, 1993, *Cell 72*:767-778). The specificity of the interactions between receptors or proteins and SH2 or PTB domains of their substrates is determined by the amino acid residues immediately surrounding the phosphorylated tyrosine residue. For example, differences in the binding affinities between SH2 domains and the amino acid dsquences surrounding the phosphotyrosine residues on particular receptors correlate with the observed differences in their substrate phosphorylation profiles (Songyang *et al.*, 1993, *Cell 72*:767-778). Observations suggest that the function of each receptor tyrosine kinase is determined not only by its pattern of expression and ligand availability but also by the array of downstream signal transduction pathways that are activated by a particular receptor as well as the timing and duration of those stimuli. Thus, phosphorylation provides an important regulatory step which determines the selectivity of signaling pathways recruited by specific growth factor receptors, as well as differentiation factor receptors.

Several receptor tyrosine kinases, and growth factors that bind thereto, have been suggested to play a role in angiogenesis, although some may promote angiogenesis indirectly (Mustonen and Alitalo, *J. Cell Biol. 129:895-898, 1995)*. One such receptor tyrosine kinase, known as "fetal liver kinase 1" (FLK-1), is a member of the type III subclass of RTKs. An alternative designation for human FLK-1 is "kinase insert domain-containing receptor" (KDR) (Terman *et al.*, *Oncogene* 6:1677-83, 1991). Another alternative designation for FLK-1/KDR is "vascular endothelial cell growth factor receptor 2" (VEGFR-2) since it binds VEGF with high affinity. The murine version of FLK-1/VEGFR-2 has also been called NYK (Oelrichs *et al*, *Oncogene*

*8*(1):11-15, 1993). DNAs encoding mouse, rat and human FLK-1 have been isolated, and the nucleotide and encoded amino acid sequences reported (Matthews *et al.*, *Proc. Natl. Acad. Sci. USA, 88:9026-30, 1991;* Terman *et al.*, 1991, *supra;* Terman *et al.*, *Biochem. Biophys. Res. Comm.* 187:1579-86, 1992; Sarzani *et al.*, *supra;* and Millauer *et al.*, *Cell* 72:835-846, 1993). Numerous studies such as those reported in Millauer *et al.*, *supra,* suggest that VEGF and FLK-1/KDR/VEGFR-2 are a ligand-receptor pair that play an important role in the proliferation of vascular endothelial cells, and formation and sprouting of blood vessels, termed vasculogenesis and angiogenesis, respectively.

Another type III subclass RTK designated *'fms*-like tyrosine kinase-1" (Flt-1) is related to FLK-1/KDR (DeVries *et al. Science* 255;989-991, 1992; Shibuya *et al.*, *Oncogene* 5:519-524, 1990). An alternative designation for flt-1 is "vascular endothelial cell growth factor receptor 1" (VEGFR-1). To date, members of the FLK-1/KDR/VEGFR-2 and flt-1/ VEGFR-1 subfamilies have been found expressed primarily on endothelial cells. These subclass members are specifically stimulated by members of the vascular endothelial cell growth factor (VEGF) family of ligands (Klagsburn and D'Amore, *Cytokine & Growth Factor Reviews 7:* 259-270, 1996). Vascular endothelial cell growth factor (VEGF), binds to Flt-1 with higher affinity than to FLK-1/KDR and is mitogenic toward vascular endothelial cells (Terman *et al.*, 1992, *supra*; Mustonen *et al. supra*; DeVries *et al.*, *supra*). Flt-1 is believed to be essential for endothelial organization during vascular development. Flt-1 expression is associated with early vascular development in mouse embryos, and with neovascularization during wound healing (Mustonen and Alitalo, *supra*). Expression of flt-1 in adult organs such as kidney glomeruli suggests an additional function for this receptor that is not related to cell growth (Mustonen and Alitalo, *supra*).

As previously stated, recent evidence suggests that VEGF plays a role in the stimulation of both normal and pathological angiogenesis (Jakeman *et al.*, *Endocrinology* 133: 848-859, 1993; Kolch *et al., Breast Cancer Research and Treatment* 36: 139-155, 1995; Ferrara *et al., Endocrine Reviews 18*(1); 4-25, 1997; Ferrara *et al., Regulation of Angiogenesis* (ed. L. D. Goldberg and E.M. Rosen), 209-232, 1997). In addition, VEGF has been implicated in the control and enhancement of vascular permeability (Connolly, *et al., J. Biol. Chem.* 264: 20017-20024, 1989; Brown

*et al., Regulation of Angiogenesis* (ed. L.D. Goldberg and E.M. Rosen), 233-269, 1997).

Different forms of VEGF arising from alternative splicing of mRNA have been reported, including the four species described by Ferrara *et al.* (*J. Cell. Biochem.* 47:211-218, 1991). Both secreted and predominantly cell-associated species of VEGF have been identified by Ferrara *et al. supra*, and the protein is known to exist in the form of disulfide linked dimers.

Several related homologs of VEGF have recently been identified. However, their roles in normal physiological and disease processes have not yet been elucidated. In addition, the members of the VEGF family are often coexpressed with VEGF in a number of tissues and are, in general, capable of forming heterodimers with VEGF. This property likely alters the receptor specificity and biological effects of the heterodimers and further complicates the elucidation of their specific functions as illustrated below (Korpelainen and Alitalo, *Curr. Opin. Cell Biol.*, 159-164, 1998 and references cited therein).

Placenta growth factor (PlGF) has an amino acid sequence that exhibits significant homology to the VEGF sequence (Park *et al.*, *J. Biol. Chem.* 269:25646-54, 1994; Maglione *et al. Oncogene* 8:925-31, 1993). As with VEGF, different species of PlGF arise from alternative splicing of mRNA, and the protein exists in dimeric form (Park *et al.*, *supra*). PlGF-1 and PlGF-2 bind to Flt-1 with high affinity, and PlGF-2 also avidly binds to neuropilin-1 (Migdal *et al.,J. Biol. Chem. 273 (35): 22272-22278)*,but neither binds to FLK-1/KDR (Park *et al.*, *supra*). PlGF has been reported to potentiate both the vascular permeability and mitogenic effect of VEGF on endothelial cells when VEGF is present at low concentrations (purportedly due to heterodimer formation) (Park *et al.*, *supra*).

VEGF-B is produced as two isoforms (167) and 185 residues) that also appear to bind Flt-1/VEGFR-1. It may play a role in the regulation of extracellular matrix degradation, cell adhesion, and migration through modulation of the expression and activity of urokinase type plasminogen activator and plasminogen activator inhibitor 1 (Pepper et al, Proc. Natl. Acad. Sci. U. S. A. (1998), 95(20): 11709-11714).

VEGF-C was originally cloned as a ligand for VEGFR-3/Flt-4 which is primarily expressed by lymphatic endothelial cells. In its fully processed form, VEGF-

C can also bind KDR/VEGFR-2 and stimulate proliferation and migration of endothelial cells *in vitro* and angiogenesis in *in vivo* models(Lymboussaki *et al, Am. J. Pathol.* (1998), 153(2): 395-403; Witzenbichler *et al, Am J. Pathol.* (1998), 153(2), 381-394). The overexpression of VEGF-C causes proliferation and enlargement of only lymphatic vessels, while blood vessels are unaffected. Unlike VEGF, the expression of VEGF-C is not induced by hypoxia (Ristimaki *et al, J. Biol. Chem.* (1998), 273(14),8413-8418).

The most recently discovered VEGF-D is structurally very similar to VEGF-C. VEGF-D is reported to bind and activate at least two VEGFRs, VEGFR-3/Flt-4 and KDR/VEGFR-2. It was originally cloned as a c-fos inducible mitogen for fibroblasts and is most prominently expressed in the mesenchymal cells of the lung and skin (Achen *et al, Proc. Natl. Acad. Sci. U. S. A.* (1998), 95(2), 548-553 and references therein).

VEGF-C and VEGF-D have been claimed to induce increases in vascular permeability *in vivo* in a Miles assay when injected into cutaneous tissue (PCT/US97/14696; WO98/07832, Witzenbichler *et al., supra*). The physiological role and significance of these ligands in modulating vascular hyperpermeability and endothelial responses in tissues where they are expressed remains uncertain.

Based upon emerging discoveries of other homologs of VEGF and VEGFRs and the precedents for ligand and receptor heterodimerization, the actions of such VEGF homologs may involve formation of VEGF ligand heterodimers, and/or heterodimerization of receptors, or binding to a yet undiscovered VEGFR (Witzenbichler *et al., supra*). Also, recent reports suggest the possible involvement of neuropilin-1 (Migdal *et al, supra*) or VEGFR-3/Flt-4 (Witzenbichler *et al., supra*), and that receptors other than KDR/VEGFR-2 are responsible for the induction of vascular permeability (Stacker, S.A., Vitali, A., Domagala, T., Nice, E., and Wilks, A.F., "Angiogenesis and Cancer" Conference, Amer. Assoc. Cancer Res., Jan. 1998, Orlando, FL; Williams, *Diabetelogia* 40: S118-120 (1997)).

*Development of Compounds to Modulate the PTKs.* In view of the surmised importance of PTKs to the control, regulation, and modulation of cell proliferation, as well as the diseases and disorders associated with abnormal cell proliferation, many attempts have been made to identify receptor and non-receptor tyrosine kinase

"inhibitors" using a variety of approaches, including the use of mutant ligands (U.S. Application No. 4,966,849), soluble receptors and antibodies (Application No. WO 94/10202; Kendall & Thomas, 1994, *Proc. Natl. Acad. Sci 90:*10705-09; Kim *et al.*, 1993, *Nature 362:*841-844), RNA ligands (Jellinek, *et al., Biochemistry 33*:10450-56; Takano, *et al.*, 1993, *Mol. Bio. Cell 4*:358A; Kinsella, *et al.* 1992, *Exp. Cell Res. 199:*56-62; Wright, *et al.*, 1992, *J. Cellular Phys. 152*:448-57) and tyrosine kinase inhibitors (WO 94/03427; WO 92/21660; WO 91/15495; WO 94/14808; U.S. Patent No. 5,330,992; Mariani, *et al.*, 1994, *Proc. Am. Assoc. Cancer Res. 35*:2268).

More recently, attempts have been made to identify small molecules which act as tyrosine kinase inhibitors. For example, bis monocyclic, bicyclic or heterocyclic aryl compounds (PCT WO 92/20642) and vinylene-azaindole derivatives (PCT WO 94/14808) have been described generally as tyrosine kinase inhibitors. Styryl compounds (U.S. Patent No. 5,217,999), styryl-substituted pyridyl compounds (U.S. Patent No. 5,302,606), certain quinazoline derivatives (EP Application No. 0 566 266 A1); Expert Opin. Ther. Pat. (1998), 8(4): 475-478, seleoindoles and selenides (PCT WO 94/03427), tricyclic polyhydroxylic compounds (PCT WO 92/21660) and benzylphosphonic acid compounds (PCT WO 91/15495) have been described as compounds for use as tyrosine kinase inhibitors for use in the treatment of cancer. Anilinocinnolines (PCT WO 97/34876) and quinazoline derivative compounds (PCT WO 97/22596; PCT WO 97/42187) have been decribed as inhibitors of angiogenesis and vascular permeability.

In addition, attempts have been made to identify small molecules which act as serine/threonine kinase inhibitors. In particular, bis(indolylmaleimide) compounds have been described as inhibiting particular PKC serine/threonine kinase isoforms whose dysfunction is associated with altered vascular permeability in VEGF-related diseases (PCT WO97/40830; PCT WO97/40831).

The identification of effective macromolecules and small organic compounds which specifically inhibit tyrosine signal transduction by modulating the activity of receptor and non-receptor tyrosine kinases to regulate and modulate abnormal or inappropriate cellular function, cell proliferation or differentiation is therefore desirable. In particular, the identification of methods and compounds that specifically inhibit the function of a tyrosine kinases which is essential for the formation of vascular

hyperpermeability leading to edema, effusions, exudates, and macromolecular extravasation and deposition as well as associated disorders would be beneficial.

## SUMMARY OF THE INVENTION

This invention is directed to the inhibition of vascular hyperpermeability by inhibiting the cellular signaling function of KDR tyrosine kinase. This invention also provides a method of inhibiting vascular hyperpermeability by selectively disrupting the catalytic kinase response of KDR/VEGFR-2 without significantly affecting the activity of Flt-1/VEGFR1 or other tyrosine kinases. Agents which function according to this method have a distinct pharmacological advantage over current therapeutic approaches encompassing materials such as steroids which are prone to numerous undesirable side-effects. These methods of the present invention are also preferred over the use of less specific kinase inhibitors, including those which inhibit multiple VEGF receptors, since these methods will not directly perturb the inportant normal physiologic function of the other kinases. As a result of the inhibition of the hyperpermeability of the vascular endothelium, the subsequent formation of edema, associated diapedesis, alterations in trans-endothelial molecular exchange, extravasation, exudates and effusions are also inhibited by the suppression of the tyrosine kinase activity of KDR. Since these latter events often lead to excessive matrix deposition, aberrant stromal proliferation and organ dysfunction, the inhibition of KDR tyrosine kinase is also useful in the treatment of numerous non-cancerous disorders that share these etiologic features. In addition, vascular hypotension that can be caused by a VEGF-related activating ligand binding to KDR tyrosine kinase receptor is also minimized by inhibiting the activity of KDR tyrosine kinase.

This invention also provides a therapeutic approach to the inhibition of vascular hyperpermeability and of the formation of edema in individuals by administering a compound that specifically inhibits the activity of KDR tyrosine kinase.

## DETAILED DESCRIPTION OF THE INVENTION

This invention discloses a method of inhibiting vascular hyperpermeability by inhibiting the cellular signaling function of KDR tyrosine kinase. This invention also discloses a method of inhibiting vascular hyperpermeability through the utilization of

agents which selectively inhibit the cellular signaling function of KDR. Through the identification and utilization of highly selective KDR inhibitors which effectively block KDR cellular signaling, and subsequently vascular hyperpermeability, according to the methods of this invention, the essential role of KDR in mediating the vascular permeability response to VEGF has been established. Such highly selective KDR inhibitors have demonstrated efficacy in modulating vascular permeability, without the need to inhibit the function of the higher affinity receptor, VEGFR-1/Flt-1. This property should afford better toleration to therapy than current therapies or treatment with agents that less selectively disrupt the function of other non-KDR kinases

KDR tyrosine kinase is activated when vascular endothelial cell growth factor (VEGF) or another activating ligand (such as HIV Tat protein, VEGF-C or VEGF-D) binds to a KDR tyrosine kinase receptor which lies on the surface of vascular endothelial cells. Although naturally occurring kinase-activating mutations and truncations have not yet been identified for KDR, they have been reported for EGFR and Tie-2 receptor kinases. Hence instances of constitutive activation of KDR are also anticipated. The KDR tyrosine kinase may also be referred to as FLK-1 tyrosine kinase, NYK tyrosine kinase or VEGFR-2 tyrosine kinase.

In addition to stimulating angiogenesis, and endothelial cell migration and proliferation, VEGF induces hypermeability of the blood vessels. As a result, fluid moves from the blood stream past the blood vessel walls into the interstitial spaces, thereby forming an area of edema. Diapedesis also often accompanies this response. Similarly, excessive vascular hyperpermeability can disrupt normal molecular exchange across the endothelium in critical tissues and organs (e.g., lung and kidney), thereby causing organ dysfunction, macromolecular extravasation, and matrix deposition often with promoted stromal proliferation. When occurring in confined compartments, the edema (e.g., cerebral edema) may lead to impaired organ function and damage.

KDR cellular signaling function can be inhibited by a number of approaches: either by blocking the production of the activating ligand, by blocking the activating ligand binding to the KDR tyrosine kinase receptor, by preventing receptor dimerization and transphosphorylation, by inhibiting the enzyme activity of the KDR tyrosine kinase (inhibiting the phosphorylation capacity of the enzyme) or by some other mechanism that interrupts its downstream signaling (D. Mukhopedhyay *et al.*, *Cancer Res. 58*:1278-

1284 (1998) and references therein). According to the method disclosed herein, such approaches which are selective for disrupting KDR cellular signaling function will reduce vascular hyperpermeability, as well as associated extravasation, subsequent edema formation and matrix deposition.

There are a variety of compounds that have the requisite KDR tyrosine kinase inhibition property. Among these compounds are antibodies (hereafter meant to include single chain antibody constructs) that bind the extracellular KDR receptor domain or cellular kinase enzyme portion or, alternatively, that bind VEGF itself. These antibodies interfere with VEGF binding to the KDR tyrosine kinase receptor and/or, importantly, with KDR tyrosine kinase cellular signaling function. Antibodies that bind to the KDR tyrosine kinase may act as VEGF antagonists or, more generally, VEGF activator antagonists. Alternatively these antibodies may block functional receptor dimerization or they may be KDR tyrosine kinase inhibitors. Antibodies that bind to VEGF or an activating ligand are neutralizing antibodies of VEGF or activating ligand. It should be noted that such VEGF neutralizing antibodies may block VEGF responses through both the KDR and the Flt-1 receptors and, typically, are specific for a single activating ligand. In most instances, the blocking of VEGF responses through the Flt-1 receptors is not necessary nor desirable. Since these VEGFRs have been reported to recognize different epitopes on VEGF, the desired specific blockade of KDR activation can be achieved through the use of antibodies that specifically bind to and "mask" the KDR-binding epitope of VEGF or other activating ligand.

Other compounds that can inhibit KDR tyrosine kinase activity, and thereby minimize vascular hyperpermeability and the formation of edema, include peptides and organic molecules. Among the peptides are the soluble extracellular domain of KDR and KDR binding fragments. Other useful peptides are mutants of VEGF or VEGF-related growth factors (e.g., VEGF-C, VEGF-D or HIV Tat protein and fusion proteins thereof) which bind and block further ligand binding to this receptor but do not stimulate dimerization, activation or KDR tyrosine kinase transphosphorylation. Such mutants may act as monomers or nonfunctional heterodimers, thereby blocking the binding of native dimeric VEGF or activating ligand. Similarly, other peptides or small molecules that block receptor dimerization and/or activation can be successfully employed. These compounds act, also, as antagonists of activating ligands or are

inhibitors of KDR tyrosine kinase activity. Preferred compounds are small organic molecules.

In addition, molecules such as KDR-specific ribozymes, antisense polynucleotides (such as antisense mRNA) or intracellular single chain antibodies ($S_cF_v$) that inhibit the biosynthesis or proper presentation of active, functional KDR tyrosine kinase will effectively block KDR-mediated responses to VEGF. These molecules can be introduced into cells preformed or their production can be induced intracellularly (e.g., through the use of appropriate adenoviral, retroviral or baculoviral vectors).

The preferred compounds of this invention have the property of inhibiting the cellular signaling function of KDR without significantly inhibiting the cellular signaling function Flt-1 (Flt-1 tyrosine kinase is also referred to as VEGFR-1 tyrosine kinase). Both KDR tyrosine kinase and flt-1 tyrosine kinase are activated by VEGF binding to KDR tyrosine kinase receptors and to Flt-1 tyrosine kinase receptors, respectively. Since Flt-1 tyrosine kinase activity may mediate important events in endothelial maintenance and vascular function, an inhibition of this enzyme activity or associated transduced signals may lead to toxic or adverse effects. At the very least, such inhibition is unnecessary for blocking the induction of vascular hyperpermeability and the formation of edema, so it is wasteful and of no value to the individual. The preferred compounds of this invention are unique because they inhibit the activity of one VEGF-receptor tyrosine kinase (KDR) that is activated by activating ligands but do not inhibit other receptor tyrosine kinases, such as Flt-1, that are also activated by certain activating ligands. The most preferred compounds of this invention are, therefore, selective in their tyrosine kinase inhibitory activity.

VEGF is known to contribute to vascular hyperpermeability and the formation of edema. VEGF is expressed by inflammatory T-cells, macrophages, neutrophils and eosinophils, etc. at sites of inflammation. The production of this factor is quickly upregulated by hypoxia, certain vasopressor hormones, growth factors, reproductive hormones and numerous inflammatory cytokines. Indeed, vascular hyperpermeability and edema that is associated with the expression or administration of many other growth factors appears to be mediated via VEGF production. Vascular hyperpermeability, associated edema, altered transendothelial exchange and macromolecular extravasation, which is often accompanied by diapedesis, can result in excessive matrix deposition,

aberrant stromal proliferation, fibrosis, etc. Hence, VEGF-mediated hyperpermeability can significantly contribute to disorders with these etiologic features. For example:

(1) VEGF is markedly increased in the epidermis of lesional psoriatic skin. This factor potently stimulates the dermal endothelial cell proliferation and microvascular hyperpermeability associated with psoriasis.

(2) Following burns and severe scalds, main organs are often damaged. This appears to be manifested by an uncontrollable "mediator disease" resulting from ischemic-reperfusion damage, swelling and edema of visceral tissues, i.e., endothelial cell damage. For burn victims, inhalation injury is one of the primary causes of mortality. The tracheobronchial epithelium sloughs and combines with a protein rich exudate to form casts of the airways that can lead to obstruction of these airways. The combination of inhalation burn and hypoxia followed by exposure to a high concentration of oxygen (in an attempt to aid the individual) can worsen the situation by causing progressive changes in the lung, such as diffuse exudative formation, hemorrhage into the trachea and edematic changes in the wall of the blood vessels. Circulating serum VEGF levels are dramatically increased (up to twentyfold) in victims following burns and multiple trauma and may be a prime mediator of these complications (Grad *et al*, *Clin. Chem. Lab. Med. 36:*379-383, 1998).

(3) Sunburns are also associated with the formation of edema. VEGF production is also known to be upregulated following UV radiation exposure. Other skin disorders where edema is produced include blistering symptomatic erythedema (acrodynia), persistent acrodema and bullous diseases such as erythema multiforme, bullous pemphigoid and dermatitis herpetiformis (i.e., conditions of acute or chronic inflammation). Edematous macules and roseacea such as that associated with telangiectasia are further disorders where edema is manifested.

(4) Enhanced microvascular permeability and edema are common characteristics of inflammatory and neoplastic disorders. Brain tumors such as gliomas, where tumoral and peritumoral brain edema and fluid filled cysts are formed, and meningiomas, with accompanying massive cerebral edema, are examples of such disorders. Locally high levels of VEGF are associated with these disorders. Induction of malignant ascites fluid and tumor effusions (especially malignant

pleural and pericardial effusions) are further examples of such edema-producing disorders and are known to involve VEGF production. Additionally, edema resulting from head trauma can produce concussions and impaired brain functions. Similarily, communicating hydrocephalus has been shown to involve cytokines such as IGF-1 and TGF-$\beta$1 known to modulate VEGF production.

(5) Edema occurs in some types of chronic inflammation such as the formation of nasal polyps, uterine cervical polyps and gastric hyperplastic polyps. In such cases, inflammatory cells have been shown to play an important role in the development of these edematous states, at least in part through the production of VEGF.

(6) Cytokine-activated eosinophils can be an important source of VEGF, thereby contributing to tissue edema formation at sites of allergic inflammation. Edema and exudates are common complications that arise during allergic and delayed-type hypersensitivity reactions; also often including anaphylaxis. VEGF is implicated especially in those reactions that are not responsive to antihistamines or aspirin, and its upregulation has been observed in cases of poison ivy, and contact dermatitis. In addition, tuberculosis, certain viral infections, angioedema, urticaria (hives) and exercise-induced anaphylaxis are examples of such allergic and delayed-type hypersensitivity reactions which may also involve VEGF. Edema is also often formed as a result of drug sensitivity or hypersensitivity reactions, or in response to the administration of VEGF-upregulating growth factor or cytokines (e.g., IGF-1, FGF-2, or IL-2). Radioanaphylaxis and radiodermatitis is associated with vascular hyperpermeability.

(7) VEGF is involved in ocular neovascularization leading to diabetic retinopathy and microangiopathy, blindness due to age-related macular degeneration and neonatal blindness resulting from hyperoxic exposure. In many instances, these conditions are preceded by macular or other ocular edema. VEGF has been identified as a prime mediator of iris, corneal and retinal neovascularization in cases of ocular ischemia and vascular edema. VEGF induced vascular hyperpermeability contributes to blood-retinal barrier breakdown in a variety of ocular disorders with extravasation and matrix deposition laying the foundations

for subsequent angiogenesis. Corneal neovascularization is a major outcome following chemical burns, corneal inflammation and edema. Recent evidence implicates VEGF in the processes following such ocular trauma. The iron chelator deferoxamine has been used in clinical treatment of cancer patients. However, this treatment often induces macular edema. The concentrations of this iron chelator that are achieved in the patients induce a 3-5 fold increase in VEGF mRNA expression in all normal and tumor cell lines studied, implicating VEGF as a likely mediator of the edema formation. Increased intraocular pressures caused by VEGF overproduction and edema can lead to inappropriate matrix depositions, ocular distortions, changes in the optic disk, defects in the field of vision and can result in glaucoma. Vascular hyperpermeability is also often associated with conjunctivitis.

(8) Chronic lung disease in neonates and adults results from both lung injury and inadequate repair processes. The production of VEGF has been reported in several animal models of lung injury. Destruction of pulmonary endothelial cells is also characteristic of hyperoxic lung injury. During recovery from hyperoxia, VEGF is upregulated by alveolar type II cells and subsequently causes pulmonary endothelial cells to proliferate and regenerate. However, this result can cause disrupted exchange across pulmonary endothelia and pulmonary edema. Asthma and bronchitis often involve bronchial vascular dilation, vascular engorgement, edema of the bronchial wall and exudates which result in thickening of airway mucosa and narrowing of the bronchial lumen. Edema with protein exudates and aberrant stromal growth are typically intertwined with these phenomena. By related processes, pulmonary edema is formed during adult respiratory distress syndrome. Causes of adult respiratory distress syndrome typically include pneumonia, inhalation of noxious substances, lung contusions, near drowning and aspiration of gastric contents.

(9) Corticosteroids, such as cortisone, hydrocortisone, dexamethasone or prednisolone, are among the most widely used therapeutants for edematous conditions. They are potent inhibitors of VEGF expression. This property is now believed to significantly contribute to the well-known antiedematous efficacy of such steroids. However, their pluripotent biological activities are

also responsible for their undesirable side-effects. Steroid hormones and their agonists and antagonists also dramatically affect the production of VEGF, especially in reproductive tissues. Endometritis and endometriosis can occur during pregnancy, the menstrual cycle or sex hormone therapy. Swelling and cramps of menstruation is associated with vascular hyperpermeability. Tamoxifen, an agent that reduces the risk of breast cancer, also increases uterine cell proliferation and tumor incidence. This steroid analog, as well as estradiol, causes uterine edema and cell proliferation which have been shown to involve local increases in VEGF production. Ovarian hyperstimulation syndrome is a serious complication affecting ovulation induction. The most severe manifestation of these syndromes take the form of massive ovarian enlargement and multiple cysts, ascites, hemoconcentration and third-space accumulation of fluid. The increased capillary permeability triggered by the release of VEGF secreted by luteinized granulosa cells, etc. of the ovaries following stimulation with human chorionic gonadotropin is believed to play a key role in these syndromes. It has been demonstrated that VEGF is overexpressed in the hyperthecotic ovarian stroma of polycystic ovaries in the Stein-Leventhal syndrome.

(10) A rapid differential induction of VEGF gene expression in both neuronal and pial cells after transient middle cerebral artery occlusion has been demonstrated in animal models of stroke. VEGF may contribute to the recovery of brain cells from ischemic insult, such as from stroke, head trauma or cerebral infarct, by potentiating neovascularization, but brain damage can also be exacerbated by the concomitant formation of brain edema. Malaria can also induce edema as a result of VEGF-induced cerebral hypoxia. Brain tumor-associated cerebral edema and fluid-filled cysts arise because tumor capillaries lack normal blood-brain barrier function. VEGF released by glioma cells *in situ* most likely accounts for the pathognomonic histopathology and clinical features of glioblastoma tumors in patients including increased cerebral edema. Carpal tunnel syndrome is accompanied by enhanced nerve hydration and, often, by subsequent increased extracellular matrix deposition (entrapment neuropathy). Increased VEGF levels in the tissues which surround the nerve can cause the

nerve entrapment by inducing vascular permeability, fluid efflux and stromal deposition into the perineural tissues.

(11) VEGF production in tissues is dramatically upregulated in response to hypoxia. Hence, the observation that in regions of necrosis, ischemia, infarct, occlusion, anemia, circulatory impairments, or other oxygen deprivation, VEGF levels are increased and vascular hyperpermeability, edema and extravasation are common. Lower oxygen pressure that is responsible for "altitude sickness" also induces rapid VEGF production which is the likely cause of life threatening cerebral and pulmonary edema (HACE and HAPE) that can occur if a person is unacclimatized.

(12) VEGF overproduction is likewise implicated in pericardial and pleural effusions caused by vascular hyperpermeability that results from radiation injury, rheumatoid diseases, lupus, myocardial infarction, trauma or drug reaction. Not surprisingly, VEGF overproduction in association with pericardial and pleural effusions is commonly observed at autopsy in patients with lung or breast carcinomas, lymphomas and leukemias. VEGF amounts are also significantly elevated in the synovial fluid of swollen joints of individuals with rheumatoid arthritis. Sprains and fractures, although associated with some swelling and vascular hyperpermeability that is beneficial in promoting angiogenesis and healing, can be accompanied by painful and excessive, undesirable edema. Similarly, VEGF involvement is anticipated in conditions such as synovitis or meniscus injury with effusion (e.g., "water-on-the-knee").

(13) Ulcerations associated with circulatory restriction (e.g. decubitus, gravitational and varicose ulcers) are also often accompanied by edema and protein exudates. Diabetic complications often arise as a result of elevational circulating glucose levels (hyperglycemia) and the formation of advanced glycation endproducts (AGE), often accompanied by impaired circulation. These conditions, either alone or in combination, are known to stimulate VEGF production and, hence, vascular hyperpermeability which can lead to numerous diabetic complications.

(14) Due to the significant constitutive production of endogenous VEGF by the kidney podocytes and the known vascular hyperpermeability effects of elevated VEGF levels, renal disorders such as microalbuminuria, proteinuria, oliguria,

electrolyte imbalance (often encountered as diabetic complications) and nephrotic syndrome (especially when hypoxia-induced following burns, shock or trauma) may be treated according to the method of this invention.

(15) Protein extravasation and diapedesis, that commonly accompanies edema and leads to excessive matrix deposition and stromal proliferation, contribute to the progression of other disorders. These disorders include hyperviscosity syndrome, liver cirrhosis, fibroses, keloid and formation of undesired scar tissue. Inhibition of VEGF-mediated hyperpermeability will impede such disease progression.

(16) Significant amounts of VEGF isoforms are known to be stored in platelets, mast cells, etc. and in extracellular matrices. In certain situations, these stores of VEGF/VPF can be rapidly liberated and thereby contribute to acute vascular hyperpermeability.

From these diverse examples, it is readily apparent that edema occurs under a variety of physiological conditions and VEGF/VPF or a related analog is strongly implicated in edema formation and extravasation. The compounds of this invention minimize the edematous state associated with macular edema, aphakic/pseudoaphakic cystoid macular edema, retinoblastoma, ocular ischemia, ocular inflammatory disease or infection, choroidal melanoma, edematous side-effects induced by iron chelation therapy, pulmonary edema, pleural effusion, pericardial effusion, myocardial infarction, rheumatoid diseases, tissue edema at sites of trauma or allergic inflammation, polyp edema at sites of chronic inflammation, cerebral edema, brain tumor fluid-filled cysts, communicating hydrocephalus, edema associated with organ damage resulting from a burn and edema resulting from an inhalation burn injury. The compounds of this invention also minimize the edematous state associated with skin burns, blisters, erythema multiforme, edematous macules and other skin disorders, brain tumors, ascites and various effusions associated with cancers, carpal tunnel syndrome, altitude "sickness", allergies and hypersensitivity reactions, radioanaphylaxis, radiodermatitis, glaucoma, conjunctivitis, adult respiratory distress syndrome, asthma, bronchitis, ovarian hyperstimulation syndrome, polycystic ovary syndrome, menstrual swelling and cramps, stroke, head trauma, cerebral infarct or occlusion, ulcerations, sprains, fractures,

effusions associated with synovitis, diabetic complications, liver cirrhosis and the administration of growth factors. The compounds of this invention can also be used to treat microalbuminuria, proteinuria, oliguria, electrolyte imbalance, nephrotic syndrome, hyperviscosity syndrome, exudates, fibroses, keloid and formation of undesired scar tissue.

The compounds of this invention can be administered in combination with one or more additional pharmaceutical agents that inhibit or prevent the production of VEGF, attenuate intracellular responses to VEFG, inhibit vascular hyperpermeability, reduce inflammation or inhibit or prevent the formation of edema. The compounds of the invention can be administered prior to, subsequent to or simultaneously with the additional pharmaceutical agent, whichever course of administration is appropriate. The additional pharmaceutical agents include but are not limited to anti-edemic steroids, NSAIDS, ras inhibitors, anti-TNF agents, anti-IL1 agents, antihistamines, PAF-antagonists, COX-1 inhibitors, COX-2 inhibitors, NO synthase inhibitors, PKC inhibitors and $PI_3$ kinase inhibitors. The compounds of the invention and the additional pharmaceutical agents act either additively or synergistically. Thus, the administration of such a combination of substances that inhibit vascular hyperpermeability and/or inhibit the formation of edema can provide greater relief from the deletrious effects of vascular hyperpermeability or edema than the administration of either substance alone.

Since the formation of edema often results from the extravasation of fluid from the bloodstream, hypotension often occurs as extravasation takes place. Hypotension can also occur as a result of VEGF or VEGF activator binding to VEGF receptors on vascular endothelial cells. The compounds of this invention minimize the development of hypotension by, it appears, inhibiting the cellular signaling function of KDR that is a consequence of VEGF (or other activating ligand) binding to this receptor. The compounds of this invention inhibit hypotension in individuals when they are administered to the individual.

**Pharmaceutical Formulations**

The compounds of this invention can be administered to a human patient by themselves or in pharmaceutical compositions where they are mixed with suitable carriers or excipient(s) at doses to treat or ameliorate vascular hyperpermeability, edema and associated disorders. Mixtures of these compounds can also be administered to the patient as a simple mixture or in suitable formulated pharmaceutical compositions. A therapeutically effective dose further refers to that amount of the compound or compounds sufficient to result in the prevention of edema, VEGF-associated hyperpermeability and/or VEGF-related hypotension progression. Techniques for formulation and administration of the compounds of the instant application may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition.

**Routes of Administration**

Suitable routes of administration may, for example, include oral, eyedrop, rectal, transmucosal, topical, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

Alternatively, one may administer the compound in a local rather than a systemic manner, for example, via injection of the compound directly into an edematous site, often in a depot or sustained release formulation.

Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with endothelial cell-specific antibody.

**Composition/Formulation**

The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of

the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained by combining the active compound with a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active

compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g. gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, e.g. bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly or by intramuscular injection). Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

An example of a pharmaceutical carrier for the hydrophobic compounds of the invention is a cosolvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The cosolvent system may be the VPD cosolvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD cosolvent system (VPD:5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This cosolvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a cosolvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the cosolvent components may be varied: for example, other low-toxicity nonpolar surfactants may be used instead of polysorbate 80; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, e.g. polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose.

Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethysulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various sustained-release materials have been established and are well known by

those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

Many of the organic molecule compounds of the invention may be provided as salts with pharmaceutically compatible counterions. Pharmaceutically compatible salts may be formed with many acids, including but not limited to hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms.

**Effective Dosage**

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. More specifically, a therapeutically effective amount means an amount effective to prevent development of or to alleviate the existing symptoms of the subject being treated. Determination of the effective amounts is well within the capability of those skilled in the art.

The effective dose of the compound inhibits the cellular signaling function of KDR sufficiently to suppress vascular hyperpermeability without causing significant adverse effects due to inhibition of the Flt-1 or other tyrosine kinase functions. Certain compounds which have such activity can be identified by *in vitro* assays that determine the dose-dependent inhibition of KDR tyrosine kinase. Preferred compounds have an $IC_{50}$ versus KDR that is significantly lower than the $IC_{50}$ against Flt-1 or other PTK's determined under similar conditions of [ATP]/$K_m$(ATP) and substrate (ideally, ~100x selective for KDR tyrosine kinase).

For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cellular assays. For example, a dose can be formulated in cellular and animal models to achieve a circulating concentration range that includes the $IC_{50}$ as determined in cellular assays (i.e., the concentration of the test compound which achieves a half-maximal inhibition of the cellular signaling function KDR, usually in response to VEGF or another activating stimulus). The determination of the cellular $IC_{50}$ in the presence of 3 to 5% serum albumin may approximate the binding effects of plasma protein on the compound. Such information can be used to more accurately determine useful doses in humans. Further, the most preferred compounds for systemic administration effectively inhibit the cellular signaling functi. KDR in intact cells at levels that are safely achievable in plasma.

A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms in a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the maximum tolerated dose (MTD) and the $ED_{50}$ (effective dose for 50% maximal response). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between MTD and $ED_{50}$. Compounds which exhibit high therapeutic indices are preferred. The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the $ED_{50}$ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g. Fingl *et al.*, 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p1). In the treatment of crises, the administration of an acute bolus or an infusion approaching the MTD may be required to obtain a rapid response.

Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the KDR modulating effects, or minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from *in vitro* data; e.g. the concentration necessary to achieve 50-90%

inhibition of KDR tyrosine kinase using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using the MEC value. Compounds should be administered using a regimen which maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90% until the desired amelioration of symptoms is achieved. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

**Packaging**

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition. Suitable conditions indicated on the label may include treatment of an edema, inhibition of vascular hyperpermeability and extravasation, stromal deposition, minimization of VEGF-related hypotension, and the like.

EXEMPLIFICATIONS

**I. *In Vitro* PTK Assays**

The following *in vitro* assays may be used to determine the level of activity and effect of the different compounds of the present invention on one or more of the PTKs. Similar assays can be designed along the same lines for other tyrosine kinases using techniques well known in the art.

A. **KDR Tyrosine Kinase Production Using Baculovirus System:**

The coding sequence for the human KDR intra-cellular domain (aa789-1354) was generated through PCR using cDNAs isolated from HUVEC cells. A poly-$His_6$ sequence was introduced at the N-terminus of this protein as well. This fragment was cloned into transfection vector pVL1393 at the Xba 1 and Not 1 site. Recombinant baculovirus (BV) was generated through co-transfection using the BaculoGold Transfection reagent (PharMingen). Recombinant BV was plaque purified and verified through Western analysis. For protein production, SF-9 cells were grown in SF-900-II medium at 2 x $10^6$/ml, and were infected at 0.5 plaque forming units per cell (MOI). Cells were harvested at 48 hours post infection.

B. **Purification of KDR**

SF-9 cells expressing $(His)_6$KDR(aa789-1354) were lysed by adding 50 ml of Triton X-100 lysis buffer (20 mM Tris, pH 8.0, 137 mM NaCl, 10% glycerol, 1% Triton X-100, 1mM PMSF, 10μg/ml aprotinin, 1 μg/ml leupeptin) to the cell pellet from 1L of cell culture. The lysate was centrifuged at 19,000 rpm in a Sorval SS-34 rotor for 30 min at 4°C. The cell lysate was applied to a 5 ml $NiCl_2$ chelating sepharose column, equilibrated with 50 mM HEPES, pH7.5, 0.3 M NaCl. KDR was eluted using the same buffer containing 0.25 M imidazole. Column fractions were analyzed using SDS-PAGE and an ELISA assay (below) which measures kinase activity. The purified KDR was exchanged into 25mM HEPES, pH7.5, 25mM NaCl, 5 mM DTT buffer and stored at -80°C.

C. **Human Tie-2 Kinase Production and Purification**

The coding sequence for the human Tie-2 intra-cellular domain (aa775-1124) was generated through PCR using cDNAs isolated from human placenta as a template. A poly-$His_6$ sequence was introduced at the N-terminus and this construct was cloned into transfection vector pVL 1939 at the Xba 1 and Not 1 site. Recombinant BV was generated through co-transfection using the BaculoGold Transfection reagent (PharMingen). Recombinant BV was plaque purified and verified through Western analysis. For protein production, SF-9 insect cells were grown in SF-900-II medium at

$2 \times 10^6$/ml, and were infected at MOI of 0.5. Purification of the His-tagged kinase used in screening was analogous to that described for KDR.

D. **Human Flt-1 Tyrosine Kinase Production and Purification**

The baculoviral expression vector pVL1393 (Phar Mingen, Los Angeles, CA) was used. A nucleotide sequence encoding poly-$His_6$ was placed 5' to the nucleotide region encoding the entire intracellular kinase domain of human Flt-1 (amino acids 786-1338). The nucleotide sequence encoding the kinase domain was generated through PCR using cDNA libraries isolated from HUVEC cells. The histidine residues enabled affinity purification of the protein as a manner analogous to that for KDR (Part B.) and ZAP70 (Part F.) SF-9 insect cells were infected at a 0.5 multiplicity and harvested 48 hours post infection.

E. **Lck and EGFR Tyrosine Kinase Sources**

Lck or truncated forms of Lck were commercially obtained (e.g. Upstate Biotechnology Inc., Saranac Lake, NY or Santa Cruz Biotechnology, Inc., Santa Cruz, CA) or were purified from known natural or recombinant sources using conventional methods. EGFR was purchased from Sigma (Cat # E-3641; ~ 500 units/50 µl) and the EGF ligand was acquired from Oncogene Research Products/Calbiochem (Cat # PF011-100).

F. **ZAP70 Tyrosine Kinase Production**

The Baculoviral expression vector pVL 1393 (Phar Mingen, Los Angeles, CA) was used. A nucleotide sequence encoding poly-$His_6$ was placed 5' to the nucleotide region encoding the entire ZAP70 (amino acids 1-619). The nucleotide sequence encoding the ZAP70 coding region was generated through PCR using cDNA libraries isolated from Jurkat immortalized T-cells. The histidine residues enabled affinity purification of the protein (see Part B.). The LVPRGS bridge constituted a recognition sequence for proteolytic cleavage by thrombin, thereby enabling removal of the affinity tag from the enzyme. SF-9 insect cells were infected at a 0.5 multiplicity and harvested 48 hours post infection.

G. **Purification of ZAP70**

SF-9 cells were lysed in a buffer containing 20 mM Tris, pH 8.0, 137 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM PMSF, 1 μg/ml leupeptin, 10 μg/ml aprotinin and 1 mM sodium orthovanadate. The soluble lysate was applied to a chelating Sepharose Hi Trap column (Pharmacia) equilibrated in 50 mM HEPES, pH 7.5, 0.3 M NaCl. The fusion protein was eluted with 250 mM imidazole. The recovered enzyme was stored in buffer containing 50 mM HEPES, pH 7.5, 50 mM NaCl and 5 mM DTT.

H. **Enzyme Linked Immunosorbent Assay (ELISA) For RTKs**

Enzyme linked immunosorbent assays (ELISA) were used to detect and measure the presence of tyrosine kinase activity. The ELISA were conducted according to known protocols which are described in, for example, Voller, *et al.*, 1980, "Enzyme-Linked Immunosorbent Assay," In: *Manual of Clinical Immunology, 2d ed.*, edited by Rose and Friedman, pp 359-371 Am. Soc. of Microbiology, Washington, D.C.

The disclosed protocol was adapted for determining activity with respect to a specific RTK. For example, a preferred protocol for conducting the ELISA experiments for KDR is provided below. Adaptation of this protocol for determining a compound's activity for other members of the RTK family, as well as non-receptor tyrosine kinases, are well within the abilities of those in the art. For purposes of determining inhibitor selectivity, a universal PTK substrate (e.g., random copolymer of poly ($Glu_4$ Tyr), 20,000-50,000 MW) was employed together with ATP (typically 5 μM) at concentrations approximately twice the apparent Km in the assay.

**KDR IN VITRO ELISA**

The following procedure was used to assay the inhibitory effect of compounds of this invention on KDR tyrosine kinase activity:

**Buffers and Solutions:**

**PGT:** Poly (Glu,Tyr) 4:1

Store powder at -20°C. Dissolve powder in phosphate buffered saline (PBS) for 50mg/ml solution. Store 1ml aliquots at -20°C. When making plates, dilute to 250µg/ml in Gibco PBS.

**Reaction Buffer:**

100mM Hepes, 20mM $MgCl_2$, 4mM $MnCl_2$, 5mM DTT, 0.02%BSA, 200µM $NaVO_4$, pH 7.10

**ATP:** Store aliquots of 100mM at -20°C. Dilute to 20µM in water

**Washing Buffer:**

PBS with 0.1% Tween 20

**Antibody Diluting Buffer:**

0.1% bovine serum albumin (BSA) in PBS

**TMB Substrate:**

mix TMB substrate and peroxide solutions 9:1 just before use or use K-Blue Substrate from Neogen

**Stop Solution:**

1M Phosphoric Acid

<u>Procedure</u>

1. **Plate Preparation:**

Dilute PGT stock (50mg/ml, frozen) in PBS to a 250µg/ml. Add 125µl per well of Corning modified flat bottom high affinity ELISA plates (Corning #25805-96). Add 125µl PBS to blank wells. Cover with sealing tape and incubate overnight 37°C. Wash 1x with 250µl washing buffer and dry for about 2hrs in 37°C dry incubator.

Store coated plates in sealed bag at 4°C until used.

2. **Tyrosine Kinase Reaction:**

-Prepare inhibitor solutions at a 4x concentration in 20% DMSO in water.

-Prepare reaction buffer

-Prepare enzyme solution so that desired units are in 50µl, e.g. for KDR make to 1 ng/µl for a total of 50ng per well in the reactions. Store on ice.

-Make 4x ATP solution to 20µM from 100mM stock in water. Store on ice

-Add 50µl of the enzyme solution per well (typically 5-50 ng enzyme/well depending on the specific activity of the kinase)

-Add 25µl 4x inhibitor

-Add 25µl 4x ATP for inhibitor assay

-Incubate for 10 minutes at room temperature

-Stop reaction by adding 50µl 0.05N HCl per well

-Wash plate

**Final Concentrations for Reaction:
ATP: 5µM
5% DMSO

3. **Antibody Binding**

-Dilute 1mg/ml aliquot of PY20-HRP (Pierce) antibody (a phosphotyrosine antibody) to 50ng/ml in 0.1% BSA in PBS by a 2 step dilution (100x, then 200x)

-Add 100µl Ab per well. Incubate 1 hr at room temp. Incubate 1hr at 4C.

-Wash 4x plate

4. **Color reaction**

-Prepare TMB substrate and add 100µl per well

-Monitor OD at 650nm until 0.6 is reached

-Stop with 1M Phosphoric acid. Shake on plate reader.

-Read OD immediately at 450nm

Optimal incubation times and enzyme reaction conditions vary slightly with enzyme preparations and are determined empirically for each lot.

Analogous assay conditions were used for Flt-1, Tie-2, EGFR and ZAP70. For Lck, the Reaction Buffer utilized was 100 mM MOPSO, pH 6.5, 4 mM $MnCl_2$, 20 mM $MgCl_2$, 5 mM DTT, 0.2% BSA, 200 mM $NaVO_4$ under the analogous assay conditions.

**PKC kinase source**

The catalytic subunit of PKC may be obtained commercially (Calbiochem).

**PKC kinase assay**

A radioactive kinase assay was employed following a published procedure (Yasuda, I., Kirshimoto, A., Tanaka, S., Tominaga, M., Sakurai, A., Nishizuka, Y. *Biochemical and Biophysical Research Communication 3*:166, 1220-1227 (1990)). Briefly, all reactions were performed in a kinase buffer consisting of 50 mM Tris-HCl pH7.5, 10mM $MgCl_2$, 2mM DTT, 1mM EGTA, 100 µM ATP, 8 µM peptide, 5% DMSO and $^{33}P$ ATP (8Ci/mM). Compound and enzyme were mixed in the reaction vessel and the reaction initiated by addition of the ATP and substrate mixture. Following termination of the reaction by the addition of 10 µl stop buffer (5 mM ATP in 75mM phosphoric acid), a portion of the mixture was spotted on phosphocellulose filters. The spotted samples were washed 3 times in 75 mM phosphoric acid at room

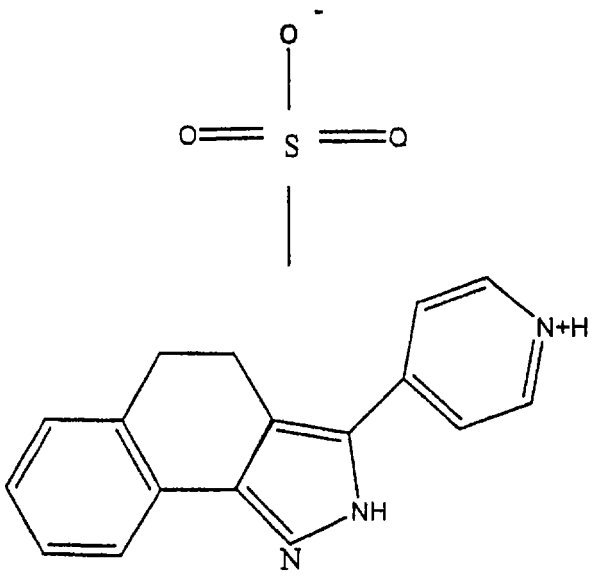
temperature for 5 to 15 minutes. Incorporation of radiolabel was quantified by liquid scintillation counting.

**Estrogen Receptor Binding Assay**

Binding of 1 nM radiolabeled 17β-estradiol to human estrogen receptor in the cytosol of MCF-7 mammary carcinoma cells was determined following incubation for 20 h at 4°C using the reaction conditions of Shein *et al.*, *Cancer Res. 45:*4192 (1985) (herein incorporated by reference). Following incubation, the cytosol fractions were mixed with a suspension of dextran-coated charcoal for 10 min at 4°C, centrifuged, and the supernatants collected. Bound radioactivity remaining in the charcoal supernatant was measured with a scintillation counter (LS 6000, Beckman) using a liquid scintillation cocktail (Formula 989 Packard). The compounds were tested simultaneously at eight concentrations in duplicate to obtain a competition curve in order to quantify the inhibitory activity. The specific radioligand binding to the estrogen receptor was defined as the difference between total binding and nonspecific binding determined in the presence of an excess of unlabeled 17β-estradiol (6 μM).

**Results**

The following inhibitory concentrations of a representative compound with the structural formula:




were obtained:

| Assay | $IC_{50}$ for Compound |
|---|---|
| KDR | 0.20 μM |
| Flt-1 | > 50.0 μM |
| Lck | > 50.0 μM |
| TIE2 | > 50.0 μM |
| ZAP70 | > 50.0 μM |
| EGFR | > 50.0 μM |
| PKC | ≥ 20 μM |
| Estrogen Receptor | >10.0 μM (< 10% inh @ 10 μM) |

These results demonstrate that compounds of the present invention and exemplified herein have notable inhibitory activity for KDR tyrosine kinase and are particularly selective as KDR tyrosine kinase inhibitors.

**II. Cellular RTK Assays**

The following cellular assay was used to determine the level of activity and effect of the different compounds of the present invention on KDR. Similar assays can be designed along the same lines for other tyrosine kinases using appropriate antibody reagents and techniques such as immunoprecipitations and Western blotting well known in the art.

A. VEGF-Induced KDR Phosphorylation in Human Umbilical Vein Endothelial Cells (HUVEC) as Measured by Western Blots.

1. HUVEC cells (from pooled donors) were purchased from Clonetics (San Diego, CA) and cultured according to the manufacturer directions. Only early passages (3-8) were used for this assay. Cells were cultured in 100 mm dishes (Falcon for tissue culture; Becton Dickinson; Plymouth, England) using complete EBM media (Clonetics).

2. For evaluating a compound's inhibitory activity, cells were trypsinized and seeded at 0.5-1.0 x $10^5$ cells/well in each well of 6-well cluster plates (Costar; Cambridge, MA).

3. 3-4 days after seeding, plates were 90-100% confluent. Medium was removed from all the wells, cells were rinsed with 5-10ml of PBS and incubated 18-24h with 5ml of EBM base media with no supplements added (i.e., serum starvation).

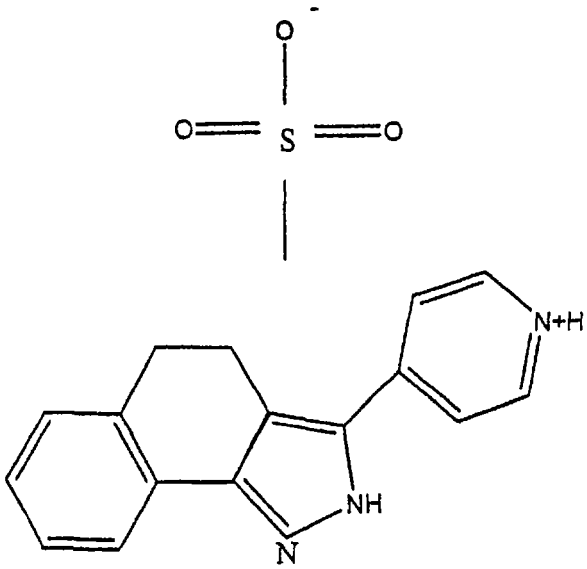
4. Serial dilutions of inhibitors were added in 1ml of EBM media (25μM, 5μM, or 1μM final concentration to cells and incubated for one hour at 37° C. Human recombinant $VEGF_{(165)}$ ( R & D Systems) was then added to all the wells in 2 ml of EBM medium at a final concentration of 50ng/ml and incubated at 37°C for 10 minutes. Control cells untreated or treated with VEGF only were used to assess background phosphorylation and phosphorylation induction by VEGF.

5. All wells were then rinsed with 5-10ml of cold PBS containing 1mM sodium orthovanadate (Sigma) and cells were lysed and scraped in 200μl of RIPA buffer (50mM Tris-HCl) pH7, 150mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 1mM EDTA) containing protease inhibitors (PMSF 1mM, aprotinin 1μg/ml, pepstatin 1μg/ml, leupeptin 1μg/ml, Na vanadate 1mM, Na fluoride 1mM) and 1μg/ml of DNase (all chemicals from Sigma Chemical Company, St Louis, MO). The lysate was spun at 14,000 rpm for 30min, to eliminate nuclei.

6. Equal amounts of proteins were then precipitated by addition of cold (-20°C) ethanol (2 volumes) for a minimum of 1 hour or a maximum of overnight. Pellets were reconstituted in Laemli sample buffer containing 5% β-mercaptoethanol (BioRad; Hercules, CA) and boiled for 5min. The proteins were resolved by polyacrylamide gel electrophoresis (6%, 1.5mm Novex, San Deigo, CA) and transferred onto a nitrocellulose membrane using the Novex system. After blocking with bovine serum albumin (3%), the proteins were probed overnight with anti-KDR polyclonal antibody (C20, Santa Cruz Biotechnology; Santa Cruz, CA) or with anti-phosphotyrosine monoclonal antibody (4G10, Upstate Biotechnology, Lake Placid, NY) at 4°C. After washing and incubating for 1 hour with HRP-conjugated F(ab)2 of goat anti-rabbit or goat-anti-mouse IgG the bands were visualized using the emission chemiluminescience (ECL) system (Amersham Life Sciences, Arlington Height, IL).

**Results**

The inhibitory concentrations of a representative compound I with the structural formula:




were:

| HUVEC | Cellular $IC_{50}$ for Compound |
|---|---|
| KDR phosphorylation | 5-10 μM |

This compound also has demonstrated KDR tyrosine kinase selectivity (See section I).

These results demonstrate that suitable compounds of the present invention have notable inhibitory activity for VEGF-induced tyrosine phosphorylation of KDR tyrosine kinase in endothelial cells.

**III. Uterine Edema Model**

This assay measures the capacity of compounds to inhibit the acute increase in uterine weight in mice which occurs in the first few hours following estrogen stimulation. This early onset of uterine weight increase is known to be due to edema caused by increased permeability of uterine vasculature. Cullinan-Bove and Koss (*Endocrinology* (1993), *133*:829-837) demonstrated a close temporal relationship of estrogen-stimulated uterine edema with increased expression of VEGF mRNA in the uterus. These results have been confirmed by the use of neutralizing monoclonal antibody to VEGF which significantly reduced the acute increase in uterine weight following estrogen stimulation (WO 97/42187). Hence, this system can serve as an *in vivo* model for inhibition of VEGF-mediated hyperpermeability and edema.

Materials: All hormones were purchased from Sigma (St. Louis, MO) or Cal Biochem (La Jolla, CA) as lyophilized powders and prepared according to supplier instructions.

Vehicle components (DMSO, Cremaphor EL) were purchased from Sigma (St. Louis, MO).

Mice (Balb/c, 8-12 weeks old) were purchased from Taconic (Germantown, NY) and housed in a pathogen-free animal facility in accordance with institutional Animal Care and Use Committee Guidelines.

Method:

Day 1: Balb/c mice were given an intraperitoneal (i.p.) injection of 12.5 units of pregnant mare's serum gonadotropin (PMSG).

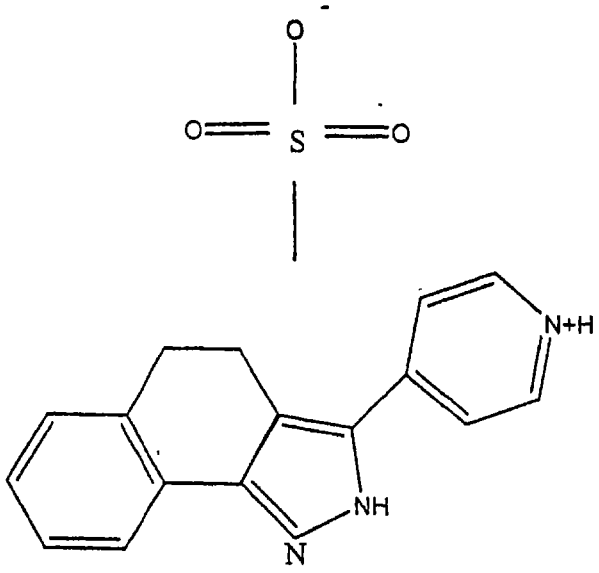
Day 3: Mice received 15 units of human chorionic gonadotropin (hCG) i.p.

Day 4: Mice were randomized and divided into groups of 5-10. Test compounds were administered by i.p., i.v. or p.o. routes depending on solubility and vehicle at doses ranging from 1-200 mg/kg. Vehicle control group received vehicle only and two groups were left untreated.

Typically thirty minutes later, experimental, vehicle and one of the untreated groups were given an i.p. injection of 17β-estradiol (500 μg/kg). After 2-3 hours, the animals were sacrificed by $CO_2$ inhalation. Following a midline incision, each uterus was isolated and removed by cutting just below the cervix and at the junctions of the uterus and oviducts. Fat and connective tissue were removed with care not to disturb the integrity of the uterus prior to weighing. Mean weights of treated groups were compared to untreated or vehicle treated groups. Significance was determined by Student's test. Non-stimulated control group was used to monitor estradiol response.

**Results**

The percent inhibition of uterine edema following estradiol stimulation for a representative compound with the structural formula:




was obtained for three routes of administration at 100 mg/kg doses.

| Administration Route | % Inhibition | p Value |
|---|---|---|
| p.o. | 17 | ns |
| i.v. | 57 | 0.01 |
| i.p. | 52 | 0.04 |

Compound I has been demonstrated herein to be KDR-selective for inhibition of kinase activity *in vitro* and efficacious in blocking cellular autophosphorylation of KDR in response to a VEGF-stimulus.

These results demonstrate that suitable compounds of the present invention such as Compound I which selectively inhibit KDR function effectively block the formation of edema. The results also demonstrate that i.v. and i.p. administration are particularly

effective for this compound. Importantly, similar anti-edemic efficacy results have also been obtained with numerous structurally-distinct selective inhibitors of KDR function.

EQUIVALENTS

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the appended claims. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the claims.

What is claimed is:

1. A use of a compound that inhibits the cellular signaling function of KDR in the manufacture of a medicament for inhibiting vascular hyperpermeability.

2. The use of Claim 1 wherein the compound that inhibits the cellular signaling function of KDR is selective for the KDR signaling function.

3. The use of Claim 1 wherein said cellular signaling function of KDR is stimulated by the binding of an activating ligand to the receptor portion of KDR.

4. The use of Claim 1 wherein said compound inhibits the catalytic kinase activity of said KDR.

5. The use of Claim 1 wherein said compound is an antagonist of KDR tyrosine kinase activation.

6. The use of Claim 1 wherein said compound selectively inhibits the phosphorylation of KDR kinase substrates.

7. The use of Claim 1 wherein said compound is selective for said KDR tyrosine kinase.

8. The use of Claim 7 wherein said compound is selected from the group consisting of peptides, antibodies and organic molecules, wherein said compound binds to said KDR tyrosine kinase.

9. The use of Claim 8 wherein the medicament inhibits the formation of a disease state selected from the group consisting of macular edema, aphakic/pseudoaphakic cystoid macular edema, retinoblastoma, ocular ischemia, ocular inflammatory disease or infection, choroidal melanoma, edematous side-effects induced by iron chelation therapy, pulmonary edema, myocardial infarction, rheumatoid diseases, anaphylaxis, tissue edema at sites of trauma and allergic inflammation, allergies, hypersensitive reactions, polyp edema at sites of chronic inflammation, cerebral edema, brain tumor fluid-filled cysts, communicating hydrocephalus, carpal tunnel syndrome, organ damage resulting from a burn, inhalation burn injury, skin burns, blistering associated with sunburn, irritation or infection, erythema multiforme,

edematous macules and other skin disorders, brain tumors, tumor effusions, lung or breast carcinomas, ascites, pleural effusions, pericardial effusions, high altitude "sickness", radioanaphylaxis, radiodermatitis, glaucoma, conjunctivitis, choroidal melanoma, adult respiratory distress syndrome, asthma, bronchitis, ovarian hyperstimulation syndrome, polycystic ovary syndrome, menstrual swelling, menstrual cramps, stroke, head trauma, cerebral infarct or occlusion, hypotension, ulcerations, sprains, fractures, effusions associated with synovitis, diabetic complications, hyperviscosity syndrome, liver cirrhosis, microalbuminuria, proteinuria, oliguria, electrolyte imbalance, nephrotic syndrome, exudates, fibroses, keloid, and the administration of growth factors.

10. The use of Claim 7 wherein adverse effects associated with an alteration in the cellular signaling function of tyrosine kinases other than KDR are avoided by said compound .

11. The use of Claim 1 wherein said compound is selected from the group consisting of single-chain antibodies, KDR-specific ribozymes and anti-sense polynucletodies, wherein said compound is introduced or produced intracellularly thereby inhibiting the proper presentation of functional KDR tyrosine kinase.

12. The use of Claim 1 wherein said medicament is manufactured in combination with a pharmaceutical agent selected from the group consisting of an anti-endemic steroid, a Ras inhibitor, anti-TNF agents, anti-IL1 agents, an antihistamine, a PAF-antagonist, a COX-1 inhibitor, a COX-2 inhibitor, a NO synthase inhibitor, a nonsteroidal anti-inflammatory agent (NSAID), a PKC inhibitor and a $PI_3$ kinase inhibitor.

13. A use of a compound that inhibits the cellular signaling function of KDR in the manufacture of a medicament for inhibiting a physiological process or state in an individual, said physiological process or state selected from the group consisting of edema formation, diapedesis, extravasation, effusion, exudation, ascites formation, matrix deposition and vascular hypotension. .

14. The use of Claim 13 wherein said compound is selective for KDR tyrosine kinase.

15. The use of Claim 14 wherein said compound is selected from the group consisting of peptides, antibodies and organic molecules, wherein said compound binds to said KDR tyrosine kinase.

16. The use of Claim 15 wherein the medicament is used for inhibiting the formation of a disease state selected from the group consisting of macular edema, aphakic/pseudoaphakic cystoid macular edema, retinoblastoma, ocular ischemia, ocular inflammatory disease or infection, choroidal melanoma, edematous side-effects induced by iron chelation therapy, pulmonary edema, myocardial infarction, rheumatoid diseases, anaphylaxis, tissue edema at sites of trauma and allergic inflammation, allergies, hypersensitive reactions, polyp edema at sites of chronic inflammation, cerebral edema, brain tumor fluid-filled cysts, communicating hydrocephalus, carpal tunnel syndrome, organ damage resulting from a burn, inhalation burn injury, skin burns, blistering associated with sunburn, irritation or infection, erythema multiforme, edematous macules and other skin disorders, brain tumors, tumor effusions, lung or breast carcinomas, ascites, pleural effusions, pericardial effusions, high altitude "sickness", radioanaphylaxis, radiodermatitis, glaucoma, conjunctivitis, choroidal melanoma, adult respiratory distress syndrome, asthma, bronchitis, ovarian hyperstimulation syndrome, polycystic ovary syndrome, menstrual swelling, menstrual cramps, stroke, head trauma, cerebral infarct or occlusion, hypotension, ulcerations, sprains, fractures, effusions associated with synovitis, diabetic complications, hyperviscosity syndrome, liver cirrhosis, microalbuminuria, proteinuria, oliguria, electrolyte imbalance, nephrotic syndrome, exudates, fibroses, keloid, and the administration of growth factors.

17. The use of Claim 13 wherein said medicament is manufactured in combination with a pharmaceutical agent selected from the group consisting of an anti-endemic steroid, a Ras inhibitor, anti-TNF agents, anti-IL1 agents, an antihistamine, a PAF-antagonist, a COX-1 inhibitor, a COX-2 inhibitor, a NO synthase inhibitor, a nonsteroidal anti-inflammatory agent (NSAID), a PKC inhibitor and a $PI_3$ kinase inhibitor.