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[Continued on next page]

(54) Title: INHIBITION OF NEOVASCULARIZATION BY INHIBITION OF PROSTANOID IP RECEPTORS

(57) Abstract: There are provided inter alia methods and compounds useful for decreasing choroidal neovascularization in a subject in need thereof.

Fig. 1A

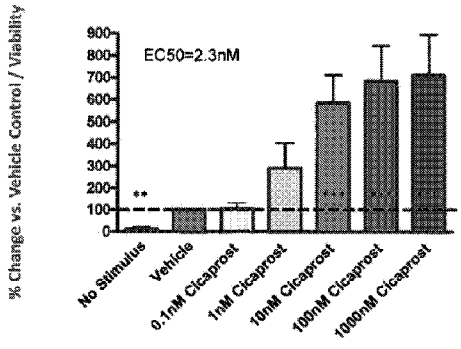
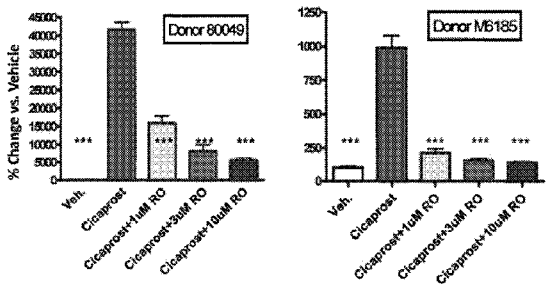


Fig. 1B



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TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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INHIBITION OF NEOVASCULARIZATION BY INHIBITION OF PROSTANOID IP RECEPTORS

By: Jenny W. Wang and David F. Woodward

RELATED APPLICATIONS

This application claims the benefit of United States Provisional Patent Application Serial No. 61/778,055 filed March 12, 2013, the disclosure of which is hereby incorporated in its entirety by reference

BACKGROUND OF THE INVENTION

Neovascularization refers generally to the formation of functional vascular (e.g., microvascular) networks. The formation may involve proliferation of blood vessels in tissue not normally containing blood vessels, or of blood vessels of a different type than found in the neovascularized tissue under normal physiological conditions. For example, choroidal neovascularization (CNV) involves the invasion of new blood vessels at the choroid. CNV can involve abnormalities in Bruch's membrane forming the innermost layer of the choroid. The resulting neovascular tissue can cause physical separation of the laminar structure of the retina and destruction of normal retinal tissue in turn causing leakage, bleeding, and blindness. Accordingly, CNV is associated with a variety of diseases of the eye including age-related macular degeneration (AMD). For example, in the so-called "wet" form of AMD, CNV develops in the choriocapillaries and subretinal space, disrupting the retinal pigment epithelium (RPE) which results in significant loss of the central vision. *See* e.g., Green, 1999, *Mol. Vis.* **5**:27. Indeed, AMD is a leading cause of blindness in older populations of developed countries. *See* e.g., Resnikof et al., 2004, *Bull. World Health Organ.* **82**:844-851.

Occlusion of retinal vessels leading to ischemia and/or hypoxia is common in CNV. *See* e.g., Michaelson I., 1948, *Trans Ophthalmol Soc UK.* **68**:137–180; Ashton N., 1957, *Am J Ophthalmol.* **44**:7–17; 6. Shimizu K, et al., 1981, *Ophthalmology* **88**:601–612. Extensive evidence on the involvement of Vascular Endothelial Growth Factor (VEGF) has been provided. For example, VEGF is upregulated in hypoxia, and levels of VEGF are increased with ischemic retinopathy. *See* e.g., Shweiki D, et al., 1992, *Nature* **359**:843–845; Plate KH, et al., 1992, *Nature* **359**:845–848; Forsythe JA, et al., 1996, *Mol Cell Biol.* **16**:4604–4613; Adamis AP, et al., 1994, *Am J Ophthalmol* **118**:445–450; 12. Aiello LP, et al., 1994, *N Engl J*

Med. **331**:1480–1487; Malecaze F, et al., 1992, *Arch Ophthalmol.* **112**:1476-1482; Pe'er J, et al., 1995, *Lab Invest.* **72**:638–645; Miller JW, et al., 1994, *Am J Pathol.* **145**:574–584; Pierce EA, et al., 1995, *Proc Natl Acad Sci USA.* **92**:905–909. Indeed, increased expression of VEGF in retinal photoreceptors of transgenic mice stimulates neovascularization within the retina, and VEGF antagonists partially inhibit retinal neovascularization in animal models. *See* e.g., Okamoto N, et al. 1997, *Am J Pathol.* **151**:281–291; Tobe T, et al., 1998, *Invest Ophthalmol Vis Sci.* **39**:180–188; Aiello LP, et al., 1995, *Proc Natl Acad Sci USA.* **92**:10457–104611 Robinson GS, et al., 1996, *Proc Natl Acad Sci USA.* **93**:4851–4856; Adamis AP, et al., 1996, *Arch Ophthalmol.* **114**:66–71. Moreover, overexpression of VEGF in the subretinal space results in neovascularization of the choroidal membranes and death of the overlying photoreceptor cells. See e.g., Spilsbury, K., et al., 2000, *Am. J. Pathology* **157**:135-144.

Accordingly, there is a need to decrease neovascularization (e.g., CNV) in subjects in need thereof. There are provided herein methods and compounds useful, *inter alia*, to decrease neovascularization.

BRIEF SUMMARY OF THE INVENTION

In a first aspect, there is provided a method for decreasing neovascularization in a subject in need thereof. The method includes administering to the subject an effective amount of an IP receptor antagonist, thereby decreasing the neovascularization.

In another aspect, there is provided a pharmaceutical composition including an IP receptor antagonist and a pharmaceutically acceptable excipient.

In another aspect, there is provided an ophthalmic pharmaceutical formulation including an IP receptor antagonist and an ophthalmic pharmaceutically acceptable excipient.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Fig. 1A: IP receptor agonist Cicaprost dose dependently induces VEGF secretion in TNFα-stimulated human macrophages. *** $p<0.01$, *** $p<0.001$ compared to the vehicle control. Average EC50=2.3nM, n=4 donors. Fig. 1B: IP receptor antagonist Formula I dose dependently inhibits 100nM Cicaprost-induced VEGF secretion in TNFα-stimulated human macrophages from two individual donors. *** $p<0.001$ compared to Cicaprost control.

Figure 2. Fig. 2 depicts that blockade of IP receptors alone is sufficient to inhibit laser-induced choroidal neovascularization (CNV). Fig. 2A: Rat vs. Rat (n=6); Fig. 2B: Eye

vs. Eye (n=12); Fig. 2C: Lesion vs. lesion (n=38). Vehicle is 50% propylene glycol in H_20. IP antagonist is Formula II.

DETAILED DESCRIPTION OF THE INVENTION

I. Methods

In a first aspect, there is provided a method for decreasing neovascularization in a subject in need thereof. The method includes administering to the subject an effective amount of an IP receptor antagonist, thereby decreasing the neovascularization.

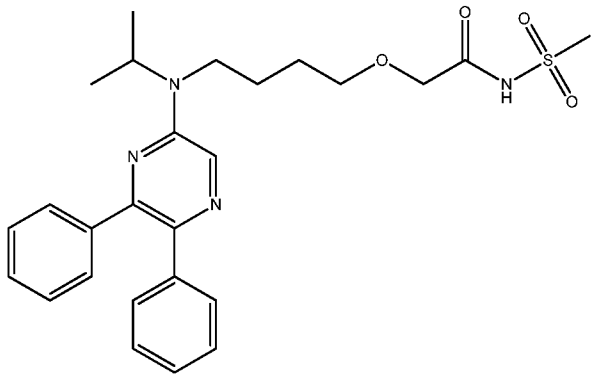
The terms "subject," "patient" and the like refer, except where indicated otherwise, to mammals such as humans and non-human primates, as well as rabbits, rats, mice, goats, pigs, and other mammalian species. The term does not necessarily indicate that the subject has been diagnosed with a particular disease but typically refers to an individual under medical supervision. In one embodiment, the subject is a human. The phrases "subject in need" and the like refer to a subject having a perceived or diagnosed disease or condition (e.g., age-related macular degeneration).

The term "neovascularization" refers in the usual and customary sense to the formation of vasculature, such as *de novo* formation of functional microvascular networks having red blood cell perfusion. In one embodiment, neovascularization is of the choroid layer of the eye. Accordingly, the terms "choroidal neovascularization," "CNV" and the like refer to the formation of vasculature in the choroid, such as functional microvascular networks having red blood cell perfusion within or associated with the choroid. Thus, in one embodiment, there is provided a method for decreasing choroidal neovascularization in a subject in need thereof. The method includes administering to the subject an effective amount of an IP receptor antagonist, thereby decreasing the neovascularization.

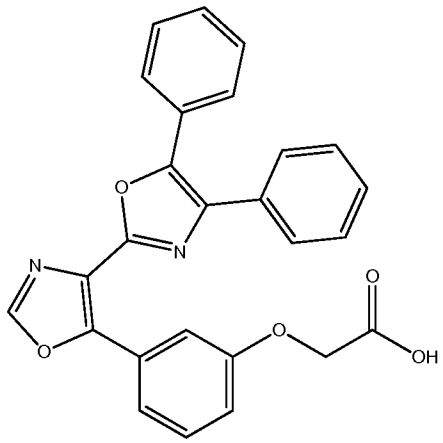
The terms "IP receptor," "prostaglandin I_2 receptor," "prostacyclin receptor," "PTGIR" and the like refer in the usual and customary sense to a receptor for prostacyclin (i.e., PGI_2, prostaglandin I_2). The natural receptors are members of the G protein-coupled receptor gene superfamily. Upon activation, an IP receptor typically causes an elevation in intracellular cAMP via direct stimulation of adenylate cyclase. Human IP receptors are well-known in the art and are discussed in detail, for example, in Boie et al., 1994, *J. Biol. Chem.* **269**:12173-12178.

Useful IP receptor antagonists include, for example, those set forth in: EP 0 902 018 A2 (e.g. 2-(arylphenyl)amino-imidazoline derivatives which are IP receptor antagonists); U.S. Pat. No. 6,184,242 (e.g. 2-(substituted-phenyl)amino-imidazoline derivatives); WO 02/070514 (e.g. alkoxycarbonylamino heteroaryl carboxylic acid derivatives) ;WO 02/070500 (e.g. alkoxycarbonylamino benzoic acid or alkoxycarbonylamino tetrazolyl phenyl derivatives); WO 02/40453 (e.g. substituted 2-phenylaminoimidazoline phenyl ketone derivatives); WO 01/68591 (e.g. carboxylic acid derivatives); U.S. 6,417,186; U.S. 6,569,860; U.S. 6,903,086; U.S. 6,693,098; U.S. 6,998,414 and U.S. 7,056,903

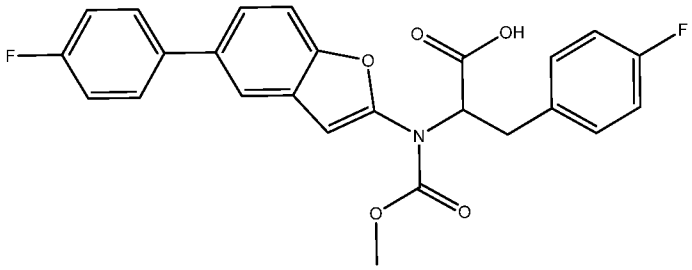
Additional IP receptor antagonists for use in the methods set forth herein are as follows. The structure of selexipag (2-(4-((5,6-diphenylpyrazin-2-yl)(isopropyl)amino) butoxy)-N-(methylsulfonyl)acetamide) is:



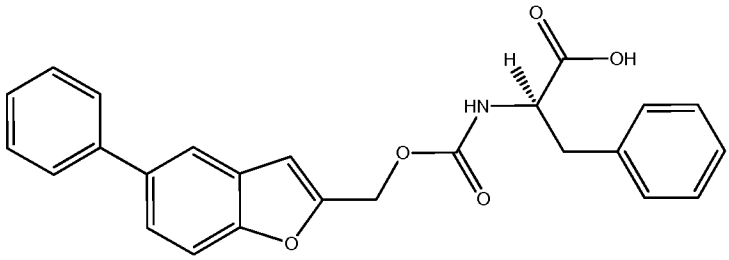
and includes pharmaceutically acceptable salts of the above. The structure of 2-(3-(4,5-diphenyl-[2,4'-bioxazol]-5'-yl)phenoxy)acetic acid is:



and includes pharmaceutically acceptable salts of the above. The structure of 3-(4-fluorophenyl)-2-((5-(4-fluorophenyl)benzofuran-2-yl)(methoxycarbonyl)amino)propanoic acid is:

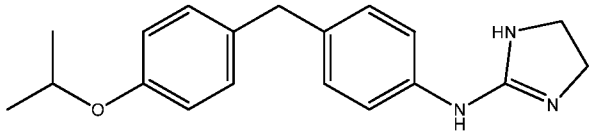


and includes pharmaceutically acceptable salts of the above. The structure of (R)-3-phenyl-2-((((5-phenylbenzofuran-2-yl)methoxy)carbonyl)amino)propanoic acid is:



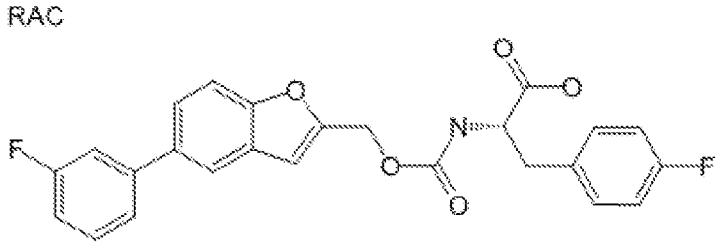
and includes pharmaceutically acceptable salts of the above.

Other useful IP receptor antagonists include the compounds of Formulas I and II below. The structure of Formula I is:



(I),

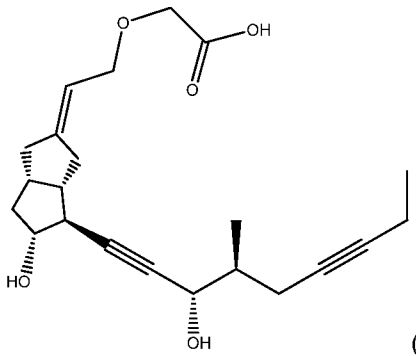
and includes pharmaceutically acceptable salts of the above. The structure of Formula II is:



(II),

and includes pharmaceutically acceptable salts of the above.

An example of an IP receptor agonist useful in the experimental work described herein is cicaprost. The structure of cicaprost is:



(III),

Cicaprost

and includes pharmaceutically acceptable salts of the above.

In one embodiment, neovascularization (e.g. choroidal neovascularization) is associated with excess levels of vascular endothelial growth factor (VEGF). The terms "excess levels" and the like in the context of concentrations of biological molecules (e.g., VEGF) refer to concentrations which are greater than concentrations found under normal physiological conditions (e.g., in the absence of neovascularization). Methods for determining the concentrations of biological molecules in the subject are well known in the art and include, e.g., biological assays, direct quantification of concentrations, and the like. Accordingly, the phrase "neovascularization is associated with excess levels of VEGF" and the like refer to neovascularization wherein the concentration of VEGF in the subject is greater than the concentration found in the absence of neovascularization. The greater concentration of VEGF can be at the site of neovascularization or in the tissue surrounding the site of neovascularization or associated with it.

The terms "vascular endothelial growth factor," "VEGF" and the like refer in the usual and customary sense to a sub-family of the platelet-derived growth factor family, which is a member of the cystine-knot family of growth factors. VEGF's include, for example, subtypes such as VEGF-A, VEGF-B, VEGF-C , VEGF-D, and the like. VEGF is a signal protein that typically stimulates vasculogenesis and angiogenesis and can contribute to disease when overexpressed. For example, overexpression of VEGF can cause vascular disease in the retina of the eye and other parts of the body. Drugs such as bevacizumab (humanized monoclonal antibody to VEGF-A) can inhibit VEGF and control or slow those diseases.

In one embodiment, administration of an effective amount of the IP receptor antagonist inhibits (e.g. decreases) the secretion of VEGF. In one embodiment, the secretion of VEGF is inhibited by 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or even greater, with respect to the secretion of VEGF in the absence of administration of the IP receptor antagonist. Thus, in one embodiment, the concentration of VEGF is decreased due to the decrease in secretion of VEGF.

In one embodiment, the neovascularization is choroidal neovascularization (CNV). Accordingly, the phrases "choroidal neovascularization is associated with excess levels of VEGF" and the like refer to choroidal neovascularization wherein the concentration of VEGF in the subject is greater than the concentration found in the absence of choroidal neovascularization. The greater concentration of VEGF can be at the site of choroidal neovascularization or in the tissue surrounding the site of choroidal neovascularization or associated with it.

In one embodiment, the choroidal neovascularization is associated with age-related macular degeneration (e.g., wet type age-related macular degeneration). The terms "age-related macular degeneration," "AMD" and the like refer to a medical condition, typically afflicting older adults (e.g., >= 50-yrs age), which results in loss of vision, especially in the macular visual field. The condition typically results from damage to the retina. In the "dry" (nonexudative) form of AMD, cellular debris (i.e., drusen) accumulates between the retina and the choroid, and the retina can become detached. In the more severe "wet" (exudative) form, blood vessels emerge from the choroid in the choriocapillaris through Bruch's membrane. This can ultimately lead to blood and protein leakage below the macula. Bleeding, leaking and scarring from these blood vessels can eventually cause irreversible damage to the photoreceptors. Moreover, the retina can become detached.

In one embodiment, the CNV is induced by exposure to a laser. In one embodiment, the IP receptor antagonist is capable of reducing VEGF levels (e.g., reducing VEGF levels in the choroid or in tissue associated with the choroid).

In one embodiment, the IP receptor antagonist has the structure of Formula (II), and the neovascularization is associated with excess levels of VEGF. In one embodiment, the neovascularization is choroidal neovascularization. In one embodiment, the choroidal neovascularization is associated with wet-type age-related macular degeneration. In one embodiment, the choroidal neovascularization is laser-induced.

II. Pharmaceutical Compositions

In another aspect, there is provided a pharmaceutical composition including an IP receptor antagonist in combination with a pharmaceutically acceptable excipient (e.g., carrier). In one embodiment, the pharmaceutical composition is an ophthalmic pharmaceutical composition. The term "ophthalmic pharmaceutical composition" refers to a pharmaceutical composition suitable for administration to the eye. . The "ophthalmic pharmaceutical composition" may be formulated for delivery of an IP receptor antagonist to the choroid of a subject.

The phrases "pharmaceutically acceptable excipient," "pharmaceutically acceptable carrier" and the like as used herein refer to pharmaceutical excipients, e.g., pharmaceutically, physiologically, acceptable organic or inorganic carrier substances suitable for administration, and which do not deleteriously react with the active agent. In one embodiment, a pharmaceutical composition can be sterilized and/or mixed with auxiliary agents such as lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, coloring, and/or aromatic substances and the like that do not deleteriously react with the compounds disclosed herein. The term "ophthalmic pharmaceutically acceptable excipient" refers to a pharmaceutically acceptable excipient suitable for administration to the eye.

The term "pharmaceutically acceptable salt" is meant to include salts of the active compounds that are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds disclosed herein contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds disclosed herein contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the

salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, oxalic, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (*see*, for example, Berge *et al.*, *Journal of Pharmaceutical Science*, **1977**, *66*, 1-19). Certain specific compounds disclosed herein contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts.

The compounds disclosed herein may exist as salts, such as with pharmaceutically acceptable acids. Examples of such salts include hydrochlorides, hydrobromides, sulfates, methanesulfonates, nitrates, maleates, acetates, citrates, fumarates, tartrates (e.g., (+)-tartrates, (-)-tartrates, or mixtures thereof including racemic mixtures), succinates, benzoates, and salts with amino acids such as glutamic acid. These salts may be prepared by methods known to those skilled in the art.

The neutral forms of the compounds are preferably regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents.

In addition to salt forms, embodiments disclosed herein provide compounds in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide certain of the the compounds disclosed herein. Additionally, prodrugs can be converted to certain compounds disclosed herein by chemical or biochemical methods in an *ex vivo* environment. For example, prodrugs can be slowly converted to certain compounds disclosed herein when placed in a transdermal patch reservoir with a suitable enzyme or chemical reagent.

Certain compounds disclosed herein can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present invention. Certain compounds disclosed herein may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

Certain compounds disclosed herein possess asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, tautomers, geometric isomers, and individual isomers are encompassed within the scope of the present invention. The compounds disclosed herein do not include those that are known in the art to be too unstable to synthesize and/or isolate.

The compounds disclosed herein may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be radiolabeled with radioactive isotopes, such as for example tritium (^{3}H), iodine-125 (^{125}I), or carbon-14 (^{14}C). All isotopic variations of the compounds disclosed herein, whether radioactive or not, are encompassed within the scope of the present invention.

In one embodiment, the pharmaceutical composition includes an IP receptor antagonist at a concentration in the range of about 0.01% to 1.00% (w/v). In one embodiment, the concentration of the IP receptor antagonist is about 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10%, 0.11%, 0.12%, 0.13%, 0.14%, 0.15%, 0.16%, 0.17%, 0.18%, 0.19%, 0.20%, 0.30%, 0.40%, 0.50%, 0.60%, 0.70%, 0.80%, 0.90%, or 1.00% (w/v), or even greater.

A. Formulations

The compounds disclosed herein can be prepared and administered in a wide variety of ophthalmic, oral, parenteral, and topical dosage formulations. Thus, the compounds disclosed herein can be administered by injection (e.g. intravenously, intramuscularly, intracutaneously, subcutaneously, intraduodenally, or intraperitoneally). Also, the compounds described herein can be administered by inhalation, for example, intranasally. Additionally, the compounds disclosed herein can be administered transdermally or by ocular instillation. Multiple routes of administration (e.g., intramuscular, oral, transdermal, ocular instillation) are contemplated that can be used to administer the compounds disclosed herein. Accordingly, embodiments of the present invention also provide pharmaceutical compositions (e.g., an ophthalmic pharmaceutical composition) which include a pharmaceutically acceptable carrier or excipient and one or more compounds (e.g., IP receptor antagonist).

In another aspect, there is provided an ophthalmic pharmaceutical formulation. An "ophthalmic pharmaceutical formulation" includes an ophthalmic pharmaceutical

composition and is formulated to be administered to the eye. In one embodiment, the ophthalmic pharmaceutical formulation is instilled in the eye. In one embodiment, the ophthalmic pharmaceutical formulation is injected into the eye, or into tissue surrounding or associated with the eye.

In one embodiment, the ophthalmic pharmaceutical formulation includes an IP receptor antagonist at a concentration in the range of about 0.01% to 1.00% (w/v). In one embodiment, the concentration of the IP receptor antagonist is about 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10%, 0.11%, 0.12%, 0.13%, 0.14%, 0.15%, 0.16%, 0.17%, 0.18%, 0.19%, 0.20%, 0.30%, 0.40%, 0.50%, 0.60%, 0.70%, 0.80%, 0.90%, or 1.00% (w/v), or even greater.

The ophthalmic pharmaceutical formulation can include buffers to adjust the pH to a desirable range for ophthalmic use. Generally, a pH of around 6-8 is desired, and in certain ophthalmic pharmaceutical formulations a pH of 7.4 is desired. Many buffers including salts of inorganic acids such as phosphate, borate, and sulfate are known. In one embodiment, the buffer maintains the pH of the ophthalmic pharmaceutical formulation in the range of about 6.5 to 7.5. In one embodiment, the buffer maintains the pH of the ophthalmic pharmaceutical formulation in the range of about 7.0 to 7.4.

In one embodiment, the ophthalmic pharmaceutical formulation includes one or more viscosity-enhancing agents, or thickening agents. Thickening agents are used for a variety of reasons, ranging from improving the form of the formulation for convenient administration to improving the contact with the eye to improve bioavailability. The viscosity-enhancing agent may comprise a polymer containing hydrophilic groups such as monosaccharides, polysaccharides, ethylene oxide groups, hydroxyl groups, carboxylic acids or other charged functional groups. While not intending to limit the scope of the invention, some examples of useful viscosity-enhancing agents are sodium carboxymethylcellulose, hydroxypropyl methylcellulose, povidone, polyvinyl alcohol, and polyethylene glycol. In one embodiment, viscosity-enhancing agents are employed at a level between about 0.01% and about 2% (w/v).

In one embodiment, the ophthalmic pharmaceutical formulation includes one or more tonicity agents useful to adjust the ophthalmic pharmaceutical formulation to the desired isotonic range. Tonicity agents are well known in the art and some examples include glycerin, mannitol, sorbitol, sodium chloride, and other electrolytes. In one embodiment, the

concentration of tonicity agent is in the range of about 0.1 to 2.00% (w/v). In one embodiment, the concentration of tonicity agent is in the range of about 1.15 to 1.30% (w/v). In one embodiment, the concentration of tonicity agent is about 0.10%, 0.20%, 0.30%, 0.40%, 0.50%, 0.60%, 0.70%, 0.80%, 0.90%, 1.00%, 1.10%, 1.20%, 1.30%, 1.40%, 1.50%, 1.60%, 1.70%, 1.80%, 1.90%, or 2.00%. In one embodiment, the concentration of tonicity agent is about 1.15%, 1.16%, 1.17%, 1.18%, 1.19%, 1.20%, 1.21%, 1.22%, 1.23%, 1.24%, 1.25%, 1.26%, 1.27%, 1.28%, 1.29%, or 1.30% (w/v).

In one embodiment, the ophthalmic pharmaceutical formulation includes a solubilizer (e.g., surfactant or other appropriate co-solvent) in order to facilitate solubilization of one or more components of the ophthalmic pharmaceutical formulation. Such solubilizers include Polysorbate 20, 60, and 80, Pluronic F-68, F-84, and P-103, cyclodextrin, hydroxy-beta-cyclodextrin, solutol, polyoxyethylene 40 stearate, and polyoxyl 35 castor oil. Such solubilizers can be employed at a level between about 0.01 % and about 2% by weight. In one embodiment, the solubilizer is present in the range of about 0.01% to 0.20% (w/v). In one embodiment, the solubilizer is present at 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.10%, 0.11%, 0.12%, 0.13%, 0.14%, 0.15%, 0.16%, 0.17%, 0.18%, 0.19%, or 0.20% (w/v). In one embodiment, the solubilizer is polysorbate 80.

In one embodiment, the ophthalmic pharmaceutical formulation includes a preservative. In one embodiment, the preservative is benzalkonium chloride, chlorine dioxide, chlorobutanol, thimerosal, phenylmercuric acetate, or phenylmercuric nitrate. In one embodiment, the preservative is present at a concentration in the range of about 0.01% to 0.05% (w/v). In one embodiment, the preservative is present at a concentration in the range of about 0.015% to 0.025% (w/v). In one embodiment, the concentration of the preservative is about 0.015%, 0.016%, 0.017%, 0.018%, 0.019%, 0.020%, 0.021%, 0.022%, 0.023%, 0.024%, or 0.025% (w/v). In one embodiment, the preservative is benzalkonium chloride.

The compositions disclosed herein may additionally include components to provide sustained release and/or comfort. Such components include high molecular weight, anionic mucomimetic polymers, gelling polysaccharides, and finely-divided drug carrier substrates. These components are discussed in greater detail in U.S. Pat. Nos. 4,911,920; 5,403,841; 5,212,162; and 4,861,760, the entire contents of each of which are incorporated herein by reference in their entirety and for all purposes.

B. Effective Dosages

Pharmaceutical compositions contemplated herein include compositions wherein the active ingredient is contained in an effective amount, i.e., in an amount effective to achieve its intended purpose. An "effective amount" is an amount sufficient to accomplish a stated purpose (e.g., achieve the effect for which it is administered, treat a disease, reduce one or more symptoms of a disease or condition). An example of an "effective amount" is an amount sufficient to contribute to the treatment, prevention, or reduction of a symptom or symptoms of a disease, which can be referred to as a "therapeutically effective amount." A "reduction" of a symptom or symptoms (and grammatical equivalents of this phrase) means decreasing of the severity or frequency of the symptom(s), or elimination of the symptom(s). The actual amount effective for a particular application will depend, *inter alia,* on the condition being treated. For example, when administered in methods to treat neovascularization (e.g., choroidal neovascularization), such compositions will contain amounts of active ingredients effective to achieve the desired result (e.g. decreasing the extent of neovascularization in a subject).

The dosage and frequency (single or multiple doses) of compounds administered can vary depending upon a variety of factors, including route of administration; size, age, sex, health, body weight, body mass index, and diet of the recipient; nature and extent of symptoms of the disease being treated (e.g., the disease responsive to an IP receptor antagonist); presence of other diseases or other health-related problems; kind of concurrent treatment; and complications from any disease or treatment regimen. Other therapeutic regimens or agents can be used in conjunction with the methods and compounds disclosed herein.

For any compound described herein or combination thereof, the therapeutically effective amounts can be initially determined from cell culture assays. Target concentrations will be those concentrations of active compound(s) that are capable of decreasing neovascularization (e.g., choroidal neovascularization) as measured, for example, using methods known in the art.

Therapeutically effective amounts for use in humans may be determined from animal models. For example, a dose for humans can be formulated to achieve a concentration that has been found to be effective in animals. The dosage in humans can be

adjusted by monitoring neovascularization (e.g., choroidal neovascularization) and adjusting the dosage upwards or downwards, as described above.

Dosages may be varied depending upon the requirements of the subject and the compound being employed. The dose administered to a subject, in the context of certain embodiments disclosed herein, should be sufficient to effect a beneficial therapeutic response in the subject over time. The size of the dose also will be determined by the existence, nature, and extent of any adverse side effects. Generally, treatment is initiated with smaller dosages, which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under circumstances is reached.

Dosage amounts and intervals can be adjusted individually to provide levels of the administered compounds effective for the particular clinical indication being treated. This will provide a therapeutic regimen that is commensurate with the severity of the individual's disease state.

Utilizing the teachings provided herein, an effective therapeutic treatment regimen can be planned that does not cause substantial toxicity and yet is entirely effective to treat the clinical symptoms demonstrated by the particular patient. This planning should involve the careful choice of active compound by considering factors such as compound potency, relative bioavailability, patient body weight, presence and severity of adverse side effects, preferred mode of administration, and the toxicity profile of the selected agent.

C. Toxicity

The ratio between toxicity and therapeutic effect for a particular compound is its therapeutic index and can be expressed as the ratio between LD_{50} (the amount of compound lethal in 50% of the population) and ED_{50} (the amount of compound effective in 50% of the population). Compounds that exhibit high therapeutic indices are preferred. Therapeutic index data obtained from cell culture assays and/or animal studies can be used in formulating a range of dosages for use in humans. The dosage of such compounds preferably lies within a range of plasma 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. See, *e.g.* Fingl *et al., In:* THE PHARMACOLOGICAL BASIS OF THERAPEUTICS, Ch.1, p.l, 1975. The exact formulation, route of administration, and dosage

can be chosen by the individual physician in view of the patient's condition and the particular method in which the compound is used.

The abbreviations used herein have their conventional meaning within the chemical and biological arts. The chemical structures and formulae set forth herein are constructed according to the standard rules of chemical valency known in the chemical arts.

III. Examples

Example 1. Investigation of VEGF Secretion and Inhibition in Culture

A TNFα-stimulated human macrophage system was employed, as known in the art, to assess the effect of IP receptor agonist and IP receptor antagonist on induction of VEGF secretion. As depicted in Fig. 1A, cicaprost dose dependently induces VEGF secretion in TNFα-stimulated human macrophages with EC_{50} = 2.3 nM.

In order to test whether a IP receptor antagonist can reverse the effect of cicaprost-induced VEGF secretion in TNFα-stimulated human macrophages, the IP receptor antagonist Formula I was employed. As depicted in Fig 1B, Formula I dose dependently inhibits the secretion of VEGF in TNFα-stimulated human macrophages which can been exposed to 100 nM cicaprost. The human macrophages had been obtained from two separate donors, as indicated in Fig. 1B.

Example 2. Investigation of Blockade of IP Receptors in Laser-Induced CNV

A laser-induced model of CNV was employed, as known in the art, to determine whether blockade of IP receptors alone is sufficient to inhibit CNV. *See* e.g., Francois et al., 1975, *Am. J. Ophthalmol.* **79**:206-210; Edelman & Castro, 2000, Exp. Eye Res. 71:523-533; Goody, R.J., et al., 2011, *Exp. Eye. Research* **92**:464-472.

Test rats were administered laser treatment after which vehicle (50% propylene glycol in H_2O) or IP receptor antagonist Formula II was administered. The area of subsequently identified CNV was quantified by methods known in the art. As shown in Fig. 2A, the area (μm^2) of CNV was significantly reduced per test subject after administration of IP receptor antagonist. As depicted in Fig. 2B, the area of CNV was also reduced as judged by a comparison of each eye. Moreover, as depicted in Fig. 2C, the area of CNV was reduced on a per lesion basis.

WHAT IS CLAIMED IS:

1. A method for decreasing neovascularization in a subject in need thereof, comprising administering to said subject an effective amount of an IP receptor antagonist, thereby decreasing said neovascularization.

2. The method of claim 1, wherein said neovascularization is associated with excess levels of vascular endothelial growth factor (VEGF).

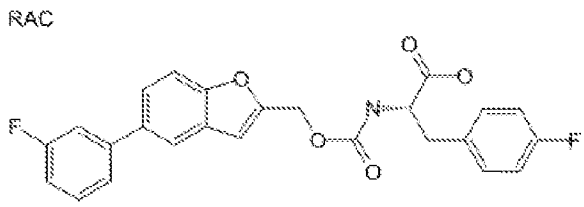
3. The method of claim 2, wherein said IP receptor antagonist inhibits secretion of VEGF in said subject.

4. The method of claim 3, wherein said neovascularization is choroidal neovascularization.

5. The method of claim 4, wherein said choroidal neovascularization is associated with wet type age-related macular degeneration.

6. The method of claim 4, wherein said choroidal neovascularization due to physical or chemical trauma or a secondary result of cardiovascular disease .

7. The method of claim 1, wherein said IP receptor antagonist has the structure of Formula II:



(II).

8. The method of claim 7, wherein said choroidal neovascularization is associated with excess levels of vascular endothelial growth factor (VEGF).

9. The method of claim 8, wherein said IP receptor antagonist inhibits secretion of VEGF in said subject.

10. The method of claim 7, wherein said neovascularization is choroidal neovascularization.

11. The method of claim 10, wherein said choroidal neovascularization is associated with wet type age-related macular degeneration.

12. The method of claim 10, wherein said choroidal neovascularization is laser induced.

13. A pharmaceutical composition comprising an IP receptor antagonist and a pharmaceutically acceptable excipient.

14. An ophthalmic pharmaceutical formulation comprising an ophthalmic pharmaceutical composition and an ophthalmic pharmaceutically acceptable excipient, said ophthalmic pharmaceutical composition comprising an IP receptor antagonist.

Fig. 1A

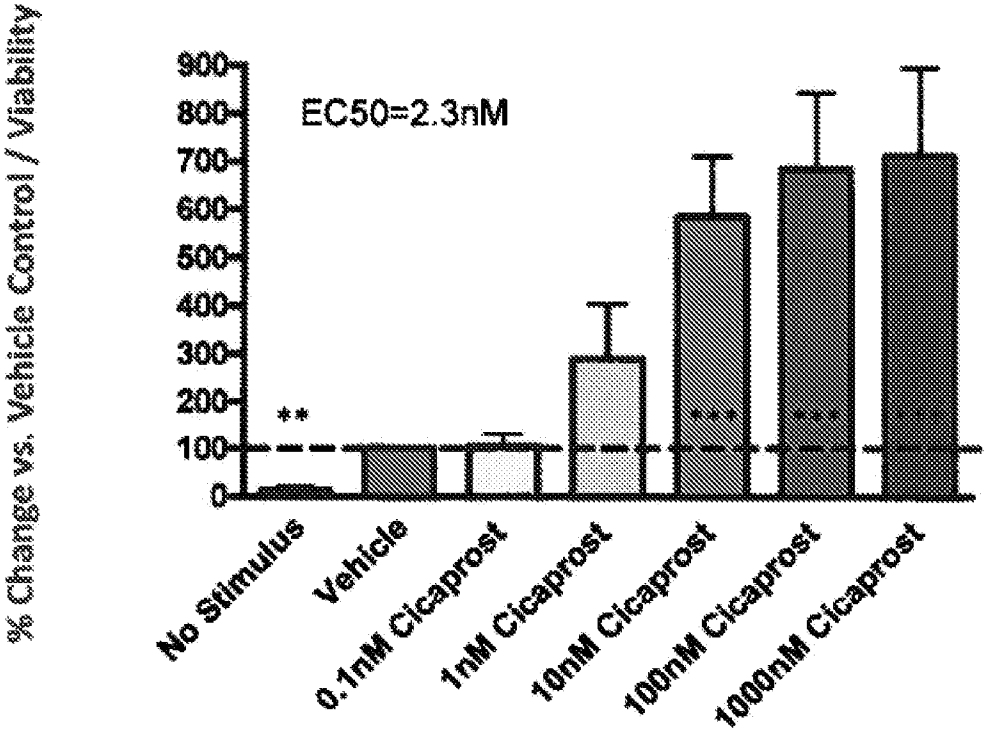

% Change vs. Vehicle Control / Viability
900
800
700
600
500
400
300
200
100
0
EC50=2.3nM
**
***
***
***
No Stimulus
Vehicle
0.1nM Cicaprost
1nM Cicaprost
10nM Cicaprost
100nM Cicaprost
1000nM Cicaprost

Fig. 1B

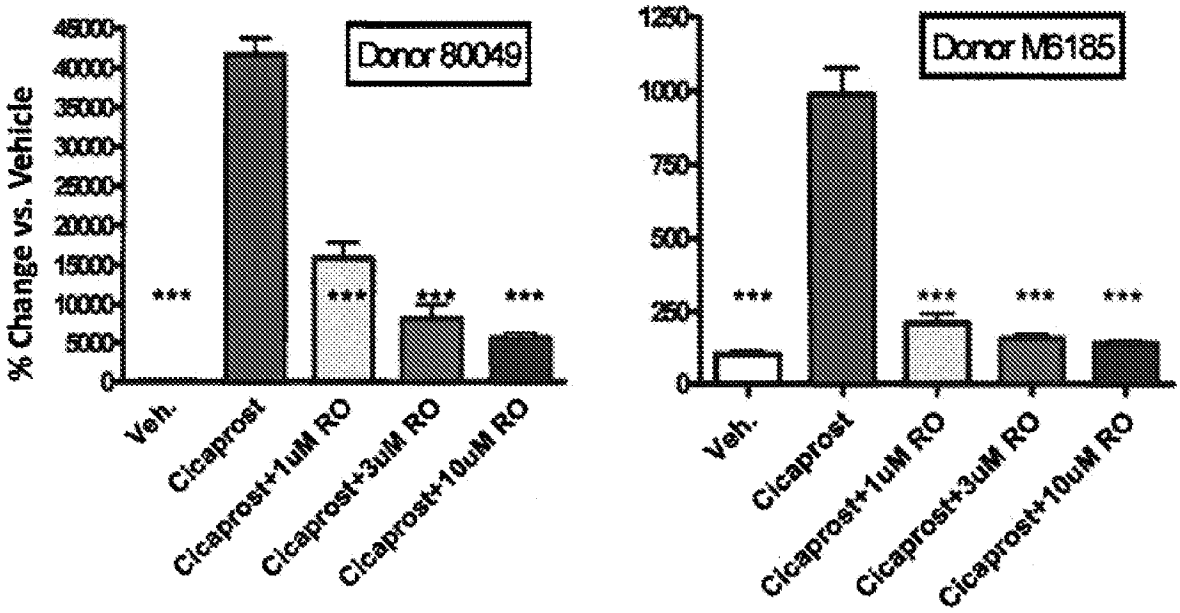

% Change vs. Vehicle
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***
***
***
***
Veh.
Cicaprost
Cicaprost+1uM RO
Cicaprost+3uM RO
Cicaprost+10uM RO
Donor M6185
***
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Cicaprost+3uM RO
Cicaprost+10uM RO

Fig. 2A

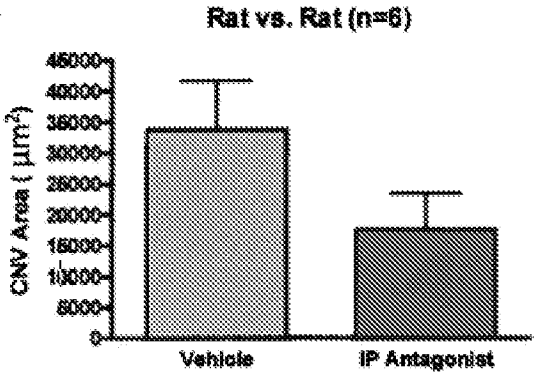


Fig. 2B

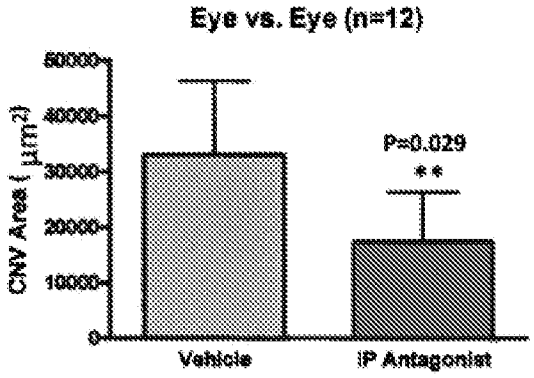
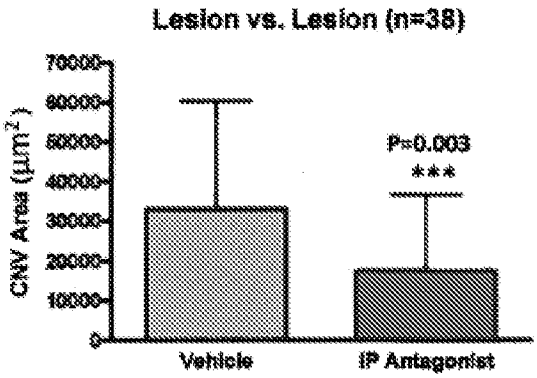


Fig. 2C



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/020830

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/343 A61K31/4168 A61K31/422 A61K31/4965 A61P27/02
A61K9/00
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	page 55, lines 9-10; example 3	1-12

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 April 2014	22/04/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Ansaldo, M

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/020830

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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