

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization

International Bureau



(10) International Publication Number

WO 2013/166449 A2

(43) International Publication Date  
7 November 2013 (07.11.2013)

(51) International Patent Classification: A61K 31/138 (2006.01)

(52) International Application Number: PCT/US2013/039562

(53) International Filing Date: 3 May 2013 (03.05.2013)

(54) Filing Language: English

(55) Publication Language: English

(56) Priority Data:

61/643,178	4 May 2012 (04.05.2012)	US
61/643,058	4 May 2012 (04.05.2012)	US
61/643,051	4 May 2012 (04.05.2012)	US
61/781,907	14 March 2013 (14.03.2013)	US

(71) Applicant: ACUCELA INC. [US/US]; 1301 Second Ave., Ste. 1900, Seattle, WA 98101 (US).

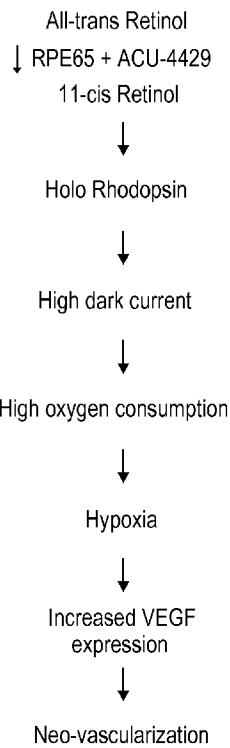
(72) Inventors: BAVIK, Claes, Olof; 20216 41st Place NE, Lake Forest Park, WA 98155 (US). HENRY, Susan, Hayes; 9425 126th Place, Kirkland, WA 98034 (US). KUBOTA, Ryo; 1301 Second Ave., Suite 1900, Seattle, WA 98101 (US). KUKSA, Vladimir, A.; 417 203rd Place SE, Bothell, WA 98101 (US).

(74) Agent: CLARK, David, L., D.; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, CA 94304 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,

[Continued on next page]

(54) Title: METHODS FOR THE TREATMENT OF DIABETIC RETINOPATHY AND OTHER OPHTHALMIC DISEASES



(57) Abstract: Methods are provided herein for the treatment of ophthalmic diseases or conditions such as an ophthalmic disease or disorder associated with diabetes in a patient. Also provided herein are methods of treating retinopathy of prematurity in a patient. Further, provided herein are methods for treating wet age-related macular degeneration in a patient. The methods comprise administration of compounds disclosed herein to a patient in need thereof that inhibit or slow one or more signs or symptoms of such conditions.

**FIG. 4B**



HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

**(84) Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,

LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Declarations under Rule 4.17:**

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))

**Published:**

- without international search report and to be republished upon receipt of that report (Rule 48.2(g))

## METHODS FOR THE TREATMENT OF DIABETIC RETINOPATHY AND OTHER OPHTHALMIC DISEASES

### CROSS REFERENCE

**[0001]** This application claims the benefit of U.S. Provisional Application No. 61/781,907, filed March 14, 2013, U.S. Provisional Application No. 61/643,178, filed May 4, 2012, U.S. Provisional Application No. 61/643,051, filed May 4, 2012, U.S. Provisional Application No. 61/643,058, filed May 4, 2012, which are incorporated herein by reference in their entirety.

### BACKGROUND OF THE INVENTION

**[0002]** Diabetic Retinopathy is a common and specific micro vascular complication of diabetes, and is the leading cause of preventable blindness in working-age people. It is identified in a third of people with diabetes and is associated with increased risk of life-threatening systemic vascular complications, including stroke, coronary heart disease, and heart failure. Optimum control of blood glucose, blood pressure, and possibly blood lipids remains the foundation for reduction of risk of retinopathy development and progression.

**[0003]** Retinopathy of prematurity (ROP) blinds between about 400-800 babies annually in the United States, and reduces vision in many thousands more world-wide. It is a growing problem in the developing world because while steady improvements in neonatal intensive care have led to an increase in the survival rate of very low birth weight infants, these are the very patients at greatest risk for ROP.

**[0004]** The retina contains photoreceptors that transduce light into a neural signal, and also has an extensive vascular supply. The clinical hallmark of ROP is abnormal retinal vasculature, which appears at the pre-term ages. This abnormal vasculature is insufficient to supply oxygen during the maturation of the rod photoreceptors, cells that are the most demanding of oxygen of any cells in the body. In the most severe ROP cases, vision loss results from retinal detachment instigated by leaky retinal blood vessels. However, milder cases of ROP, the retinal vascular abnormalities usually resolve without treatment, but the patients nevertheless suffer a range of lifelong visual impairments even with optimal optical correction.

**[0005]** Age-related macular degeneration (AMD) is the major cause of severe visual loss in the United States for individuals over the age of 55. AMD occurs in either an atrophic or (less commonly) an exudative form. In exudative AMD, blood vessels grow from the choriocapillaris through defects in Bruch's membrane, and in some cases the underlying retinal pigment epithelium (choroidal neovascularization or angiogenesis). Organization of serous or hemorrhagic exudates escaping from these vessels results in fibrovascular scarring of the macular region with attendant degeneration of the neuroretina, detachment and tears of the retinal pigment epithelium, vitreous hemorrhage and permanent

loss of central vision. This process is responsible for more than 80% of cases of significant visual loss in patients with AMD.

**[0006]** Choroidal neovascularization (CNV) has proven recalcitrant to treatment in most cases. Laser treatment can ablate CNV and help to preserve vision in selected cases not involving the center of the retina, but this is limited to only about 10% of the cases. Unfortunately, even with successful laser photocoagulation, the neovascularization recurs in about 50-70% of eyes (50% over 3 years and >60% at 5 years). (Macular Photocoagulation Study Group, Arch. Ophthalmol. 204:694-701 (1986)). In addition, many patients who develop CNV are not good candidates for laser therapy because the CNV is too large for laser treatment, or the location cannot be determined so that the physician cannot accurately aim the laser.

**[0007]** Retinal neovascularization (RNV) develops in numerous retinopathies associated with retinal ischemia, such as sickle cell retinopathy, Eales disease, ocular ischemic syndrome, carotid cavernous fistula, familial exudative vitreoretinopathy, hyperviscosity syndrome, idiopathic occlusive arteriolitis, radiation retinopathy, retinal vein occlusion, retinal artery occlusion, retinal embolism. Retinal neovascularization can also occur with inflammatory diseases (birdshot retinochoroidopathy, retinal vasculitis, sarcoidosis, toxoplasmosis, and uveitis), choroidal melanoma, chronic retinal detachment, incontinentia pigmenti, and rarely in retinitis pigmentosa.

**[0008]** A factor common to almost all RNV is retinal ischemia, which releases diffusible angiogenic factors (such as VEGF). The neovascularization begins within the retina and then breaches the retinal internal limiting membrane. The new vessels grow on the inner retina and the posterior surface of the vitreous after it has detached (vitreous detachment). Neovascularization may erupt from the surface of the optic disk or the retina. RNV commonly progresses to vitreoretinal neovascularization. Iris neovascularization often follows retinal neovascularization.

## SUMMARY OF THE INVENTION

**[0009]** Provided herein are methods for treating various ophthalmic diseases or conditions such as an ophthalmic disease or disorder associated with diabetes in a patient. Also provided herein is a method of treating retinopathy of prematurity in a patient. Further, provided herein is a method for treating wet age-related macular degeneration in a patient.

**[0010]** In one aspect, herein is a method of treating retinopathy of prematurity in an immature eye by administering a Visual Cycle Modulation (VCM) compound to a patient in need thereof. The methods described herein relate to the administration of compounds described herein that are visual cycle modulators (VCM) that reduce or suppress energy-demanding processes in rod photoreceptors. In one embodiment, the VCM compound is administered orally.

**[0011]** In another aspect, described herein is a method of improving rod-mediated retinal function by administering a VCM compound to a patient with an immature retina. The methods described herein reduce rod energy demand in the developing retina, whereby rod-mediated retinal function is improved upon retinal maturity relative to a patient not treated with the agent.

**[0012]** In another aspect, described herein is a method of modulating the visual cycle by administering to a patient in need thereof a composition comprising a compound described herein, where modulation of the visual cycle treats retinopathy of prematurity.

**[0013]** Also described herein is a method for improving function and/or suppressing the visual cycle in a developing rod cell, by contacting the cell with a VCM compound that suppresses energy demand in the rod cell. In one embodiment of such methods, the treatment is administered locally to the eye. In another embodiment such methods, the treatment is administered at a site distant from the eye or systemically.

**[0014]** In one embodiment, a patient to be treated with a compound described herein is administered one or more additional compounds or treatments. For example, in one embodiment, the patient is treated with supplemental oxygen.

**[0015]** In a further aspect is a method for treating wet age-related macular degeneration in a patient comprising administering to the patient a therapeutically effective amount of a Visual Cycle Modulation (VCM) compound.

**[0016]** Patients to be treated include humans as well as non-humans (e.g., domestic or wild animals)

**[0017]** In one embodiment, the composition of the VCM compound is administered orally. Compositions may be administered one or more times. Administration may occur more than once per day, once per day, every other day, every week, or every month.

**[0018]** In such methods, treatment results in improvement of one or more symptoms of the patient.

Symptoms that may be improved by such methods include, but are not limited to, bleeding, leaking, scarring, damage to the photoreceptors, vision loss, or a combination thereof.

**[0019]** In one embodiment is a method for reducing or inhibiting vascularization (e.g., neovascularization) in a patient comprising administering to the patient a therapeutically effective amount of a Visual Cycle Modulation (VCM) compound. In one embodiment, the vascularization is associated with choroidal neovascularization. In one embodiment, the vascularization is associated with retinal neovascularization. The inhibition or reduction in vascularization can be, for example, at least about 1%, 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

**[0020]** In one embodiment is a method for treating choroidal neovascularization in a patient comprising administering to the patient a therapeutically effective amount of a Visual Cycle Modulation (VCM) compound.

**[0021]** One embodiment described herein is a method for protecting an eye during medical procedures requiring exposure of the eye to bright light, to laser light, procedures resulting in prolonged and/or excessive dilation of the pupil, or that otherwise sensitize the eye to light, the method comprising administration of a composition comprising a compound described herein to a patient in need thereof. The compounds described herein, at sufficient dosages, inhibit the visual cycle by at least 50%. Thus, in some embodiments, an effective dose inhibits the visual cycle in the eye of the subject undergoing the medical procedure by at least 50%, by at least 75%, or by at least 90%. Furthermore, the duration of the inhibition also depends on the dose. Thus, in one embodiment, the inhibition continues for at least one

hour, for at least 2 hours, for at least 4 hours, for at least 8 hours, for at least 12 hours, for at least 24 hours, or for at least 48 hours. Finally, the compounds herein are reversible inhibitors of the visual cycle, and thus the subjects visual cycle returns to normal within 3 half-lives. In one embodiment, the compound used with such aforementioned medical procedures is emixustat.

**[0022]** In another aspect are dosing schedules (e.g., number of administrations per day) for the treatment of the ophthalmic diseases and conditions described herein. In one embodiment, the compound is administered once daily (which includes multiple sub-doses of the compound administered at approximately the same time); in another embodiment, the compound is administered once every two days (which includes multiple sub-doses of the compound administered at approximately the same time); and in another embodiment, the compound is administered once every three days or more (which includes multiple sub-doses of the compound administered at approximately the same time).

**[0023]** In another aspect are dosing schedules (e.g., variations between dose amounts of subsequent administrations) for the treatment of the ophthalmic diseases and conditions described herein. In one embodiment, the compound is administered on day 1 at a dose level higher than that administered on following days (e.g., a loading dose). In another embodiment, the compound is administered on day 1 at a dose level two times that administered on following days. In another embodiment, the compound is administered on day 1 at a dose level three times that administered on following days.

**[0024]** In another aspect are dosing schedules (e.g., time of day when compound is administered) for the treatment of the ophthalmic diseases and conditions described herein. In one embodiment, the compound is administered in the morning; in another embodiment, the compound is administered in the evening; in another embodiment, the compound is administered upon waking; and in another embodiment, the compound is administered prior to going to sleep. In one embodiment, the compound is administered as a controlled release formulation in the evening. In another embodiment, the compound is administered prior to eating, or alternatively during a meal, or alternatively, subsequent to a meal. In some embodiments, such a meal is breakfast; in other embodiments, such a meal is lunch; in yet other embodiments, such a meal is dinner/supper.

**[0025]** In one aspect the daily dose of (R)-3-amino-1-(3-(cyclohexylmethoxy)phenyl)propan-1-ol is about 4 mg to about 100 mg. In another aspect the daily dose of (R)-3-amino-1-(3-(cyclohexylmethoxy)phenyl)propan-1-ol is about 2 mg; about 5 mg; about 7 mg; about 10 mg; about 15 mg; about 20 mg; about 40 mg; about 60 mg; about 75 mg; or about 100 mg.

**[0026]** Inhibition of the visual cycle is determined, in some embodiments, by an ERG. Information regarding doses of the compounds described herein, sufficient to inhibit the visual cycle to at least 50%, as well as methods for determining visual cycle inhibition in a subject (including ERG) are described in US Patent Application Publication US 2011/0003895, which incorporated herein by reference for such disclosure.

**[0027]** In one embodiment, the composition is administered orally prior to the medical procedure. In one embodiment, the composition is administered 24 hours and/or 48 hours after the medical procedure.

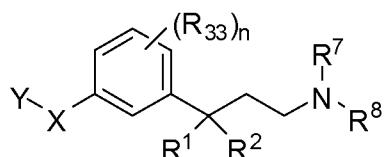
**[0028]** In one embodiment, the composition of the VCM compound is administered orally. Compositions may be administered one or more times. Administration may occur more than once per day, once per day, every other day, every week, or every month.

**[0029]** In such methods, treatment results in improvement of one or more symptoms of the patient. Symptoms that may be improved by such methods include, but are not limited to, defects in Bruch's membrane, increases in amount of ocular vascular endothelial growth factor (VEGF), myopia, myopic degeneration, deterioration of central vision, metamorphopsia, color disturbances, hemorrhaging of blood vessels, or a combination thereof.

**[0030]** In one embodiment is a method for treating retinal neovascularization in a patient comprising administering to the patient a therapeutically effective amount of a Visual Cycle Modulation (VCM) compound.

**[0031]** In one embodiment, the retinal neovascularization is associated with one or more retinopathies including, but not limited to, sickle cell retinopathy, Eales disease, ocular ischemic syndrome, carotid cavernous fistula, familial exudative vitreoretinopathy, hyperviscosity syndrome, idiopathic occlusive arteriolitis, radiation retinopathy, retinal vein occlusion, retinal artery occlusion, retinal embolism, birdshot retinochoroidopathy, retinal vasculitis, sarcoidosis, toxoplasmosis, uveitis, choroidal melanoma, chronic retinal detachment, incontinentia pigmenti, and retinitis pigmentosa.

**[0032]** In another aspect is a method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient, comprising administering to the patient a therapeutically effective amount of a composition comprising a compound of Formula (A), or tautomer, stereoisomer, geometric isomer, N-oxide or a pharmaceutically acceptable salt thereof:



Formula (A)

wherein,

X is selected from  $-C(R^9)=C(R^9)-$ ,  $-C\equiv C-$ ,  $-C(R^9)_2-O-$ ,  $-C(R^9)_2-C(R^9)_2-$ ,  $-C(R^9)_2-S-$ ,  $-C(R^9)_2-S(O)_2-$ , or  $-C(R^9)_2-NR^9$ ;

Y is selected from:

- a) substituted or unsubstituted carbocyclyl, optionally substituted with  $C_1-C_4$  alkyl, halogen, -OH, or  $C_1-C_4$  alkoxy;
- b) substituted or unsubstituted carbocyclylalkyl, optionally substituted with  $C_1-C_4$  alkyl, halogen, -OH, or  $C_1-C_4$  alkoxy;
- c) substituted or unsubstituted aralkyl, optionally substituted with  $C_1-C_4$  alkyl, halogen, -OH, or  $C_1-C_4$  alkoxy; or
- d) substituted or unsubstituted  $C_3-C_{10}$  alkyl, optionally substituted with halogen, -OH, or  $C_1-C_4$  alkoxy;

$R^1$  is hydrogen and  $R^2$  is hydroxyl; or  $R^1$  and  $R^2$  form an oxo;  
 $R^7$  is hydrogen;  
 $R^8$  is hydrogen or  $CH_3$ ;  
each  $R^9$  independently hydrogen, or substituted or unsubstituted  $C_1$ - $C_4$  alkyl;  
each  $R^{33}$  is independently selected from halogen or substituted or unsubstituted  $C_1$ - $C_4$  alkyl, and  $n$  is 0, 1, 2, 3, or 4.

**[0033]** Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein  $n$  is 0, 1, or 2.

**[0034]** Another embodiment provides the method wherein  $X$  is  $-C(R^9)=C(R^9)-$ . Another embodiment provides the method wherein  $X$  is  $-C\equiv C-$ . Another embodiment provides the method wherein  $X$  is  $-C(R^9)_2-O-$ . Another embodiment provides the method wherein  $X$  is  $-C(R^9)_2-C(R^9)_2-$ . Another embodiment provides the method wherein  $X$  is  $-C(R^9)_2-S-$ . Another embodiment provides the method wherein  $X$  is  $-C(R^9)_2-S(O)_2-$ . Another embodiment provides the method wherein  $X$  is  $-C(R^9)_2-NR^9-$ .

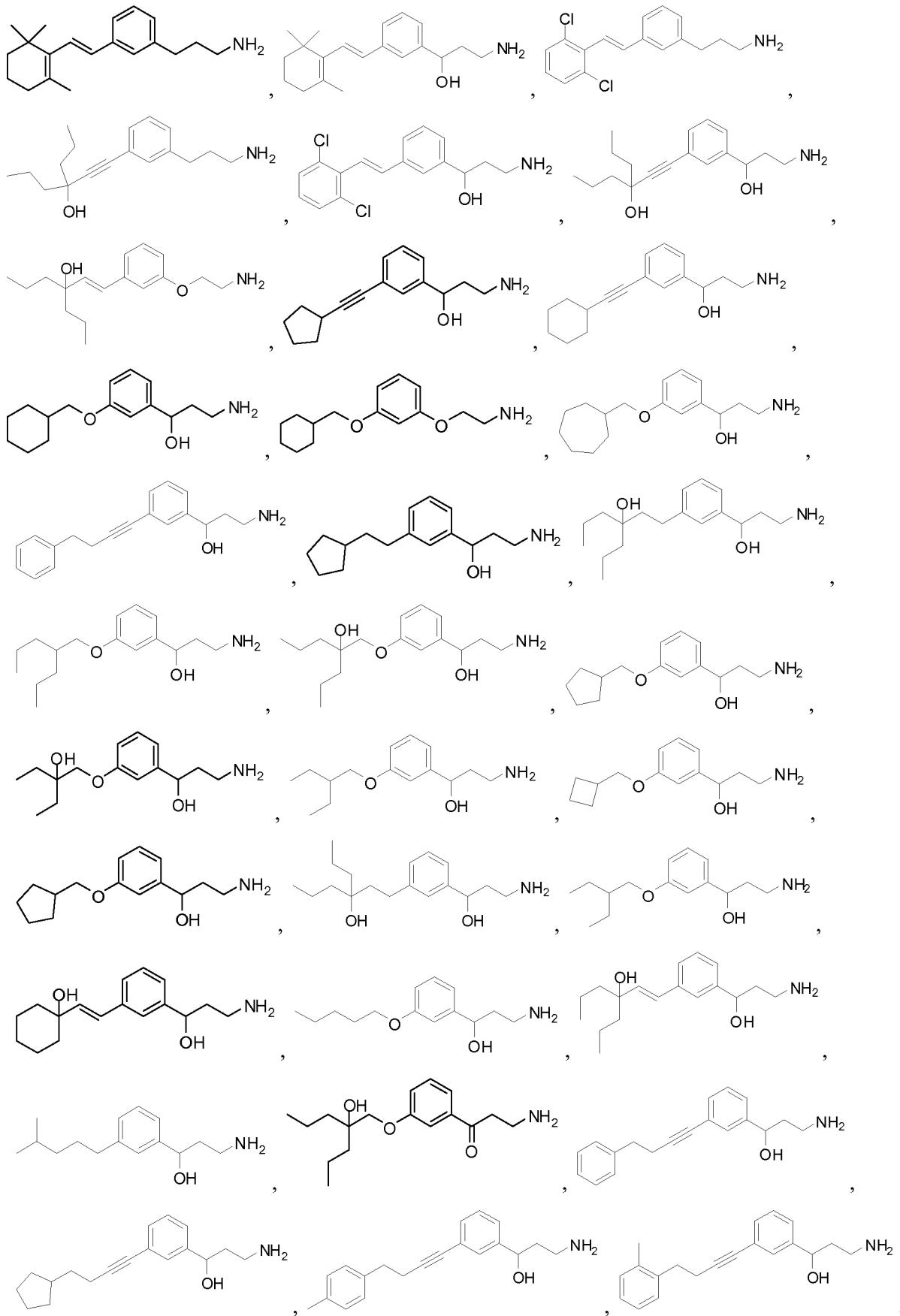
**[0035]** Another embodiment provides the method wherein  $Y$  is substituted or unsubstituted carbocyclyl, or substituted or unsubstituted  $C_3$ - $C_{10}$  alkyl. Another embodiment provides the method wherein  $Y$  is substituted or unsubstituted carbocyclyl. Another embodiment provides the method wherein the substituted or unsubstituted carbocyclyl is a substituted or unsubstituted 4-, 5-, 6-, or 7-membered ring. Another embodiment provides the method wherein the substituted or unsubstituted carbocyclyl is a 6-membered ring. Another embodiment provides the method wherein the substituted or unsubstituted 6-membered ring is a substituted or unsubstituted cyclohexyl. Another embodiment provides the method wherein the substituted or unsubstituted 6-membered ring is a substituted or unsubstituted cyclohexyl and  $X$  is  $-C(R^9)_2-O-$ .

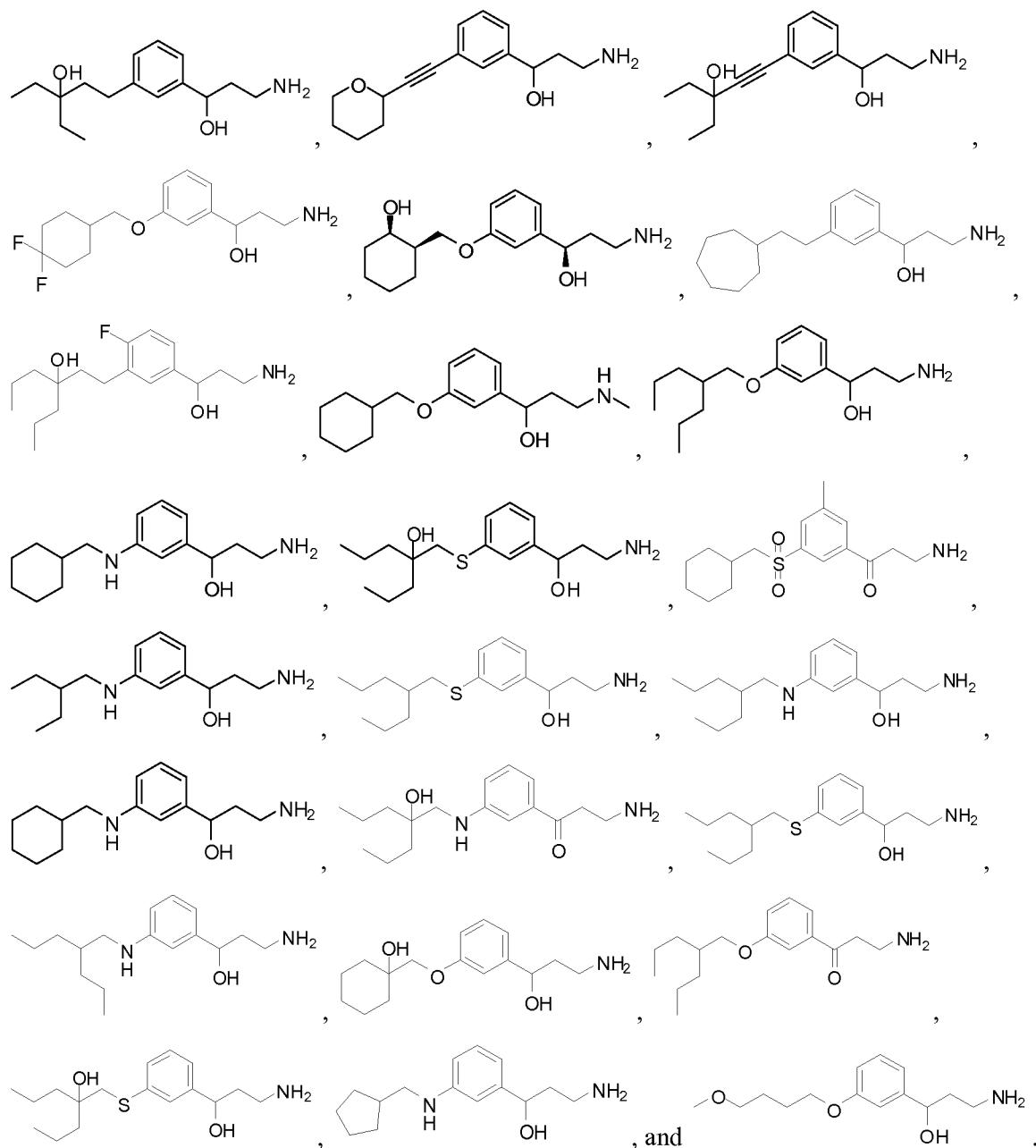
**[0036]** Another embodiment provides the method wherein  $Y$  is substituted or unsubstituted  $C_3$ - $C_{10}$  alkyl. Another embodiment provides the method wherein the substituted or unsubstituted  $C_3$ - $C_{10}$  alkyl is a substituted or unsubstituted  $C_3$ - $C_6$  alkyl. Another embodiment provides the method wherein the substituted  $C_3$ - $C_6$  alkyl is substituted with an  $C_1$ - $C_2$  alkoxy group. Another embodiment provides the method wherein the substituted  $C_3$ - $C_6$  alkyl is  $-CH_2CH_2CH_2OCH_3$ .

**[0037]** Another embodiment provides the method wherein  $R^1$  is hydrogen and  $R^2$  is hydroxyl. Another embodiment provides the method wherein  $R^1$  and  $R^2$  form an oxo. Another embodiment provides the method wherein  $R^8$  is hydrogen. Another embodiment provides the method wherein  $R^8$  is methyl. Another embodiment provides the method wherein  $R^1$  is hydrogen,  $R^2$  is hydroxyl and  $X$  is  $-C(R^9)_2-O-$ .

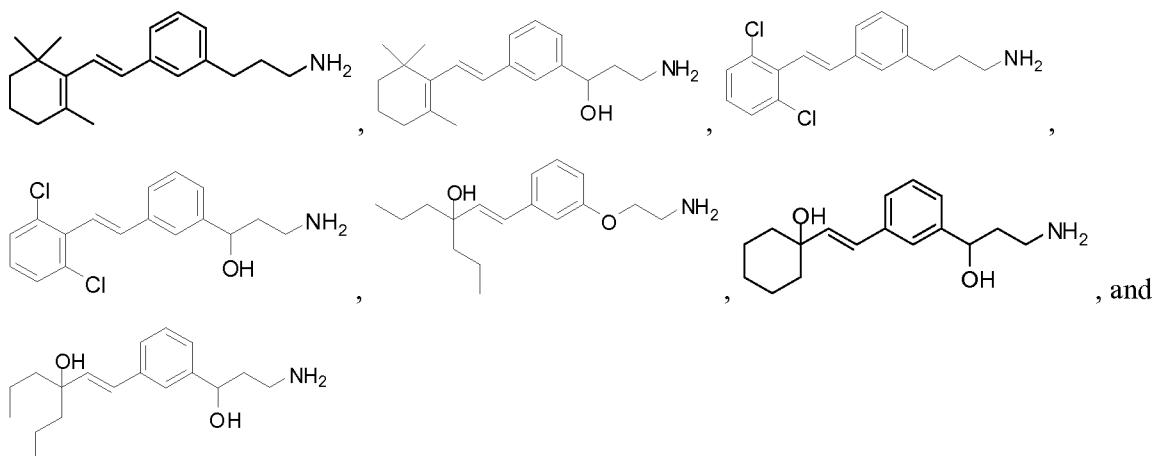
**[0038]** One embodiment provides a method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient comprising administering to the patient a therapeutically effective amount of a composition comprising a compound,

or tautomer, stereoisomer, geometric isomer, N-oxide or a pharmaceutically acceptable salt thereof, selected from:

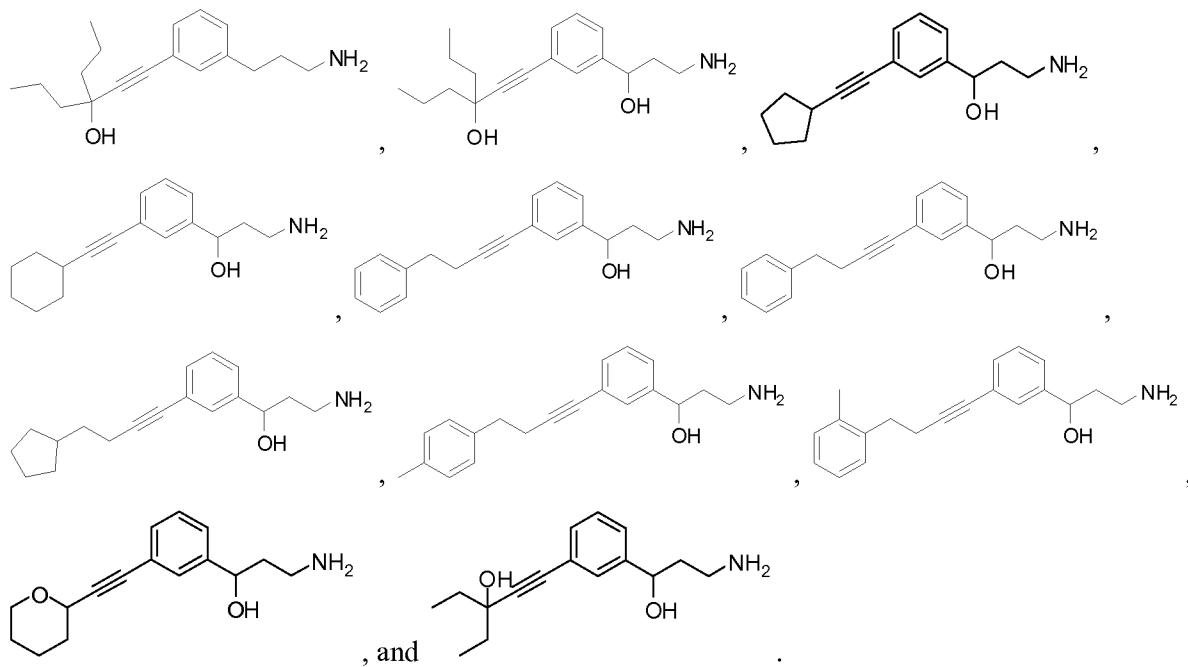




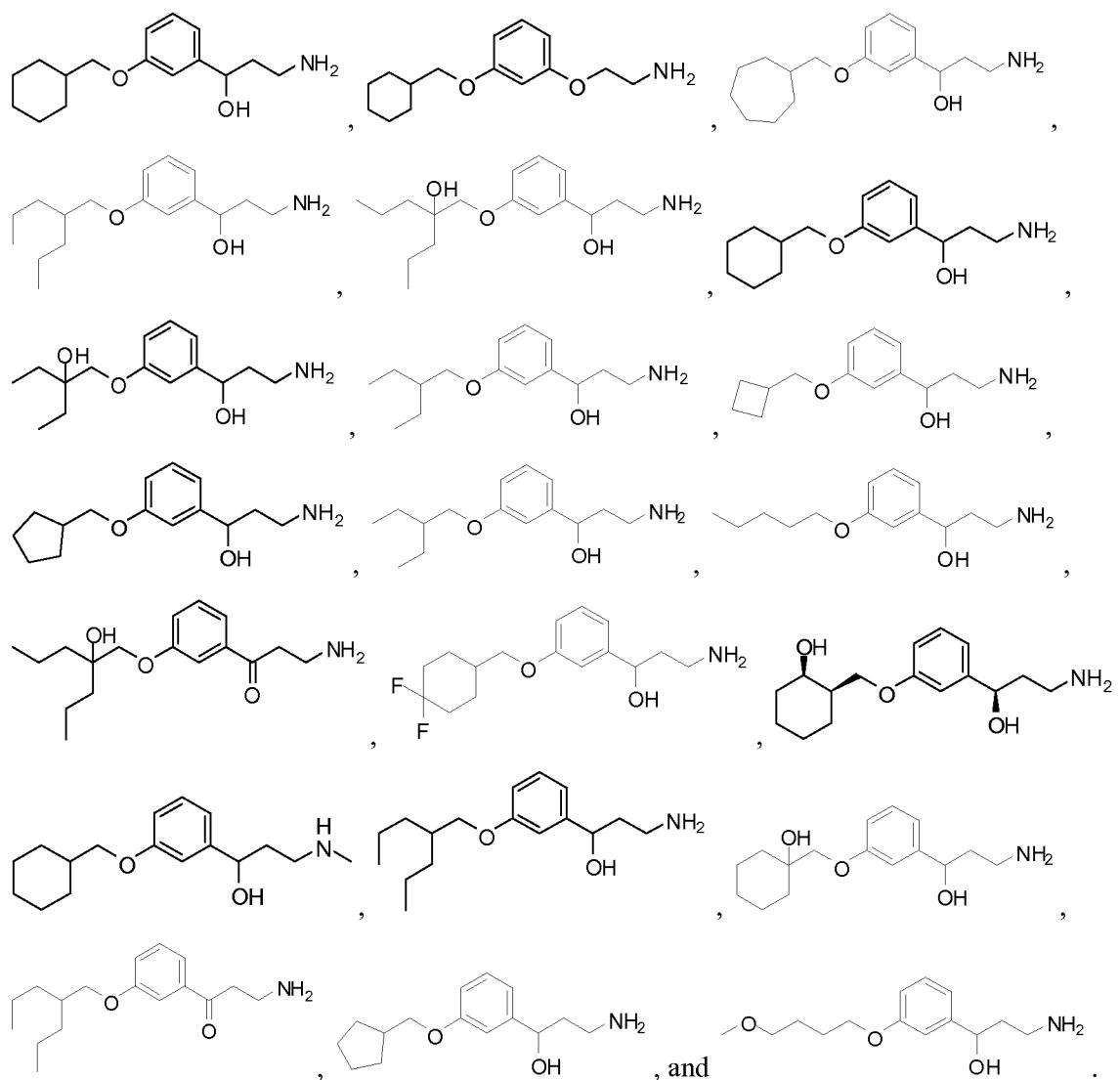
**[0039]** Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient, wherein the composition comprises a compound, or stereoisomer, geometric isomer, N-oxide or a pharmaceutically acceptable salt thereof, selected from:



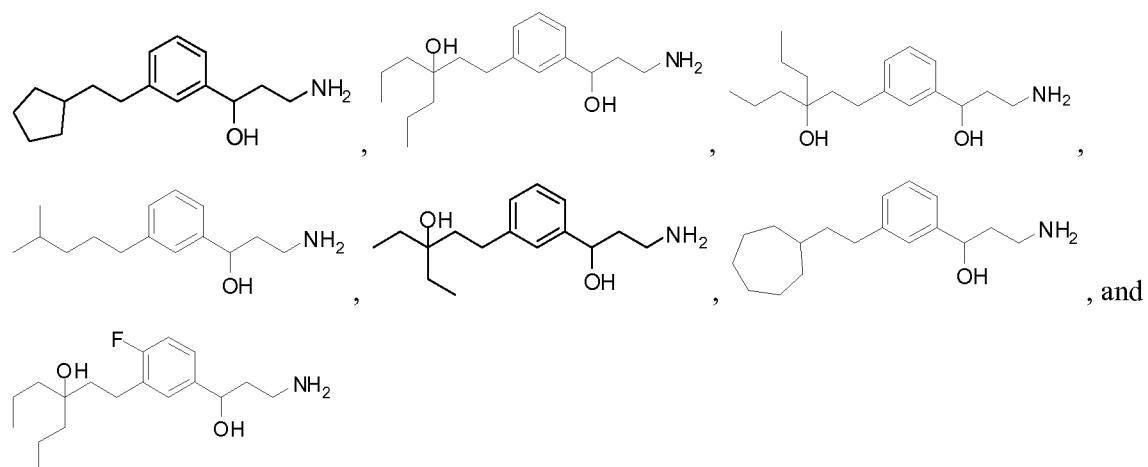
**[0040]** Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein the composition comprises a compound, or stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, selected from:



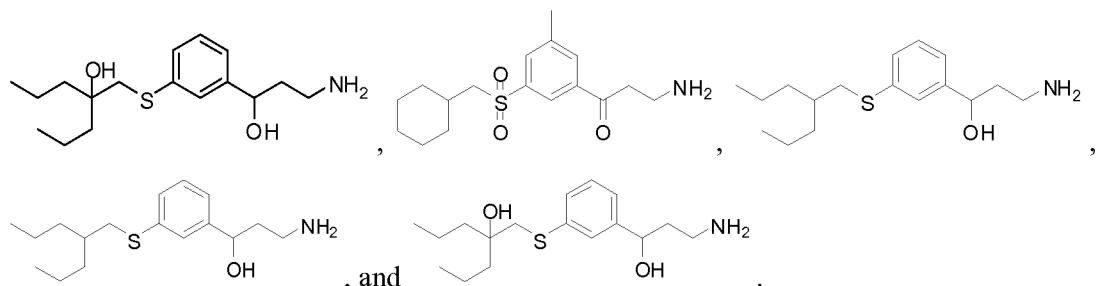
**[0041]** Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein the composition comprises a compound, or tautomer, stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, selected from:



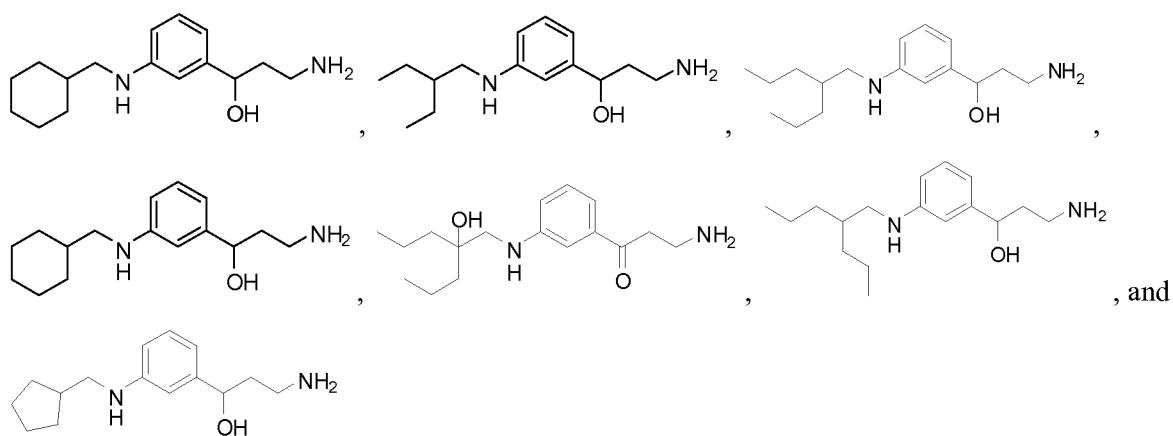
**[0042]** Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein the composition comprises a compound, or stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, selected from:



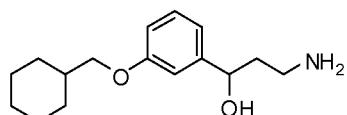
[0043] Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein the composition comprises a compound, or tautomer, stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, selected from:



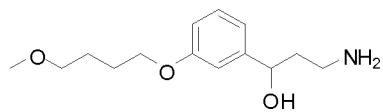
[0044] Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein the composition comprises a compound, or tautomer, stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, selected from:



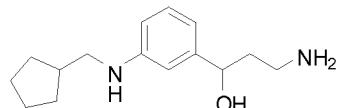
[0045] Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein the composition comprises a compound, or stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, having the structure:



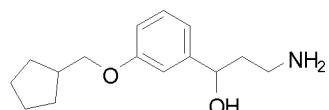
[0046] Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein the composition comprises a compound, stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, having the structure:



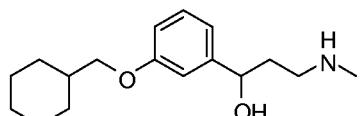
**[0047]** Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein the composition comprises a compound, or stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, having the structure:



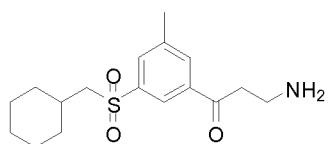
**[0048]** Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein the composition comprises a compound, or stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, having the structure:



**[0049]** Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein the composition comprises a compound, or stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, having the structure:

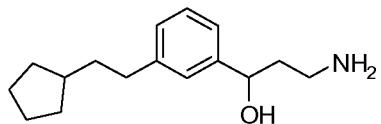


**[0050]** Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein the composition comprises a compound, or stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, having the structure:



**[0051]** Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient

wherein the composition comprises a compound, or stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, having the structure:



**[0052]** Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein the composition is administered to the patient orally. Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient, wherein the composition is administered once per day. Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient, wherein treatment results in improvement of central vision in the patient.

**[0053]** Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient further comprising administering one or more additional therapeutic regimens. Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient; treating or preventing retinopathy of prematurity in a patient; or treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein said one or more therapeutic regimens is laser therapy, cryotherapy, fluorescein angiography, vitrectomy, corticosteroids, anti-vascular endothelial growth factor (VEGF) treatment, vitrectomy for persistent diffuse diabetic macular edema, pharmacologic vitreolysis in the management of diabetic retinopathy, fibrates, renin-angiotensin system (ras) blockers, peroxisome proliferator-activated receptor gamma agonists, Anti-Protein Kinase C (PKC), islet cell transplantation, therapeutic oligonucleotides, growth hormone and insulin growth factor (IGF), control of systemic factors or a combination thereof.

**[0054]** Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient wherein the ophthalmic disease or disorder associated with diabetes is diabetic retinopathy. Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient wherein the ophthalmic disease or disorder associated with diabetes is non-proliferative diabetic retinopathy. Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient wherein the ophthalmic disease or disorder associated with diabetes is proliferative diabetic retinopathy. Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient wherein the

ophthalmic disease or disorder associated with diabetes is diabetic maculopathy. Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient wherein the ophthalmic disease or disorder associated with diabetes is diabetic macular edema. Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient wherein the ophthalmic disease or disorder associated with diabetes is neovascular glaucoma. Another embodiment provides the method for treating an ophthalmic disease or disorder associated with diabetes in a patient wherein the ophthalmic disease or disorder associated with diabetes is macular ischemia.

#### **INCORPORATION BY REFERENCE**

**[0055]** All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

**[0056]** The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

**[0057]** **Figure 1** is a graph depicting the timeline for Groups 1-3 as described in Example 3.

**[0058]** **Figure 2** is a graph depicting the timeline for Group 4 as described in Example 3.

**[0059]** **Figure 3** is a graph depicting the timeline for Groups 5-6 as described in Example 3.

**[0060]** **Figure 4A** depicts the Visual Cycle, which shows the biochemical conversion of visually active retinoids in the retina. **Figure 4B** illustrates a possible means of action of ACU-4429.

**[0061]** **Figure 5** is a graph depicting ACU-4429 Phase 1a data of mean oral pharmacokinetic (PK) profiles.

**[0062]** **Figure 6** is a graph depicting ACU-4429 Phase 1a Rod ERG Suppression.

**[0063]** **Figure 7** is a graph depicting Phase 1b PK Data.

**[0064]** **Figure 8** provides the timeline for an experiment to test if ACU-4935 reduced VEGF up-regulation caused by hypoxic conditions.

**[0065]** **Figure 9** is a graph illustrating VEGF Protein Expression caused by hypoxic conditions after treatment with ACU-4935.

**[0066]** **Figure 10** is a graph illustrating VEGF mRNA levels caused by hypoxic conditions after treatment with ACU-4935.

**[0067]** **Figure 11:** Mean Concentration Time Profiles for Blood or Plasma (**Figure 11A**) or in Eye Tissue (**Figure 11B**).

**[0068]** **Figure 12:** Metabolite radioprofiles at 4 hours post-dose on day 7 as described in Example 10.

**Figure 12A** provides the results of G4 M Day 8 4H Plasma. **Figure 12B** provides the results of G3 M 4H Retinal Pigmented Epithelium.

[0069] **Figure 13** is a graph illustrating mean cumulative percentage of radioactive dose recovered as described in Example 10.

[0070] **Figure 14:** Visual cycle modulators (VCMs), such as ACU-4420 and ACU-4935, inhibit the visual cycle isomerase, thereby mimicking a state of constitutive phototransduction and decreasing the dark current.

[0071] **Figure 15:** Illustrates the protocol for treatment of 129 SvE mouse pups (PO) with ACU-4420 and ACU-4935.

[0072] **Figures 16A-16B** demonstrate that VCMs inhibit neovascularization. **Figure 16A** depicts isolectin staining of flatmount preparations of retina. Neovascular areas are outlined in red. **Figure 16B** is a histogram comparing % neovascularization in the various treatment groups. **Figures 16C-16F** demonstrate that ACU-4429 inhibited neovascularization and 11-cis-RAL in a dose-dependent manner. **Figures 16C and 16D** show that ACU-4429 decreased 11-cis-RAL concentrations in eyes and, therefore, visual cycle isomerase activity in a dose dependent manner (ED50 0.88 mg/kg). The difference between ACU-4429 and vehicle was statistically significant ( $P < 0.01$ ). **Figures 16E and 16F** show neovascularization in left eyes (measured in isolectin-stained flatmount preparations) decreased in a dose-dependent manner with ACU-4429; this decrease is significant at 3.0 and 10.0 mg/kg, by 1-way-ANOVA comparison of vehicle (water) at 21% O<sub>2</sub>, vehicle (water) at 75% O<sub>2</sub>, and ACU-4429 treatments.

[0073] **Figure 17** is a diagram of the neural retina and its vascular supplies (not to scale). The layers of the neural retina (ganglion cell, inner plexiform, inner nuclear, outer plexiform, outer nuclear) are indicated. Blood flow through the choroidal vessels is swift. The retinal vasculature, visible by ophthalmoscopy, lies among the ganglion cells on the vitreal surface of the retina and extends capillary networks deep into the post-receptor layers. The caliber of the retinal arterioles adjusts to perturbations in blood oxygen levels (“autoregulation”).

[0074] **Figure 18** illustrates logistic growth curve showing human rhodopsin content (Fulton *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (1999) 40: 1878-1883) as a function of age. The arrow indicates the age of ROP onset in preterm infants (Palmer *et al.* *Ophthalmology*, (1991) 98:1628-1640).

[0075] **Figure 19** is a rat model of retinopathy of prematurity. (a) Scanning laser ophthalmoscope (SLO) images obtained using blue (488 nm) laser stimulation (Seeliger *et al.*, *Vision Res.*, (2005) 45: 3512-9) after injection of fluorescein in 22 day old control and ROP rats. (Pigmented rats are used to facilitate SLO imaging.) The integrated curvature of each retinal arteriole is expressed as a proportion of the mean (ICA) in the control. The higher ICA value for the ROP rat reflects the greater tortuosity of its arterioles. The choroidal appearance is similar in the control and ROP fundi. (b) Sample electroretinographic (ERG) responses to full-field stimuli in control and ROP rats. Both rats are tested with the same flash intensities, as indicated. The vertical grey lines indicate the time at which the flash is presented.

[0076] **Figure 20** illustrates features of the experimental paradigm. The ambient oxygen and light cycle were tightly controlled and synchronized. Dosing with the VCM is designed to target the rapid growth

phase of the developmental increase in rhodopsin in the retina (arrows). Area in dashed line box indicate the three test windows.

[0077] **Figure 21** provides pictures of H&E staining of paraffin sections (from example 7, chronic light induce CNV). The outer nuclear layer is thinnest in sections from eyes of animals treated with light and vehicle.

[0078] **Figure 22** is a graph depicting the number of rows of nuclei in the outer nuclear layer in H&E sections from animals treated with ambient light and 3000 lux plus vehicle or ACU-4429. Data are mean  $\pm$  SEM.

[0079] **Figure 23** is a graph depicting number of vessels crossing layers/sections.

#### DETAILED DESCRIPTION OF THE INVENTION

[0080] The present disclosure relates to methods for treating diabetic retinopathy. As used herein, “Diabetic retinopathy” refers to changes in the retina due to the micro vascular changes seen in diabetes. The blood vessels that supply oxygen to the retina of the eye are damaged due to long-term high levels of blood sugar (hyperglycemia). The disease generally develops slowly over a period of months but over time, diabetic retinopathy can get worse and cause vision loss. Diabetic retinopathy usually affects both eyes. Diabetic retinopathy progresses from mild non-proliferative abnormalities, characterized by increased vascular permeability, to moderate and severe non-proliferative diabetic retinopathy (NPDR), characterized by vascular closure, to proliferative diabetic retinopathy (PDR), characterized by the growth of new blood vessels on the retina and posterior surface of the vitreous. Macular edema, characterized by retinal thickening from leaky blood vessels, can develop at all stages of retinopathy. Furthermore conditions such as pregnancy, puberty, blood glucose control, hypertension, and cataract surgery can accelerate these changes.

[0081] Non-proliferative diabetic retinopathy, proliferative diabetic retinopathy and diabetic maculopathy are the three main types of diabetic retinopathy.

[0082] **Non-Proliferative Diabetic Retinopathy (NPDR)** is considered as the early stage of retinopathy and is the most common seen in diabetics. The tiny blood vessels in the retina are only mildly affected, but may form bulges (micro aneurysms) and connections with each other (intraretinal micro vascular anomalies) and/or leak fluid (edema), protein deposits (exudates) and blood (hemorrhage). Another typical sign of non-proliferative diabetic retinopathy (NPDR) is the presence of puffy white patches on the retina (cotton wool spots). These changes can occur anywhere throughout the retina, including the macula.

[0083] There are three stages of non-proliferative diabetic retinopathy which are detailed below:

[0084] **(1) Mild Non-proliferative Diabetic Retinopathy:** At this earliest stage, at least one micro aneurysm may occur. Micro aneurysms are small areas of balloon-like swelling in the retina's blood vessels.

[0085] **(2) Moderate Non-proliferative Diabetic Retinopathy:** As the disease progresses, some blood vessels that nourish the retina are blocked.

**[0086] (3) Severe Non-proliferative Diabetic Retinopathy:** Many more blood vessels are blocked, depriving several areas of the retina of blood supply. These areas of the retina send signals to the body to grow new blood vessels for nourishment.

**[0087]** Non-proliferative diabetic retinopathy should not cause any problems to the patient, as the vision remains normal as long as the macula is not affected. However, as the symptoms of diabetic retinopathy are generally not visible in this stage, it is recommended that regular retinal screening eye tests should be done to monitor the signs of progression to more serious stages of retinopathy.

**[0088] Proliferative Diabetic Retinopathy (PDR):** This stage comes after severe non-proliferative diabetic retinopathy and is characterized by the growth of abnormal new blood vessels in the eye. When the diabetes causes the blood vessels to become blocked, parts of the eye and retina develop ischemia, as they become starved of oxygen and nutrients. The eye tries to respond to this condition, by growing a new blood supply to the oxygen starved areas. Unfortunately, fragile new blood vessels that bleed easily are formed instead. This process is called neo-vascularization. These abnormal new blood vessels grow in the wrong place on the surface of the retina and into the vitreous gel. Vitreous hemorrhage occurs when these new blood vessels bleed into the vitreous cavity. The blood blocks light that enters the eye from reaching the retina. The amount of sight loss can be mild to severe, and depends on how much blood is in the eye. The vision might slowly improve as the hemorrhage gradually clears over several months.

**[0089]** Abnormal new vessels also cause the formation of scar tissue which pulls on the retina and may result in tractional retinal detachment. The retinal detachment can affect any part of the retina. If it affects the macula, the patient might lose his/her central vision and it can be treated only with surgery.

**[0090] Diabetic Maculopathy:** Diabetic maculopathy is the most common cause of visual loss in diabetes. It occurs when the macula becomes affected by the retinopathy changes caused by diabetes. The macula is located at the center of the retina and is important for central vision and for seeing fine details clearly. Therefore, the central vision and ability to see detail will be affected in the patients that develop diabetic maculopathy. For instance, the affected individuals might find it difficult to recognize faces in the distance or to read small prints. The amount of sight loss may be mild to severe. However, even in the worst cases, the peripheral (side) vision that allows the individual to get around at home and outside will remain unaffected.

**[0091]** Diabetic retinopathy (DR) is an ocular disorder characterized by excessive angiogenesis that develops in diabetes due to thickening of capillary basement membranes, and lack of contact between pericytes and endothelial cells of the capillaries. Loss of pericytes increases leakage of the capillaries and leads to breakdown of the blood-retina barrier. Diabetic retinopathy is the result of microvascular retinal changes. Hyperglycemia-induced pericyte death and thickening of the basement membrane lead to incompetence of the vascular walls. These damages change the formation of the blood-retinal barrier and also make the retinal blood vessels become more permeable. Small blood vessels – such as those in the eye – are especially vulnerable to poor blood sugar (blood glucose) control. An over-accumulation of glucose and/or fructose damages the tiny blood vessels in the retina. Macular edema can also develop

when the damaged blood vessels leak fluid and lipids onto the macula. These fluids make the macula swell, which blurs vision. This damage also results in a lack of oxygen at the retina.

[0092] As the disease progresses, the lack of oxygen in the retina stimulates angiogenesis along the retina and in the clear, gel-like vitreous humor that fills the inside of the eye. Without timely treatment, these new blood vessels can bleed, cloud vision, and destroy the retina. Fibrovascular proliferation can also cause tractional retinal detachment. The new blood vessels can also grow into the angle of the anterior chamber of the eye and cause neovascular glaucoma.

[0093] Vision loss from diabetic maculopathy occurs in 2 ways.

[0094] **Diabetic macular edema** (DME) is the swelling and thickening of the macula. This is due to fluid leakage from the retinal blood vessels in the macula. The vision becomes blurry because the structure and function of the macular photoreceptor cells becomes disrupted. Vision loss from macular edema can be controlled with laser and injections into the eyeball.

[0095] Macular ischemia occurs when the tiny retinal blood vessels (capillaries) to the macula close up. The vision becomes blurry because the macula does not receive enough blood supply for it to work properly. Unfortunately, there are no effective treatments for macular ischemia. Macular edema is due to leakage of fluid from the retinal blood vessels. Hard exudates are the yellowish deposits seen on the retina. They are caused by leakage of protein material.

[0096] The following medical conditions are some of the possible causes of diabetic retinopathy.

[0097] Diabetes: Prolonged hyperglycemia (high blood glucose levels) affects the anatomy and function of retinal capillaries. The excess glucose is converted into sorbitol when it is diverted to alternative metabolic pathways. Sorbitol leads to death or dysfunction of the pericytes of the retinal capillaries. This weakens the capillary walls allowing for the formation of micro aneurysms, which are the earliest signs of diabetic retinopathy. The weak capillary walls can also be responsible for increased permeability and the exudates. Due to the predisposition to increased platelet aggregation and adhesion (blood clot formation) as a result of diabetes, the capillary circulation becomes sluggish or even totally impaired by an occlusion. This can also contribute to the development of diabetic retinopathy.

[0098] Type 1 and Type 2 diabetes: Individuals diagnosed with type 1 diabetes, are considered insulin-dependent as they require injections or other medications to supply the insulin that the body is unable to produce on its own. Due to lack of insulin the blood sugar is unregulated and levels are too high. Individuals with type 2 diabetes are considered non-insulin-dependent or insulin-resistant. The individuals affected with this type of diabetes, produce enough insulin but the body is unable to make proper use of it. The body then compensates by producing even more insulin, which can cause an accompanying abnormal increase in blood sugar levels. All people with Type I diabetes (juvenile onset) and with Type II diabetes (adult onset) are at risk of developing diabetic retinopathy. However, people with Type 1 diabetes are more likely to cause retinopathy compared to type 2 diabetes.

[0099] Diabetes mellitus type 1 and Diabetes mellitus type 2: People with Diabetes mellitus type 1 and type 2 are at increased risk of developing diabetic retinopathy.

**[00100]** Excessive alcohol: Alcohol if used to extreme reduces Vitamin B12 and thiamine levels. However, alcohol consumption alone is not associated with diabetic retinopathy, the consumption of empty calories from alcohol makes adhering to a calorie-restricted diabetic diet very difficult and it is unclear that what effect moderate alcohol has on retinopathy.

**[00101]** Hypertension and other vascular risk factors such as obesity and dyslipidaemia can influence the onset and progression of retinopathy.

**[00102]** High cholesterol: Cholesterol can exacerbate retinopathy by hardening of large artery blood vessels and can cause damage to the small blood vessels of the eye.

**[00103]** Renal disease, as evidenced by proteinuria and elevated urea/creatinine levels, is an excellent predictor of the presence of retinopathy.

**[00104]** Pregnancy: It can exacerbate existing retinopathy though probably not cause it directly. Women with diabetes have a slightly higher risk during pregnancy. It is recommended that all pregnant women with diabetes have dilated eye examinations each trimester to protect their vision.

**[00105]** Kidney impairment: Associated with diabetic retinopathy, though it appears that diabetic retinopathy leads to kidney impairment rather than *vice versa*.

**[00106]** Chromosome 15q deletion: A rare chromosomal disorder involving deletion of genetic material from the long arm of chromosome 15.

**[00107]** It is thought that intraocular surgery may possibly increase the risk of progression of diabetic retinopathy.

**[00108]** There are often no symptoms in the earliest stages of non-proliferative diabetic retinopathy. The signs and symptoms of diabetic retinopathy are commonly presented as the disease progresses toward advanced or proliferative diabetic retinopathy. The diagnostic signs of diabetic retinopathy include one or more of the following: changes in the blood vessels; retinal swelling (macular edema); pale deposits on the retina; damaged nerve tissue; visual appearance of leaking blood vessels; loss of central or peripheral vision; temporary or permanent vision loss; development of a scotoma or shadow in the field of view; spotty, blurry, hazy or double vision; eye pain; near vision problems unrelated to presbyopia; spots or dark strings floating in the vision (floaters); impaired color vision; vision loss; a dark or blind spot in the central vision; poor or reduced night vision; venous dilation and intraretinal micro vascular abnormalities; in the advanced stage of retinopathy tiny blood vessels grow along the retina, in the clear, gel-like vitreous humor that fills the inside of the eye; nerve damage (neuropathy) affecting ocular muscles that control eye movements; involuntary eye movement (nystagmus); fluctuating and progressive deterioration of vision; macular edema; macular ischemia; traction retinal detachment; sudden, severe painless vision loss; increased vascular permeability, leading to edema; endothelial cell proliferation; flashes of light (photopsias) or defects in the field of vision; presence of abnormal blood vessels on the iris (rubeosis or nvi), cataract (associated with diabetes) and vitreous cells (blood in the vitreous or pigmented cells if there is a retinal detachment with hole formation); micro aneurysms - physical weakening of the capillary walls which predisposes them to leakages; hard exudates - precipitates of lipoproteins/other proteins leaking from retinal blood vessels; haemorrhages - rupture of weakened capillaries, appearing as small dots/larger blots or 'flame'

haemorrhages that track along nerve-fiber bundles in superficial retinal layers (the haemorrhage arises from larger and more superficial arterioles); cotton wool spots - build-up of axonal debris due to poor axonal metabolism at the margins of ischaemic infarcts; and neo-vascularization - an attempt (by residual healthy retina) to revascularize hypoxic retinal tissue.

**[00109]** The present disclosure also relates to the methods of using visual cycle modulation (VCM) compounds to treat retinopathy of prematurity (ROP). The work described herein provides the first demonstration of an effect of systemic treatment with a non-retinoid VCM on a retinopathy in an immature eye. One key element of this process is a high O<sub>2</sub> content when subjects are new-born is the key element. Premature infants are put into a high oxygen atmosphere to support the immature lung function where the high oxygen concentration suppresses the normal development of retinal vasculature. When the infant is returned to normal air, the retina becomes ischemic due to the under developed vasculature. The ischemia triggers VEGF expression and neo-vascularization. *See*, for example, Figure 4B. VCMs work by increasing apo-rhodopsin that reduces the dark current and hence oxygen consumption.

**[00110]** Described herein are VCM compounds for the treatment or prevention of diseases or disorders of the retina, and particularly, VCM compounds for the treatment or prevention of retinal diseases or disorders related to or involving vascular abnormalities, such as, for example, ROP. The methods described herein relate to the administration of the VCM compounds that modulate the visual cycle.

**[00111]** As a system, the mammalian retina is subject to diseases that affect the balanced interconnection of the neural retina and the vasculature that nourishes it; visual loss occurs when this balance is disturbed. Diseases such as photoreceptor degenerations that primarily affect the neural retina also affect the retinal vasculature. Diseases that are clinically characterized by abnormality in the choroidal or retinal vasculature, such as ROP, also affect the retinal neurons. These conditions all involve hypoxic ischemic disorders of neural tissue. Photoreceptors are specialized cells that have the highest oxygen requirements of any cell in the body (Steinberg, R., *Invest. Ophthalmol. Vis. Sci.*, (1987) 28: 1888-1903), which plays a role in all hypoxic ischemic diseases of the retina.

**[00112]** In normal development, as the rod photoreceptors differentiate and begin to produce rhodopsin (the molecule responsible for the capture of light); their extraordinarily high oxygen demands render the retina hypoxic, driving the growth of the retinal blood vessels. However, in ROP, supplemental oxygen administered for the acute cardiopulmonary care of the prematurely born infant renders the retina hyperoxic, interrupting normal vascular growth and leaving the peripheral retina avascular. Upon cessation of the supplemental oxygen, the peripheral retina becomes hypoxic. Hypoxia instigates a molecular cascade that leads to the formation of the abnormal retinal blood vessels that are clinically used to diagnose ROP. Even though a premature infant is subjected to high ambient oxygen, immature lungs and other medical complications often lead to fluctuations in blood oxygen and, consequently, to episodes of both hypoxia and hyperoxia at the retina which affect the sensitive photoreceptors. The developing neural retina and its vasculature are under cooperative molecular control, and the vascular abnormalities of ROP are related to the function of the neural retina. Recent studies have found that the

degree of dysfunction of the rods in ROP helps predict the degree of abnormality observed in the retinal vasculature, but the degree of abnormality observed in the retinal vasculature may not help predict the degree of dysfunction of the rods in ROP. Thus, the rods cause ROP.

[00113] As used herein, an “immature retina” refers to a retina of a preterm infant or a retina of similar morphology/function to that of a pre-term infant retina. An immature retina can be characterized by the presence of poorly developed or disorganized blood vessels with or without the presence of scar tissue. In general, a human preterm infant is one born at 37 weeks gestation, or earlier. Conversely, the term “retinal maturity” refers to a retina of a full-term infant or a retina of similar morphology/function to that of a full-term infant.

[00114] As used herein, the phrases “reduces rod energy demand” or “suppresses rod energy demand” refer to a reduction in oxygen demand of a rod cell of at least 10%; preferably the reduction of oxygen demand of a rod cell is at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or more. In general, it is preferred that the oxygen demand of a rod cell is maintained below the level necessary to induce pathological angiogenesis (*i.e.*, blood vessel growth) or vascular abnormalities.

[00115] As used herein, the term “vascular abnormalities” is used to refer to an abnormal or pathological level of vascular blood vessel growth (*e.g.*, angiogenesis) or morphology (*e.g.*, tortuosity) that does not permit proper development of the retina to “retinal maturity” as that term is used herein. One of skill in the art can titrate the amount of agent administered or the timing of administration to maintain the growth and morphology of blood vessels below that of pathological blood vessel growth as assessed by, for example, Laser Doppler Blood Flow analysis. In an alternative embodiment, the level of tortuosity of retinal blood vessels is used to assess the degree of pathological blood vessel morphology and/or growth. Methods for measuring tortuosity are further described herein.

[00116] As used herein, the term “supplemental oxygen” refers to a concentration of oxygen above that of ambient air (*i.e.*, about 20-21%) that is necessary to maintain blood oxygen levels in a subject at a desired level. In general, supplemental oxygen is supplied in a clinical setting to maintain a blood oxygen level of 100% as assessed using, for example, transcutaneous oxygen monitoring. Monitoring blood oxygen levels and altering the level of “supplemental oxygen” to maintain, for example, a 100% blood oxygen level is a standard procedure in a clinical setting (*e.g.*, a neonatal intensive care unit) and is well known to those of skill in the art of medicine.

### **Vascular and Neural Diseases of the Retina**

[00117] Despite advancements in the medical management of neovascular diseases of the retina, such as retinopathy of prematurity (ROP), retinal neurovascular diseases remain the leading cause of blindness worldwide.

[00118] For ROP, current treatment is photocoagulation of the peripheral vasculature, which carries its own negative consequences, and experimental approaches such as treatment with anti-angiogenic pharmaceuticals, that have unknown efficacy. Because rod photoreceptors are unique to the eye and have among the highest oxygen requirements of any cell in the body, they may play a role in hypoxic ischemic

neovascular retinal diseases (Arden *et al.*, *Br J Ophthalmol* (2005) 89:764; and Fulton *et al.*, *Doc Ophthalmol*, (2009) 118(1):55-61). Rat models of ROP provide an *in vivo* system in which the relation of the photoreceptors to the retinal vasculature can be studied and manipulated.

**[00119]** Abnormal retinal function is a feature of neovascular retinal diseases. (Fulton *et al.*, *Doc Ophthalmol*, (2009) 118(1):55-61). Vision loss in neovascular retinal disease results from blood vessel abnormalities and the severity of lifelong retinal dysfunction that persists after the blood vessel abnormalities resolve is related to the severity of the antecedent vascular disease (Fulton *et al.*, *Arch Ophthalmol* (2001) 119:499). Data from rat models of ROP, however, show that dysfunction of the rod photoreceptors precedes the vascular abnormalities by which ROP is conventionally defined and predicts their severity (Reynaud, and Dorey, *Invest Ophthalmol Vis Sci* (1994) 35:3169; Akula, *Invest Ophthalmol Vis Sci* (2007) 48: 4351). Abnormalities in vascular morphology are the main diagnostic criterion of ROP; however, ROP is mainly a disorder of the neural retina with secondary vascular abnormalities. The appearance of the vascular abnormalities that characterize acute ROP is coincident with developmental elongation of the rod photoreceptors' outer segments and accompanying increase in the retinal content of rhodopsin (Lutty *et al.*, *Mol Vis* (2006) 12: 532; and Dembinska *et al.*, *Invest Ophthalmol Vis Sci* (2002) 43:2481).

### **Rod Cell Physiology and Metabolism**

**[00120]** The rods perform three linked, metabolically demanding processes: generation of the dark current, maintenance of the visual pigment (the visual cycle), and outer segment turnover, all of which ensue concomitant to developmental elongation of the rod outer segments (ROS) and increase of the rhodopsin content of the eye. The signal transduction mechanism of the rods is physiologically unique. In darkness, sodium and other cations intromitted through cyclic guanosine monophosphate (cGMP) gated channels in the ROS are expelled by pumps in the rod inner segment (RIS) so rapidly that a volume equal to the entire cytosol is circulated every half minute (Hagins, *et al.*, *Proc Natl Acad Sci USA* (1989) 86:1224). The molecular cascade initiated by photon capture by rhodopsin following a flash of light and leading to a reduction of cGMP leads the dark current to decay following the form of a delayed Gaussian that can be described by an intrinsic amplification constant, A (Lamb and EPugh, *J Physiol* (1992) 449: 719; and Pugh and Lamb, *Biochem Biophys Acta* (1993) 1141:111).

**[00121]** Following photon capture, rhodopsin's chromophore (retinol) undergoes an isomeric change which frees it from opsin and initiates phototransduction. Spent chromophore is passed from the ROS to the retinal pigment epithelium (RPE) where it undergoes a series of transformations before being returned to the ROS through the apical processes of the RPE as retinol again. There it becomes covalently linked to its active-site lysine in opsin, becoming rhodopsin again and completing the visual cycle (R. R. Rando, *Chem Rev* (2001) 101:1881). The rate-limiting step in the visual cycle mediated by the isomerohydrolase enzyme complex, RPE65 (Moiseyev *et al.*, *Proc Natl Acad Sci USA* (2005) 102:12413). Other byproducts of photo-transduction in the ROS are expelled through a process of circadian shedding of the ROS tips; each RPE cell phagocytizes thousands of disks shed from 30-50 embedded rods each day (R. W. Young, *J Cell Biol* (1967) 33:61). Controlled down-regulation of the

visual cycle through targeted inhibition of RPE65 activity lowers the flux of retinoids through the ROS/RPE complex; this would render the rods less vulnerable to insult from hyperoxia and hypoxia (Wellard *et al.*, *Vis Neurosci* (2005) 22:501) by reducing their metabolic demands. It may also slow phagocytosis and thus lengthen the rod outer segments.

### Translation from Animal Models to Patients

[00122] Photoreceptors are nestled closely to the choroidal vasculature. Highly organized post-receptor retinal neurons form layers that are supplied by the retinal vessels. Although the choroid is the principal supply to the photoreceptors, degeneration of the photoreceptors is, nonetheless, associated with attenuation of the retinal arterioles (Hansen *et al.*, *Vision Research*, 48(3):325-31 (2008)). Because the photoreceptor layer is such an extraordinary oxygen sink, while not wishing to be bound by theory, it is presumed that, as photoreceptors degenerate, their metabolic demands wane and the retinal vasculature becomes attenuated consequent to the neural retina's chronic lower requirement for oxygen (Hansen *et al.*, *Vision Research*, 48(3):325-31 (2008)).

[00123] A tight link between the photoreceptors and the retinal vascular network is evident in the developing retina. Post-receptor cells differentiate before the photoreceptors, which are the last retinal cells to mature. As the formation of rod outer segments advances in a posterior to peripheral gradient, so too does vascular coverage. Thus, concurrent and cooperative development of the neural and vascular components characterizes normal retinal maturation. In preterm infants, the age of onset of ROP is around the age of rapid developmental increase in rod outer segment length and consequent increase in rhodopsin content. In addition to immature photoreceptors and retinal vasculature, the preterm infant has immature lungs that create a precarious respiratory status with attendant risk of hypoxic injury to immature cells. Clinically, this is countered by administration of supplemental oxygen, but both high and low oxygen levels are known to injure the immature photoreceptors (Fulton *et al.* *Invest. Ophthalmol. Vis. Sci.*, (1999) 40: 168-174; and Wellard *et al.*, *Vis. Neurosci.*, (2005) 22: 501-507).

[00124] Rat models of ROP are induced by rearing pups in habitats with alternating periods of relatively high and low oxygen during the critical period of rod outer segment elongation (Akula *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2007) 48: 4351-9; Akula *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2007) 48: 5788-97; Dembinska *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2001) 42: 1111-1118; Liu *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2006) 47: 5447-52; Liu *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2006) 47: 2639-47; Penn *et al.*, *Invest. Ophthalmol. Vis. Sci.*, 1995. 36: 2063-2070). Following induction, abnormalities of the retinal vasculature ensue, as do abnormalities of the structure and function of the neural retina (Fulton *et al.* *Invest. Ophthalmol. Vis. Sci.*, (1999) 40: 168-174; Akula *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2007) 48: 4351-9; Akula *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2007) 48: 5788-97; Dembinska *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2001) 42: 1111-1118; Liu *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2006) 47: 5447-52; Liu *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2006) 47: 2639-47; Reynaud *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (1995) 36:2071-2079). The abnormalities in the morphology of the retinal vasculature and in the function of the neural retina in ROP rats are similar to those found in pediatric ROP patients (Dembinska *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2001) 42: 1111-1118; Liu *et al.*, *Invest. Ophthalmol. Vis. Sci.*,

(2006) 47: 5447-52; Liu *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2006) 47: 2639-47; Reynaud *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (1995) 36:2071-2079; Barnaby, A. M., *Invest. Ophthalmol. Vis. Sci.*, (2007). 48:4854-60; Fulton *et al.*, *Arch. Ophthalmol.*, (2001)119: 499-505; Gelman, R., *Invest. Ophthalmol. Vis. Sci.*, (2005) 46(12): 4734-4738; Moskowitz *et al.*, *Optometry & Vision Science*, (2005) 82: 307-317; Fulton, A. B., *Invest. Ophthalmol. Vis. Sci.*, 49(2):814-9 (20089)). Thus, rat models can be extrapolated to human treatment.

**[00125]** Albino rat models of ROP are used to study the neural and vascular characteristics of the retina during development (Akula *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2007) 48: 4351-9; Akula *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2007) 48: 5788-97; Liu, K., *Invest. Ophthalmol. Vis. Sci.*, (2006) 47: 5447-52; Liu *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2006) 47: 2639-47). Different schedules of oxygen exposure induce a range of effects on the retinal vasculature and the neural retina that model the gamut of retinopathy, mild to severe, observed in human ROP cases. The oxygen exposures are timed to impact the retina during the ages when the rod outer segments are elongating and the rhodopsin content of the retina is increasing. Longitudinal measures of electroretinographic (ERG) responses and retinal vascular features are obtained in infant (about 20 day old), adolescent (about 30 day old), and adult (about 60 day old) rats.

#### **Assessment of Neural Function**

**[00126]** ERG is used to characterize neural function. ERG responses to full-field stimuli over a range of intensities are recorded from the dark-adapted animal as previously described in detail (Akula *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2007) 48: 4351-9). To summarize rod photoreceptor activity, a model of the activation of phototransduction is fit to the a-waves and the resulting sensitivity (SROD) and saturated amplitude (RROD) parameters are calculated. Post-receptor activity is represented by the b-wave. The stimulus/response functions are summarized by the saturated amplitude (Vmax) and the stimulus producing a half-maximum response (log s); these parameters are derived from the Michaelis-Menten function fit to the b-wave amplitudes (Hood Birch, *Invest. Ophthalmol. Vis. Sci.*, (1994) 35: 2948-2961; Lamb, and Pugh, *J. Physiol. (Lond)*. (1992) 449: 719-758; Pugh. and Lamb, *Biochim. Biophys. Acta*, 1993. 1141: 111-149; Pugh and Lamb, in *Handbook of biological physics*. Volume 3 (2000), Elsevier Science. p. 183-255; Akula *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2007) 48: 4351-9).

#### **Assessment of Vascular Characteristics**

**[00127]** Retinal vascular parameters are derived using image analysis software and may be applied to digital fundus photographs (Akula *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2007) 48: 4351-9; Martinez-Perez, M. E., (2001), Imperial College: London; Martinez-Perez *et al.*, *Trans. Biomed. Eng.*, (2002) 49: 912-917). Integrated curvature (IC), which agrees well with subjective assessment of vascular tortuosity reported by experienced clinicians, may be used to specify the vascular status of each fundus (Gelman, R. M. *Invest. Ophthalmol. Vis. Sci.*, (2005) 46(12): 4734-4738). Both arterioles and venules are significantly affected by ROP. It has been found, however, that the arterioles are markedly affected while the venules are less so; therefore, the arteriolar parameter ICA is used in the analyses described herein (Akula *et al.*, *Ophthalmol. Vis. Sci.*, (2007) 48: 4351-9; Liu *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2006) 47: 5447-52;

Liu *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2006) 47: 2639-47; Gelman, R., M. *Invest. Ophthalmol. Vis. Sci.*, (2005) 46(12): 4734-4738).

### **Relation of Retinal Sensitivity and Vasculature**

[00128] Rod photoreceptor sensitivity (SROD) at a young age (20 days) is used to predict retinal vascular outcome as specified by ICA. Better sensitivity at an early age is associated with better (less tortuous) vascular outcome (Akula *et al.*, *Invest. Ophthalmol. Vis. Sci.*, (2007) 48: 4351-9). After cessation of the inducing oxygen exposure, recovery of post-receptor neural retinal sensitivity (b-wave log s) recovers and vascular tortuosity decreases. The regulation of developing retinal neurons and blood vessels takes place under the cooperative control of several growth factors, such as vascular endothelial growth factor (VEGF), semaphorin, and their neuropilin receptors (Gariano *et al.*, *Gene Expression Patterns*, (2006) 6: 187-192). In rat models of ROP, expression of these growth factors has been found to be altered (Mocko *et al.*, *ARVO Abstract*, (2008)).

[00129] Described herein are also methods for treating wet aged-related macular degeneration in a patient comprising administration to the patient a therapeutically effective amount of a Visual Cycle Modulation (VCM) compound.

### **Visual Cycle Modulation**

[00130] As used herein, “Visual Cycle Modulation” (VCM) refers to the biological conversion of a photon into electrical signal in the retina. (See, e.g., Figures 1A and 1B). The retina contains light-receptor cells known as “rods” (responsible for night vision) and “cones” (responsible for day vision). Rod cells are much more numerous and active than cones. Rod over-activity creates the build-up of toxins in the eye, whereas cones provide the vast majority of our visual information – including color. VCM essentially “slows down” the activity of the rods and reduces the metabolic load and oxygen consumption in the retina. Figure 4B illustrates one means by which a VCM affects the visual cycle.

[00131] VCM compounds useful to improved outcomes in ROP are disclosed herein. VCM compounds are administered alone or with one or more additional compounds/treatments including, but not limited to, pharmaceutical treatments that reduce the energy demand of the rod photoreceptors can reduce inappropriate vascular proliferation, and environmental treatments that increase the light to which a patient is exposed. Due to the physiology of the rod photoreceptors, metabolic demand is highest in low light situations; thus, exposure to increased light can reduce metabolic demand, thereby mitigate the manifestation of ROP.

### **Macular Degeneration**

[00132] Macular Degeneration refers to the loss of photoreceptors in the portion of the central retina, termed the macula, responsible for high-acuity vision. Degeneration of the macula is associated with abnormal deposition of extracellular matrix components and other debris in the membrane between the retinal pigment epithelium and the vascular choroid. This debris-like material is termed drusen. Drusen is observed with a funduscopic eye examination. Normal eyes may have maculas free of drusen, yet drusen may be abundant in the retinal periphery. The presence of soft drusen in the macula, in the absence of any loss of macular vision, is considered an early stage of AMD.

### **Age-related Macular Degeneration**

[00133] Age-related Macular Degeneration (AMD) refers to a disease that causes abnormality in the macula of the retina; it is the leading cause of vision loss in Europe and the United States. In Japan, the disease is also steadily increasing because of the aging population. The macula is located in the center of the retina, and the region is densely populated with cone cells among the photoreceptor cells. Rays of light coming from outside are refracted by the cornea and crystalline lens, and then converge on the macula, the central fovea in particular. The ability to read letters depends on the function of this area. In age-related macular degeneration, the macula, which is an important area as described above, degenerates with age and results in visual impairment, mainly in the form of image distortion (anorthopia) and central scotoma.

[00134] Central geographic atrophy, the “dry” form of advanced AMD, results from atrophy to the retinal pigment epithelial layer below the retina, which causes vision loss through loss of photoreceptors (rods and cones) in the central part of the eye. Neovascular or exudative AMD, the “wet” form of advanced AMD, causes vision loss due to abnormal blood vessel growth (choroidal neovascularization) in the choriocapillaris, through Bruch's membrane, ultimately leading to blood and protein leakage below the macula. Bleeding, leaking, and scarring from these blood vessels eventually cause irreversible damage to the photoreceptors and rapid vision loss if left untreated. The wet form of age-related macular degeneration is a disease with a poor prognosis, which results in rapid and severe visual impairment. The major pathological condition is choroidal neovascularization.

[00135] Age-related macular degeneration (AMD) is one of the leading causes of blindness in the developed world. The approval of the macromolecules LUCENTIS®, AVASTIN®, and MACUGEN® has improved the treatment options available for AMD patients. LUCENTIS® is a Fab and AVASTIN® is a monoclonal antibody. They both bind vascular endothelial growth factor (VEGF) and may be used to treat AMD; however, only a minority of treated patients experiences a significant improvement in visual acuity.

### **Choroidal Neovascularization**

[00136] Choroidal Neovascularization (CNV) refers to the creation of new blood vessels in the choroid layer of the eye. CNV can occur rapidly in individuals with defects in Bruch's membrane, the innermost layer of the choroid. It is also associated with excessive amounts of vascular endothelial growth factor (VEGF). As well as in wet AMD, CNV can also occur frequently with the rare genetic disease pseudoxanthoma elasticum and rarely with the more common optic disc drusen. CNV has also been associated with extreme myopia or malignant myopic degeneration, where in choroidal neovascularization occurs primarily in the presence of cracks within the retinal (specifically) macular tissue known as lacquer cracks.

[00137] CNV can create a sudden deterioration of central vision, noticeable within a few weeks. Other symptoms which can occur include metamorphopsia, and color disturbances. Hemorrhaging of the new blood vessels can accelerate the onset of symptoms of CNV.

[00138] CNV can be detected by measuring the Preferential Hyperacuity Perimeter. On the basis of fluorescein angiography, CNV may be described as classic or occult. PHP is a specialized perimeter that applies principles of both static and automated perimetry to detect defects in the visual field. Rather than measuring peripheral visual fields, PHP relies on the concept of hyperacuity to measure subtle differences in the central and paracentral fields. Hyperacuity is the ability to discern a subtle misalignment of an object. Hyperacuity, or Vernier acuity, has a threshold of 3 to 6 seconds of arc in the fovea. Therefore, hyperacuity's threshold is approximately 10 fold lower than that required for optimal resolution of an object, which is 30 to 60 seconds of arc in the fovea.

[00139] Choroidal neovascularization (CNV) commonly occurs in macular degeneration in addition to other ocular disorders and is associated with proliferation of choroidal endothelial cells, overproduction of extracellular matrix, and formation of a fibrovascular subretinal membrane. Retinal pigment epithelium cell proliferation and production of angiogenic factors appears to effect choroidal neovascularization.

[00140] Current standard of care in retinology today are intravitreal injections of anti-VEGF drugs to control neovascularization and reduce the area of fluid below the retinal pigment epithelium. These drugs are commonly known as AVASTIN® and LUCENTIS®, and although their effectiveness has been shown to significantly improve visual prognosis with CNV, the recurrence rate for these neovascular areas remains high. Individuals with CNV should be aware that they are at a much greater risk (25%) of developing CNV in fellow eye, this according to the American Academy of Ophthalmology and further supported by clinical reports.

[00141] In "wet" (also known as "neovascular") Age-Related Macular Degeneration, CNV is treated with photodynamic therapy coupled with a photosensitive drug such as verteporfin. Verteporfin, a benzoporphyrin derivative, is an intravenous lipophilic photosensitive drug with an absorption peak of 690 nm. This drug was first approved by the Food and Drug Administration (FDA) on April 12, 2000, and subsequently, approved for inclusion in the United States Pharmacopoeia on July 18, 2000, meeting Medicare's definition of a drug when used in conjunction with ocular photodynamic therapy (see § 80.2, "Photodynamic Therapy") when furnished intravenously incident to a physician's service. For patients with age-related macular degeneration, Verteporfin is only covered with a diagnosis of neovascular age-related macular degeneration (ICD-9-CM 362.52) with predominately classic subfoveal choroidal neovascular (CNV) lesions (where the area of classic CNV occupies > 50 percent of the area of the entire lesion) at the initial visit as determined by a fluorescein angiogram (CPT code 92235). Subsequent follow-up visits will require a fluorescein angiogram prior to treatment. OPT with verteporfin is covered for the above indication and will remain non-covered for all other indications related to AMD (see § 80.2). OPT with Verteporfin for use in non-AMD conditions is eligible for coverage through individual contractor discretion+. Verteporfin is given intravenously. It is then activated in the eye by a laser light. The drug destroys the new blood vessels, and prevents any new vessels forming by forming thrombi.

[00142] Anti-VEGF drugs, such as pegaptanib and ranibizumab, are also used to treat CNV. Anti-VEGFs bind to and inactivate VEGF.

[00143] CNV refers to ectopic growth of choroidal vessels, penetrating through Bruch's membrane and retinal pigment epithelia. In wet age-related macular degeneration, hemorrhage and leakage of plasma components comprising fat from the premature vascular plexus is the direct cause of the rapid functional impairment of the neural retina. CNV is thought to be induced by inflammatory cells mainly comprising macrophages that infiltrate to phagocytose drusen accumulated at the subretinal macular area.

Inflammatory cells such as macrophages are also sources of production of angiogenic factors, such as vascular endothelial growth factor (VEGF), and they function to enhance neovascularization at sites of inflammation. This process is called "inflammatory neovascularization". Meanwhile, drusen comprise advanced glycation end-products (AGE) and amyloid beta, which are substances that stimulate VEGF production; these substances stimulate retinal pigment epithelia that have migrated to engulf drusen, resulting in VEGF secretion, and this is thought to be another possible mechanism by which CNV develops. Diseases involving CNV include myopic choroidal neovascularization and idiopathic choroidal neovascularization as well as age-related macular degeneration. Development of diseases involving CNV can sometimes be ascribed to angioid streaks, injury, uveitis, or such. Tissue damage mainly of the Bruch's membrane and retinal pigment epithelia in the subretinal macular area, and the subsequent inflammation, have been suggested to be involved in the mechanism of CNV onset in these diseases, as well as in age-related macular degeneration.

#### **Medical Procedures Requiring Prolonged Eye Exposure**

[00144] Most eye operations, surgeries, procedures, and examinations require the exposure of direct bright light aimed at the eye(s) and in many cases this exposure is prolonged; the compounds disclosed herein are useful for limiting or otherwise preventive unwanted damage to the eye by such exposure.

[00145] Some medical procedures are aimed at correcting structural defects of an eye.

[00146] Refractive eye surgery involves various methods of surgical remodeling of the cornea or cataract (e.g. radial keratotomy uses spoke-shaped incisions made with a diamond knife). In some instances, excimer lasers are used to reshape the curvature of the cornea. In some instances, successful refractive eye surgery reduces or cures common vision disorders such as myopia, hyperopia and astigmatism, as well as degenerative disorders like keratoconus. Other types of refractive eye surgeries include keratomileusis (a disc of cornea is shaved off, quickly frozen, lathe-ground, then returned to its original power), automated lamellar keratoplasty (ALK), laser assisted in-situ keratomileusis (LASIK), intraLASIK, laser assisted sub-epithelial keratomileusis (LASEK aka Epi-LASIK), photorefractive keratectomy, laser thermal keratoplasty, conductive keratoplasty, limbal relaxing incisions, astigmatic keratotomy, radial keratotomy, mini asymmetric radial keratotomy, hexagonal keratotomy, epikeratophakia, intracorneal ring or ring segment implant (Intacs), contact lens implant, presbyopia reversal, anterior ciliary sclerotomy, laser reversal of presbyopia, scleral expansion bands, and Karmra inlay.

[00147] Corneal surgery includes but is not limited to corneal transplant surgery, penetrating keratoplasty, keratoprostheses, phototherapeutic keratectomy, pterygium excision, corneal tattooing, and osteo-odonto-keratoprostheses (OOKP). In some instances, corneal surgeries do not require a laser. In

other instances, corneal surgeries use a laser (e.g., phototherapeutic keratectomy, which removes superficial corneal opacities and surface irregularities). In some instances, patients are given dark eyeglasses to protect their eyes from bright lights after these procedures.

[00148] Some procedures are aimed at removing defective components or fluids from the eye.

[00149] Cataract surgery involves surgical removal of the lens and replacement with a plastic intraocular lens. Typically, a light is used to aid the surgeon.

[00150] There are various types of glaucoma surgery that facilitate the escape of excess aqueous humor from the eye to lower intraocular pressure. In some instances, these medical procedures use a laser (e.g., laser trabeculoplasty applies a laser beam to burn areas of the trabecular meshwork, located near the base of the iris, to increase fluid outflow; laser peripheral iridotomy applies a laser beam to selectively burn a hole through the iris near its base; etc.). Canaloplasty is an advanced, nonpenetrating procedure designed to enhance drainage through the eye's natural drainage system utilizing microcatheter technology in a simple and minimally invasive procedure. Other medical procedures used for the treatment of glaucoma include lasers, non-penetrating surgery, guarded filtration surgery, and seton valve implants.

[00151] Vitreo-retinal surgery includes vitrectomy (e.g., anterior vitrectomy and pars plana vitrectomy). In some instances, vitreo-retinal surgery is used for preventing or treating vitreous loss during cataract or corneal surgery, removing misplaced vitreous tissue in conditions such as aphakia pupillary block glaucoma, removing vitreous opacities and membranes through an incision, retinal detachment repair (using ignipuncture, a scleral buckle, or laser photocoagulation, pneumatic retinopexy, retinal cryopexy, or retinal cryotherapy), macular hole repair, partial lamellar sclerouvectomy, partial lamellar sclerocyclochoroidectomy, partial lamellar sclerochoroidectomy, posterior sclerotomy, radial optic neurotomy, and macular translocation surgery. Pan retinal photocoagulation (PRP), a type of photocoagulation laser therapy often used in the treatment of diabetic retinopathy, is aimed at treating vitreous hemorrhaging, bleeding in the eye from injuries, retinal tears, subarachnoidal bleedings, or blocked blood vessels. In some instances, photocoagulation with a laser shrinks unhealthy blood vessels or seals retinal holes once blood is removed.

[00152] Some medical procedures address structures or features that support eye function or eye appearance. Eye muscle surgery typically corrects strabismus and includes the following: loosening and weakening procedures (e.g., recession, myectomy, myotomy, tenectomy, tenotomy, tightening, etc.), strengthening procedures (e.g., resection, tucking, movement of an eye muscle from its original place of attachment on the eyeball to a more forward position, etc.); transposition and repositioning procedures, and adjustable suture surgery (e.g., methods of reattaching an extraocular muscle by means of a stitch that can be shortened or lengthened within the first post-operative day, to obtain better ocular alignment).

[00153] Oculoplastic surgery, or oculoplastics, is the subspecialty of ophthalmology that deals with the reconstruction of the eye and associated structures, including eyelid surgery, repair of tear duct obstructions, orbital fracture repairs, removal of tumors in and around the eyes, and facial rejuvenation procedures including laser skin resurfacing, eye lifts, brow lifts, facelifts, Botox injections, ultrapel microdermabrasion, and liposuction. Some eye procedures improve the lacrimal apparatus including

dacryocystorhinostomy, canaliculodacryocystostomy, canaliculotomy, dacryoadenectomy, dacryocystectomy and dacryocystostomy.

### Visual Cycle Modulation Compounds

[00154] As used in the specification and appended claims, unless specified to the contrary, the following terms have the meaning indicated below.

[00155] As used herein and in the appended claims, the singular forms "a," "and," and "the" include plural references unless the context clearly dictates otherwise. Thus, for example, reference to "a compound" includes a plurality of such compounds, and reference to "the cell" includes reference to one or more cells (or to a plurality of cells) and equivalents thereof known to those skilled in the art, and so forth. Also, for example, references to "the method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth. When ranges are used herein for physical properties, such as molecular weight, or chemical properties, such as chemical formulae, all combinations and sub-combinations of ranges and specific embodiments therein are intended to be included. The term "about" when referring to a number or a numerical range means that the number or numerical range referred to is an approximation within experimental variability (or within statistical experimental error), and thus the number or numerical range may vary between 1% and 15% of the stated number or numerical range. The term "comprising" (and related terms such as "comprise" or "comprises" or "having" or "including") is not intended to exclude that in other certain embodiments, for example, an embodiment of any composition of matter, composition, method, or process, or the like, described herein, may "consist of" or "consist essentially of" the described features.

[00156] "Amino" refers to the  $-\text{NH}_2$  radical.

[00157] "Cyano" refers to the  $-\text{CN}$  radical.

[00158] "Nitro" refers to the  $-\text{NO}_2$  radical.

[00159] "Oxa" refers to the  $-\text{O-}$  radical.

[00160] "Oxo" refers to the  $=\text{O}$  radical.

[00161] "Thioxo" refers to the  $=\text{S}$  radical.

[00162] "Imino" refers to the  $=\text{N-H}$  radical.

[00163] "Hydrazino" refers to the  $=\text{N-NH}_2$  radical.

[00164] "Alkyl" refers to a straight or branched hydrocarbon chain radical consisting solely of carbon and hydrogen atoms, containing no unsaturation, having from one to fifteen carbon atoms (e.g.,  $\text{C}_1\text{-C}_{15}$  alkyl). In certain embodiments, an alkyl comprises one to thirteen carbon atoms (e.g.,  $\text{C}_1\text{-C}_{13}$  alkyl). In certain embodiments, an alkyl comprises one to eight carbon atoms (e.g.,  $\text{C}_1\text{-C}_8$  alkyl). In other embodiments, an alkyl comprises five to fifteen carbon atoms (e.g.,  $\text{C}_5\text{-C}_{15}$  alkyl). In other embodiments, an alkyl comprises five to eight carbon atoms (e.g.,  $\text{C}_5\text{-C}_8$  alkyl). The alkyl is attached to the rest of the molecule by a single bond, for example, methyl (Me), ethyl (Et), *n*-propyl, 1-methylethyl (*iso*-propyl), *n*-butyl, *n*-pentyl, 1,1-dimethylethyl (*t*-butyl), 3-methylhexyl, 2-methylhexyl, and the like. Unless stated otherwise specifically in the specification, an alkyl group is optionally substituted by one or

more of the following substituents: halo, cyano, nitro, oxo, thioxo, trimethylsilanyl, -OR<sup>a</sup>, -SR<sup>a</sup>, -OC(O)-R<sup>a</sup>, -N(R<sup>a</sup>)<sub>2</sub>, -C(O)R<sup>a</sup>, -C(O)OR<sup>a</sup>, -C(O)N(R<sup>a</sup>)<sub>2</sub>, -N(R<sup>a</sup>)C(O)OR<sup>a</sup>, -N(R<sup>a</sup>)C(O)R<sup>a</sup>, -N(R<sup>a</sup>)S(O)<sub>t</sub>R<sup>a</sup> (where t is 1 or 2), -S(O)<sub>t</sub>OR<sup>a</sup> (where t is 1 or 2) and -S(O)<sub>t</sub>N(R<sup>a</sup>)<sub>2</sub> (where t is 1 or 2) where each R<sup>a</sup> is independently hydrogen, alkyl, fluoroalkyl, carbocyclyl, carbocyclylalkyl, aryl, aralkyl, heterocyclyl, heterocyclylalkyl, heteroaryl or heteroarylalkyl.

**[00165]** "Alkenyl" refers to a straight or branched hydrocarbon chain radical group consisting solely of carbon and hydrogen atoms, containing at least one double bond, and having from two to twelve carbon atoms. In certain embodiments, an alkenyl comprises two to eight carbon atoms. In other embodiments, an alkenyl comprises two to four carbon atoms. The alkenyl is attached to the rest of the molecule by a single bond, for example, ethenyl (*i.e.*, vinyl), prop-1-enyl (*i.e.*, allyl), but-1-enyl, pent-1-enyl, penta-1,4-dienyl, and the like. Unless stated otherwise specifically in the specification, an alkenyl group is optionally substituted by one or more of the following substituents: halo, cyano, nitro, oxo, thioxo, trimethylsilanyl, -OR<sup>a</sup>, -SR<sup>a</sup>, -OC(O)-R<sup>a</sup>, -N(R<sup>a</sup>)<sub>2</sub>, -C(O)R<sup>a</sup>, -C(O)OR<sup>a</sup>, -C(O)N(R<sup>a</sup>)<sub>2</sub>, -N(R<sup>a</sup>)C(O)OR<sup>a</sup>, -N(R<sup>a</sup>)C(O)R<sup>a</sup>, -N(R<sup>a</sup>)S(O)<sub>t</sub>R<sup>a</sup> (where t is 1 or 2), -S(O)<sub>t</sub>OR<sup>a</sup> (where t is 1 or 2) and -S(O)<sub>t</sub>N(R<sup>a</sup>)<sub>2</sub> (where t is 1 or 2) where each R<sup>a</sup> is independently hydrogen, alkyl, fluoroalkyl, carbocyclyl, carbocyclylalkyl, aryl, aralkyl, heterocyclyl, heterocyclylalkyl, heteroaryl or heteroarylalkyl.

**[00166]** "Alkynyl" refers to a straight or branched hydrocarbon chain radical group consisting solely of carbon and hydrogen atoms, containing at least one triple bond, having from two to twelve carbon atoms. In certain embodiments, an alkynyl comprises two to eight carbon atoms. In other embodiments, an alkynyl has two to four carbon atoms. The alkynyl is attached to the rest of the molecule by a single bond, for example, ethynyl, propynyl, butynyl, pentynyl, hexynyl, and the like. Unless stated otherwise specifically in the specification, an alkynyl group is optionally substituted by one or more of the following substituents: halo, cyano, nitro, oxo, thioxo, trimethylsilanyl, -OR<sup>a</sup>, -SR<sup>a</sup>, -OC(O)-R<sup>a</sup>, -N(R<sup>a</sup>)<sub>2</sub>, -C(O)R<sup>a</sup>, -C(O)OR<sup>a</sup>, -C(O)N(R<sup>a</sup>)<sub>2</sub>, -N(R<sup>a</sup>)C(O)OR<sup>a</sup>, -N(R<sup>a</sup>)C(O)R<sup>a</sup>, -N(R<sup>a</sup>)S(O)<sub>t</sub>R<sup>a</sup> (where t is 1 or 2), -S(O)<sub>t</sub>OR<sup>a</sup> (where t is 1 or 2) and -S(O)<sub>t</sub>N(R<sup>a</sup>)<sub>2</sub> (where t is 1 or 2) where each R<sup>a</sup> is independently hydrogen, alkyl, fluoroalkyl, carbocyclyl, carbocyclylalkyl, aryl, aralkyl, heterocyclyl, heterocyclylalkyl, heteroaryl or heteroarylalkyl.

**[00167]** "Alkylene" or "alkylene chain" refers to a straight or branched divalent hydrocarbon chain linking the rest of the molecule to a radical group, consisting solely of carbon and hydrogen, containing no unsaturation and having from one to twelve carbon atoms, for example, methylene, ethylene, propylene, *n*-butylene, and the like. The alkylene chain is attached to the rest of the molecule through a single bond and to the radical group through a single bond. The points of attachment of the alkylene chain to the rest of the molecule and to the radical group can be through one carbon in the alkylene chain or through any two carbons within the chain. Unless stated otherwise specifically in the specification, an alkylene chain is optionally substituted by one or more of the following substituents: halo, cyano, nitro, aryl, cycloalkyl, heterocyclyl, heteroaryl, oxo, thioxo, trimethylsilanyl, -OR<sup>a</sup>, -SR<sup>a</sup>, -OC(O)-R<sup>a</sup>, -N(R<sup>a</sup>)<sub>2</sub>, -C(O)R<sup>a</sup>, -C(O)OR<sup>a</sup>, -C(O)N(R<sup>a</sup>)<sub>2</sub>, -N(R<sup>a</sup>)C(O)OR<sup>a</sup>, -N(R<sup>a</sup>)C(O)R<sup>a</sup>, -N(R<sup>a</sup>)S(O)<sub>t</sub>R<sup>a</sup> (where t is 1 or 2), -S(O)<sub>t</sub>OR<sup>a</sup> (where t is 1 or 2) and -S(O)<sub>t</sub>N(R<sup>a</sup>)<sub>2</sub> (where t is 1 or 2) where each R<sup>a</sup> is

independently hydrogen, alkyl, fluoroalkyl, carbocyclyl, carbocyclylalkyl, aryl, aralkyl, heterocyclyl, heterocyclylalkyl, heteroaryl or heteroarylalkyl.

**[00168]** "Alkenylene" or "alkenylene chain" refers to a straight or branched divalent hydrocarbon chain linking the rest of the molecule to a radical group, consisting solely of carbon and hydrogen, containing at least one double bond and having from two to twelve carbon atoms, for example, ethenylene, propenylene, *n*-butenylene, and the like. The alkenylene chain is attached to the rest of the molecule through a double bond or a single bond and to the radical group through a double bond or a single bond. The points of attachment of the alkenylene chain to the rest of the molecule and to the radical group can be through one carbon or any two carbons within the chain. Unless stated otherwise specifically in the specification, an alkenylene chain is optionally substituted by one or more of the following substituents: halo, cyano, nitro, aryl, cycloalkyl, heterocyclyl, heteroaryl, oxo, thioxo, trimethylsilyl, -OR<sup>a</sup>, -SR<sup>a</sup>, -OC(O)-R<sup>a</sup>, -N(R<sup>a</sup>)<sub>2</sub>, -C(O)R<sup>a</sup>, -C(O)OR<sup>a</sup>, -C(O)N(R<sup>a</sup>)<sub>2</sub>, -N(R<sup>a</sup>)C(O)OR<sup>a</sup>, -N(R<sup>a</sup>)C(O)R<sup>a</sup>, -N(R<sup>a</sup>)S(O)<sub>t</sub>R<sup>a</sup> (where t is 1 or 2), -S(O)<sub>t</sub>OR<sup>a</sup> (where t is 1 or 2) and -S(O)<sub>t</sub>N(R<sup>a</sup>)<sub>2</sub> (where t is 1 or 2) where each R<sup>a</sup> is independently hydrogen, alkyl, fluoroalkyl, cycloalkyl, cycloalkylalkyl, aryl (optionally substituted with one or more halo groups), aralkyl, heterocyclyl, heterocyclylalkyl, heteroaryl or heteroarylalkyl, and where each of the above substituents is unsubstituted unless otherwise indicated.

**[00169]** "Aryl" refers to a radical derived from an aromatic monocyclic or multicyclic hydrocarbon ring system by removing a hydrogen atom from a ring carbon atom. The aromatic monocyclic or multicyclic hydrocarbon ring system contains only hydrogen and carbon from six to eighteen carbon atoms, where at least one of the rings in the ring system is fully unsaturated, *i.e.*, it contains a cyclic, delocalized (4n+2)  $\pi$ -electron system in accordance with the Hückel theory. Aryl groups include, but are not limited to, groups such as phenyl, fluorenyl, and naphthyl. Unless stated otherwise specifically in the specification, the term "aryl" or the prefix "ar-" (such as in "aralkyl") is meant to include aryl radicals optionally substituted by one or more substituents independently selected from alkyl, alkenyl, alkynyl, halo, fluoroalkyl, cyano, nitro, optionally substituted aryl, optionally substituted aralkyl, optionally substituted aralkenyl, optionally substituted aralkynyl, optionally substituted carbocyclyl, optionally substituted carbocyclylalkyl, optionally substituted heterocyclyl, optionally substituted heterocyclylalkyl, optionally substituted heteroaryl, optionally substituted heteroarylalkyl, -R<sup>b</sup>-OR<sup>a</sup>, -R<sup>b</sup>-OC(O)-R<sup>a</sup>, -R<sup>b</sup>-N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-C(O)R<sup>a</sup>, -R<sup>b</sup>-C(O)OR<sup>a</sup>, -R<sup>b</sup>-C(O)N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-O-R<sup>c</sup>-C(O)N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-N(R<sup>a</sup>)C(O)OR<sup>a</sup>, -R<sup>b</sup>-N(R<sup>a</sup>)C(O)R<sup>a</sup>, -R<sup>b</sup>-N(R<sup>a</sup>)S(O)<sub>t</sub>R<sup>a</sup> (where t is 1 or 2), -R<sup>b</sup>-S(O)<sub>t</sub>OR<sup>a</sup> (where t is 1 or 2) and -R<sup>b</sup>-S(O)<sub>t</sub>N(R<sup>a</sup>)<sub>2</sub> (where t is 1 or 2), where each R<sup>a</sup> is independently hydrogen, alkyl, fluoroalkyl, cycloalkyl, cycloalkylalkyl, aryl (optionally substituted with one or more halo groups), aralkyl, heterocyclyl, heterocyclylalkyl, heteroaryl or heteroarylalkyl, each R<sup>b</sup> is independently a direct bond or a straight or branched alkylene or alkenylene chain, and R<sup>c</sup> is a straight or branched alkylene or alkenylene chain, and where each of the above substituents is unsubstituted unless otherwise indicated.

**[00170]** "Aralkyl" refers to a radical of the formula -R<sup>c</sup>-aryl where R<sup>c</sup> is an alkylene chain as defined above, for example, benzyl, diphenylmethyl and the like. The alkylene chain part of the aralkyl

radical is optionally substituted as described above for an alkylene chain. The aryl part of the aralkyl radical is optionally substituted as described above for an aryl group.

[00171] "Aralkenyl" refers to a radical of the formula  $-R^d\text{-aryl}$  where  $R^d$  is an alkenylene chain as defined above. The aryl part of the aralkenyl radical is optionally substituted as described above for an aryl group. The alkenylene chain part of the aralkenyl radical is optionally substituted as defined above for an alkenylene group.

[00172] "Aralkynyl" refers to a radical of the formula  $-R^e\text{-aryl}$ , where  $R^e$  is an alkynylene chain as defined above. The aryl part of the aralkynyl radical is optionally substituted as described above for an aryl group. The alkynylene chain part of the aralkynyl radical is optionally substituted as defined above for an alkynylene chain.

[00173] "Carbocyclyl" refers to a stable non-aromatic monocyclic or polycyclic hydrocarbon radical consisting solely of carbon and hydrogen atoms, which may include fused or bridged ring systems, having from three to fifteen carbon atoms. In certain embodiments, a carbocyclyl comprises three to ten carbon atoms. In other embodiments, a carbocyclyl comprises five to seven carbon atoms. The carbocyclyl is attached to the rest of the molecule by a single bond. Carbocyclyl may be saturated, (*i.e.*, containing single C-C bonds only) or unsaturated (*i.e.*, containing one or more double bonds or triple bonds.) A fully saturated carbocyclyl radical is also referred to as "cycloalkyl." Examples of monocyclic cycloalkyls include, *e.g.*, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. An unsaturated carbocyclyl is also referred to as "cycloalkenyl." Examples of monocyclic cycloalkenyls include, *e.g.*, cyclopentenyl, cyclohexenyl, cycloheptenyl, and cyclooctenyl. Polycyclic carbocyclyl radicals include, for example, adamantyl, norbornyl (*i.e.*, bicyclo[2.2.1]heptanyl), norbornenyl, decalinyl, 7,7-dimethyl-bicyclo[2.2.1]heptanyl, and the like. Unless otherwise stated specifically in the specification, the term "carbocyclyl" is meant to include carbocyclyl radicals that are optionally substituted by one or more substituents independently selected from alkyl, alkenyl, alkynyl, halo, fluoroalkyl, oxo, thioxo, cyano, nitro, optionally substituted aryl, optionally substituted aralkyl, optionally substituted aralkenyl, optionally substituted aralkynyl, optionally substituted carbocyclyl, optionally substituted carbocyclylalkyl, optionally substituted heterocyclyl, optionally substituted heterocyclylalkyl, optionally substituted heteroaryl, optionally substituted heteroarylalkyl,  $-R^b\text{-OR}^a$ ,  $-R^b\text{-SR}^a$ ,  $-R^b\text{-OC(O)-R}^a$ ,  $-R^b\text{-N(R}^a\text{)}_2$ ,  $-R^b\text{-C(O)R}^a$ ,  $-R^b\text{-C(O)OR}^a$ ,  $-R^b\text{-C(O)N(R}^a\text{)}_2$ ,  $-R^b\text{-O-R}^c\text{-C(O)N(R}^a\text{)}_2$ ,  $-R^b\text{-N(R}^a\text{)C(O)OR}^a$ ,  $-R^b\text{-N(R}^a\text{)C(O)R}^a$ ,  $-R^b\text{-N(R}^a\text{)S(O)}_t\text{R}^a$  (where  $t$  is 1 or 2),  $-R^b\text{-S(O)}_t\text{OR}^a$  (where  $t$  is 1 or 2) and  $-R^b\text{-S(O)}_t\text{N(R}^a\text{)}_2$  (where  $t$  is 1 or 2), where each  $R^a$  is independently hydrogen, alkyl, fluoroalkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heterocyclyl, heterocyclylalkyl, heteroaryl or heteroarylalkyl, each  $R^b$  is independently a direct bond or a straight or branched alkylene or alkenylene chain, and  $R^c$  is a straight or branched alkylene or alkenylene chain, and where each of the above substituents is unsubstituted unless otherwise indicated.

[00174] "Carbocyclylalkyl" refers to a radical of the formula  $-R^c\text{-carbocyclyl}$  where  $R^c$  is an alkylene chain as defined above. The alkylene chain and the carbocyclyl radical is optionally substituted as defined above.

[00175] "Halo" or "halogen" refers to bromo, chloro, fluoro or iodo substituents.

[00176] "Fluoroalkyl" refers to an alkyl radical, as defined above, that is substituted by one or more fluoro radicals, as defined above, for example, trifluoromethyl, difluoromethyl, 2,2,2-trifluoroethyl, 1-fluoromethyl-2-fluoroethyl, and the like. The alkyl part of the fluoroalkyl radical may be optionally substituted as defined above for an alkyl group.

[00177] "Heterocyclyl" refers to a stable 3- to 18-membered non-aromatic ring radical that comprises two to twelve carbon atoms and from one to six heteroatoms selected from nitrogen, oxygen and sulfur. Unless stated otherwise specifically in the specification, the heterocyclyl radical is a monocyclic, bicyclic, tricyclic or tetracyclic ring system, which may include fused or bridged ring systems. The heteroatoms in the heterocyclyl radical may be optionally oxidized. One or more nitrogen atoms, if present, are optionally quaternized. The heterocyclyl radical is partially or fully saturated. The heterocyclyl may be attached to the rest of the molecule through any atom of the ring(s). Examples of such heterocyclyl radicals include, but are not limited to, dioxolanyl, thienyl[1,3]dithianyl, decahydroisoquinolyl, imidazolinyl, imidazolidinyl, isothiazolidinyl, isoxazolidinyl, morpholinyl, octahydroindolyl, octahydroisoindolyl, 2-oxopiperazinyl, 2-oxopiperidinyl, 2-oxopyrrolidinyl, oxazolidinyl, piperidinyl, piperazinyl, 4-piperidonyl, pyrrolidinyl, pyrazolidinyl, quinuclidinyl, thiazolidinyl, tetrahydrofuryl, trithianyl, tetrahydropyranyl, thiomorpholinyl, thiamorpholinyl, 1-oxo-thiomorpholinyl, and 1,1-dioxo-thiomorpholinyl. Unless stated otherwise specifically in the specification, the term "heterocyclyl" is meant to include heterocyclyl radicals as defined above that are optionally substituted by one or more substituents selected from alkyl, alkenyl, alkynyl, halo, fluoroalkyl, oxo, thioxo, cyano, nitro, optionally substituted aryl, optionally substituted aralkyl, optionally substituted aralkenyl, optionally substituted aralkynyl, optionally substituted carbocyclyl, optionally substituted carbocyclylalkyl, optionally substituted heterocyclyl, optionally substituted heterocyclylalkyl, optionally substituted heteroaryl, optionally substituted heteroarylalkyl,  $-R^b-OR^a$ ,  $-R^b-SR^a$ ,  $-R^b-OC(O)-R^a$ ,  $-R^b-N(R^a)_2$ ,  $-R^b-C(O)R^a$ ,  $-R^b-C(O)OR^a$ ,  $-R^b-C(O)N(R^a)_2$ ,  $-R^b-O-R^c-C(O)N(R^a)_2$ ,  $-R^b-N(R^a)C(O)OR^a$ ,  $-R^b-N(R^a)C(O)R^a$ ,  $-R^b-N(R^a)S(O)_tR^a$  (where t is 1 or 2),  $-R^b-S(O)_tOR^a$  (where t is 1 or 2) and  $-R^b-S(O)_tN(R^a)_2$  (where t is 1 or 2), where each  $R^a$  is independently hydrogen, alkyl, fluoroalkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heterocyclyl, heterocyclylalkyl, heteroaryl or heteroarylalkyl, each  $R^b$  is independently a direct bond or a straight or branched alkylene or alkenylene chain, and  $R^c$  is a straight or branched alkylene or alkenylene chain, and where each of the above substituents is unsubstituted unless otherwise indicated.

[00178] "*N*-heterocyclyl" or "*N*-attached heterocyclyl" refers to a heterocyclyl radical as defined above containing at least one nitrogen and where the point of attachment of the heterocyclyl radical to the rest of the molecule is through a nitrogen atom in the heterocyclyl radical. An *N*-heterocyclyl radical is optionally substituted as described above for heterocyclyl radicals. Examples of such *N*-heterocyclyl radicals include, but are not limited to, 1-morpholinyl, 1-piperidinyl, 1-piperazinyl, 1-pyrrolidinyl, pyrazolidinyl, imidazolinyl, and imidazolidinyl.

**[00179]** "C-heterocyclyl" or "C-attached heterocyclyl" refers to a heterocyclyl radical as defined above containing at least one heteroatom and where the point of attachment of the heterocyclyl radical to the rest of the molecule is through a carbon atom in the heterocyclyl radical. A C-heterocyclyl radical is optionally substituted as described above for heterocyclyl radicals. Examples of such C-heterocyclyl radicals include, but are not limited to, 2-morpholiny, 2- or 3- or 4-piperidiny, 2-piperaziny, 2- or 3-pyrrolidiny, and the like.

**[00180]** "Heterocyclalkyl" refers to a radical of the formula  $-R^c\text{-heterocyclyl}$  where  $R^c$  is an alkylene chain as defined above. If the heterocyclyl is a nitrogen-containing heterocyclyl, the heterocyclyl is optionally attached to the alkyl radical at the nitrogen atom. The alkylene chain of the heterocyclalkyl radical is optionally substituted as defined above for an alkylene chain. The heterocyclyl part of the heterocyclalkyl radical is optionally substituted as defined above for a heterocyclyl group.

**[00181]** "Heteroaryl" refers to a radical derived from a 3- to 18-membered aromatic ring radical that comprises two to seventeen carbon atoms and from one to six heteroatoms selected from nitrogen, oxygen and sulfur. As used herein, the heteroaryl radical may be a monocyclic, bicyclic, tricyclic or tetracyclic ring system, wherein at least one of the rings in the ring system is fully unsaturated, *i.e.*, it contains a cyclic, delocalized  $(4n+2)\pi$ -electron system in accordance with the Hückel theory. Heteroaryl includes fused or bridged ring systems. The heteroatom(s) in the heteroaryl radical is optionally oxidized. One or more nitrogen atoms, if present, are optionally quaternized. The heteroaryl is attached to the rest of the molecule through any atom of the ring(s). Examples of heteroaryls include, but are not limited to, azepinyl, acridinyl, benzimidazolyl, benzindolyl, 1,3-benzodioxolyl, benzofuranyl, benzoazazolyl, benzo[d]thiazolyl, benzothiadiazolyl, benzo[b][1,4]dioxepinyl, benzo[b][1,4]oxazinyl, 1,4-benzodioxanyl, benzonaphthofuranyl, benzoxazolyl, benzodioxolyl, benzodioxinyl, benzopyranyl, benzopyranonyl, benzofuranyl, benzofuranonyl, benzothienyl (benzothiophenyl), benzothieno[3,2-d]pyrimidinyl, benzotriazolyl, benzo[4,6]imidazo[1,2-a]pyridinyl, carbazolyl, cinnolinyl, cyclopenta[d]pyrimidinyl, 6,7-dihydro-5H-cyclopenta[4,5]thieno[2,3-d]pyrimidinyl, 5,6-dihydrobenzo[h]quinazolinyl, 5,6-dihydrobenzo[h]cinnolinyl, 6,7-dihydro-5H-benzo[6,7]cyclohepta[1,2-c]pyridazinyl, dibenzofuranyl, dibenzothiophenyl, furanyl, furanonyl, furo[3,2-c]pyridinyl, 5,6,7,8,9,10-hexahydrocycloocta[d]pyrimidinyl, 5,6,7,8,9,10-hexahydrocycloocta[d]pyridazinyl, 5,6,7,8,9,10-hexahydrocycloocta[d]pyridinyl, isothiazolyl, imidazolyl, indazolyl, indolyl, indazolyl, isoindolyl, indolinyl, isoindolinyl, isoquinolyl, indolizinyl, isoxazolyl, 5,8-methano-5,6,7,8-tetrahydroquinazolinyl, naphthyridinyl, 1,6-naphthyridinonyl, oxadiazolyl, 2-oxoazepinyl, oxazolyl, oxiranyl, 5,6,6a,7,8,9,10,10a-octahydrobenzo[h]quinazolinyl, 1-phenyl-1H-pyrrolyl, phenazinyl, phenothiazinyl, phenoxazinyl, phthalazinyl, pteridinyl, purinyl, pyrrolyl, pyrazolyl, pyrazolo[3,4-d]pyrimidinyl, pyridinyl, pyrido[3,2-d]pyrimidinyl, pyrido[3,4-d]pyrimidinyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrrolyl, quinazolinyl, quinoxalinyl, quinolinyl, isoquinolinyl, tetrahydroquinolinyl, 5,6,7,8-tetrahydroquinazolinyl,

5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-d]pyrimidinyl,  
6,7,8,9-tetrahydro-5H-cyclohepta[4,5]thieno[2,3-d]pyrimidinyl,  
5,6,7,8-tetrahydropyrido[4,5-c]pyridazinyl, thiazolyl, thiadiazolyl, triazolyl, tetrazolyl, triazinyl, thieno[2,3-d]pyrimidinyl, thieno[3,2-d]pyrimidinyl, thieno[2,3-c]pridinyl, and thiophenyl (*i.e.* thienyl). Unless stated otherwise specifically in the specification, the term "heteroaryl" is meant to include heteroaryl radicals as defined above which are optionally substituted by one or more substituents selected from alkyl, alkenyl, alkynyl, halo, fluoroalkyl, haloalkenyl, haloalkynyl, oxo, thioxo, cyano, nitro, optionally substituted aryl, optionally substituted aralkyl, optionally substituted aralkenyl, optionally substituted aralkynyl, optionally substituted carbocyclyl, optionally substituted carbocyclylalkyl, optionally substituted heterocyclyl, optionally substituted heterocyclylalkyl, optionally substituted heteroaryl, optionally substituted heteroarylalkyl, -R<sup>b</sup>-OR<sup>a</sup>, -R<sup>b</sup>-SR<sup>a</sup>, -R<sup>b</sup>-OC(O)-R<sup>a</sup>, -R<sup>b</sup>-N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-C(O)R<sup>a</sup>, -R<sup>b</sup>-C(O)OR<sup>a</sup>, -R<sup>b</sup>-C(O)N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-O-R<sup>c</sup>-C(O)N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-N(R<sup>a</sup>)C(O)OR<sup>a</sup>, -R<sup>b</sup>-N(R<sup>a</sup>)C(O)R<sup>a</sup>, -R<sup>b</sup>-N(R<sup>a</sup>)S(O)<sub>t</sub>R<sup>a</sup> (where t is 1 or 2), -R<sup>b</sup>-S(O)<sub>t</sub>OR<sup>a</sup> (where t is 1 or 2) and -R<sup>b</sup>-S(O)<sub>t</sub>N(R<sup>a</sup>)<sub>2</sub> (where t is 1 or 2), where each R<sup>a</sup> is independently hydrogen, alkyl, fluoroalkyl, cycloalkyl, cycloalkylalkyl, aryl, aralkyl, heterocyclyl, heterocyclylalkyl, heteroaryl or heteroarylalkyl, each R<sup>b</sup> is independently a direct bond or a straight or branched alkylene or alkenylene chain, and R<sup>c</sup> is a straight or branched alkylene or alkenylene chain, and where each of the above substituents is unsubstituted unless otherwise indicated.

**[00182]** "N-heteroaryl" refers to a heteroaryl radical as defined above containing at least one nitrogen and where the point of attachment of the heteroaryl radical to the rest of the molecule is through a nitrogen atom in the heteroaryl radical. An N-heteroaryl radical is optionally substituted as described above for heteroaryl radicals.

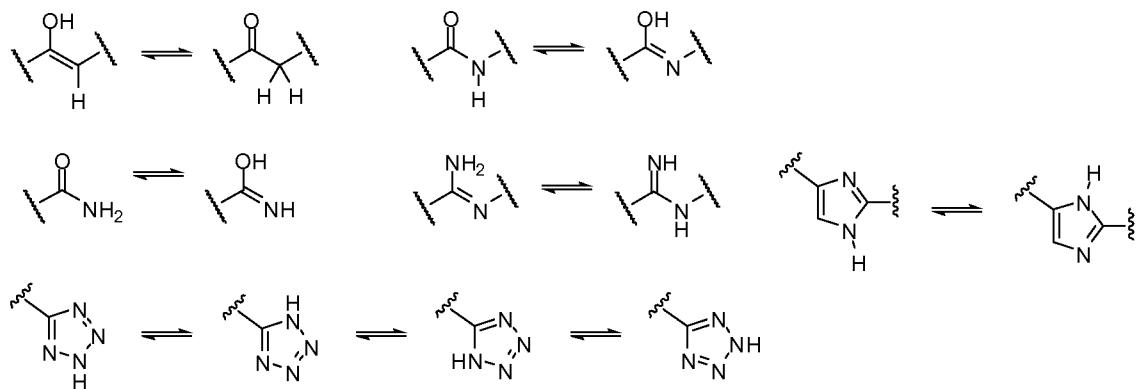
**[00183]** "C-heteroaryl" refers to a heteroaryl radical as defined above and where the point of attachment of the heteroaryl radical to the rest of the molecule is through a carbon atom in the heteroaryl radical. A C-heteroaryl radical is optionally substituted as described above for heteroaryl radicals.

**[00184]** "Heteroarylalkyl" refers to a radical of the formula -R<sup>c</sup>-heteroaryl, where R<sup>c</sup> is an alkylene chain as defined above. If the heteroaryl is a nitrogen-containing heteroaryl, the heteroaryl is optionally attached to the alkyl radical at the nitrogen atom. The alkylene chain of the heteroarylalkyl radical is optionally substituted as defined above for an alkylene chain. The heteroaryl part of the heteroarylalkyl radical is optionally substituted as defined above for a heteroaryl group.

**[00185]** The compounds, or their pharmaceutically acceptable salts may contain one or more asymmetric centers and may thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that may be defined, in terms of absolute stereochemistry, as (R)- or (S)- or, as (D)- or (L)- for amino acids. When the compounds described herein contain olefinic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both *E* and *Z* geometric isomers (*e.g.*, *cis* or *trans*.) Likewise, all possible isomers, as well as their racemic and optically pure forms, and all tautomeric forms are also intended to be included.

**[00186]** A "stereoisomer" refers to a compound made up of the same atoms bonded by the same bonds but having different three-dimensional structures, which are not interchangeable. It is therefore contemplated that various stereoisomers and mixtures thereof and includes "enantiomers," which refers to two stereoisomers whose molecules are nonsuperimposeable mirror images of one another.

**[00187]** The compounds presented herein may exist as tautomers. A "tautomer" refers to a proton shift from one atom of a molecule to another atom of the same molecule, accompanied by an isomerization of an adjacent double bond. In bonding arrangements where tautomerization is possible, a chemical equilibrium of the tautomers will exist. All tautomeric forms of the compounds disclosed herein are contemplated. The exact ratio of the tautomers depends on several factors, including temperature, solvent, and pH. Some examples of tautomeric interconversions include:



**[00188]** "Optional" or "optionally" means that a subsequently described event or circumstance may or may not occur and that the description includes instances when the event or circumstance occurs and instances in which it does not. For example, "optionally substituted aryl" means that the aryl radical may or may not be substituted and that the description includes both substituted aryl radicals and aryl radicals having no substitution.

**[00189]** "Pharmaceutically acceptable salt" includes both acid and base addition salts. A pharmaceutically acceptable salt of any one of the substituted heterocyclic amine derivative compounds described herein is intended to encompass any and all pharmaceutically suitable salt forms. Preferred pharmaceutically acceptable salts of the compounds described herein are pharmaceutically acceptable acid addition salts and pharmaceutically acceptable base addition salts.

**[00190]** "Pharmaceutically acceptable acid addition salt" refers to those salts which retain the biological effectiveness and properties of the free bases, which are not biologically or otherwise undesirable, and which are formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, hydroiodic acid, hydrofluoric acid, phosphorous acid, and the like. Also included are salts that are formed with organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, alkanedioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, etc. and include, for example, acetic acid, trifluoroacetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic

acid, and the like. Exemplary salts thus include sulfates, pyrosulfates, bisulfates, sulfites, bisulfites, nitrates, phosphates, monohydrogenphosphates, dihydrogenphosphates, metaphosphates, pyrophosphates, chlorides, bromides, iodides, acetates, trifluoroacetates, propionates, caprylates, isobutyrates, oxalates, malonates, succinate suberates, sebacates, fumarates, maleates, mandelates, benzoates, chlorobenzoates, methylbenzoates, dinitrobenzoates, phthalates, benzenesulfonates, toluenesulfonates, phenylacetates, citrates, lactates, malates, tartrates, methanesulfonates, and the like. Also contemplated are salts of amino acids, such as arginates, gluconates, and galacturonates (see, for example, Berge S.M. et al., "Pharmaceutical Salts," *Journal of Pharmaceutical Science*, 66:1-19 (1997), which is hereby incorporated by reference in its entirety). Acid addition salts of basic compounds may be prepared by contacting the free base forms with a sufficient amount of the desired acid to produce the salt according to methods and techniques with which a skilled artisan is familiar.

**[00191]** "Pharmaceutically acceptable base addition salt" refers to those salts that retain the biological effectiveness and properties of the free acids, which are not biologically or otherwise undesirable. These salts are prepared from addition of an inorganic base or an organic base to the free acid. Pharmaceutically acceptable base addition salts may be formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Salts derived from inorganic bases include, but are not limited to, sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Salts derived from organic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, for example, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, diethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, *N,N*-dibenzylethylenediamine, chloroprocaine, hydrabamine, choline, betaine, ethylenediamine, ethylenediamine, *N*-methylglucamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, *N*-ethylpiperidine, polyamine resins and the like. See Berge et al., *supra*.

**[00192]** As used herein, "treatment" or "treating," or "palliating" or "ameliorating" are used interchangeably herein. These terms refers to an approach for obtaining beneficial or desired results including but not limited to therapeutic benefit and/or a prophylactic benefit. By "therapeutic benefit" is meant eradication or amelioration of the underlying disorder being treated. Also, a therapeutic benefit is achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the underlying disorder such that an improvement is observed in the patient, notwithstanding that the patient may still be afflicted with the underlying disorder. For prophylactic benefit, the compositions may be administered to a patient at risk of developing a particular disease, or to a patient reporting one or more of the physiological symptoms of a disease, even though a diagnosis of this disease may not have been made.

**[00193]** "Prodrug" is meant to indicate a compound that may be converted under physiological conditions or by solvolysis to a biologically active compound described herein. Thus, the term "prodrug" refers to a precursor of a biologically active compound that is pharmaceutically

acceptable. A prodrug may be inactive when administered to a subject, but is converted *in vivo* to an active compound, for example, by hydrolysis. The prodrug compound often offers advantages of solubility, tissue compatibility or delayed release in a mammalian organism (see, e.g., Bundgard, H., Design of Prodrugs (1985), pp. 7-9, 21-24 (Elsevier, Amsterdam).

**[00194]** A discussion of prodrugs is provided in Higuchi, T., et al., "Pro-drugs as Novel Delivery Systems," A.C.S. Symposium Series, Vol. 14, and in Bioreversible Carriers in Drug Design, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987, both of which are incorporated in full by reference herein.

**[00195]** The term "prodrug" is also meant to include any covalently bonded carriers, which release the active compound *in vivo* when such prodrug is administered to a mammalian subject. Prodrugs of an active compound, as described herein, may be prepared by modifying functional groups present in the active compound in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to the parent active compound. Prodrugs include compounds wherein a hydroxy, amino or mercapto group is bonded to any group that, when the prodrug of the active compound is administered to a mammalian subject, cleaves to form a free hydroxy, free amino or free mercapto group, respectively. Examples of prodrugs include, but are not limited to, acetate, formate and benzoate derivatives of alcohol or amine functional groups in the active compounds and the like.

#### **Compositions and Modes of Administration**

**[00196]** In some embodiments, the compounds described herein are formulated as a pharmaceutically acceptable composition when combined with an acceptable carrier or excipient.

**[00197]** Thus, in some embodiments, compositions include, in addition to active ingredient, an acceptable excipient, carrier, buffer, stabilizer or other materials known in the art for use within a composition to be administered to a patient. Such materials are non-toxic and do not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material depends on the route of administration.

**[00198]** Acceptable carriers and their formulations are and generally described in, for example, Remington' pharmaceutical Sciences (18th Edition, ed. A. Gennaro, Mack Publishing Co., Easton, PA 1990).

**[00199]** Compositions are formulated to be compatible with a particular route of administration in mind. Thus, compositions include carriers, diluents, or excipients suitable for administration by various routes.

**[00200]** A "therapeutically effective amount" of a composition to be administered is the minimum amount necessary to prevent, ameliorate, or treat a disease or disorder. The composition is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of compound present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages. Generally, alleviation or treatment of a disease or disorder involves the lessening of one or more symptoms or medical problems associated with the disease or disorder.

**[00201]** Compounds described herein are administered in any way suitable to effectively achieve a desired therapeutic effect in the eye. Thus, methods of administration include without limitation, topical, intraocular (including intravitreal), transdermal, oral, intravenous, subconjunctival, subretinal, or peritoneal routes of administration.

**[00202]** Administration techniques that can be employed with the compounds and methods are known in the art and described herein, *e.g.*, as discussed in Goodman and Gilman, *The Pharmacological Basis of Therapeutics*, current *ed.*; Pergamon; and Remington's, *Pharmaceutical Sciences* (current edition), Mack Publishing Co., Easton, Pa. In certain embodiments, the compounds and compositions described herein are administered orally.

**[00203]** Liquid formulation dosage forms for oral administration may be aqueous suspensions such as, for example, pharmaceutically acceptable aqueous oral dispersions, emulsions, solutions, elixirs, gels, and syrups. See, *e.g.*, Singh *et al.*, *Encyclopedia of Pharmaceutical Technology*, 2nd Ed., pp. 754-757 (2002). In addition to the compound, a liquid dosage form optionally includes a pharmaceutically acceptable carrier or excipient suitable for oral administration, and, optionally, one or more additives, such as: (a) disintegrating agents; (b) dispersing agents; (c) wetting agents; (d) preservatives, (e) viscosity enhancing agents, (f) sweetening agents, and/or (g) flavoring agents. In some embodiments, the aqueous dispersions further include a crystal-forming inhibitor.

**[00204]** In one embodiment, emulsifying and/or suspending agents, together with diluents such as water, ethanol, propylene glycol, glycerin and various combinations thereof, may be added to the compositions.

**[00205]** Water may be added (*e.g.*, 5%) as a means of simulating long-term storage in order to determine characteristics such as shelf-life or the stability of formulations over time. Anhydrous compositions and dosage forms may be prepared using anhydrous or low moisture containing ingredients and low moisture or low humidity conditions. Compositions and dosage forms which contain lactose can be made anhydrous if substantial contact with moisture and/or humidity during manufacturing, packaging, and/or storage is expected. An anhydrous composition may be prepared and stored such that its anhydrous nature is maintained. Accordingly, anhydrous compositions may be packaged using materials known to prevent exposure to water such that they can be included in suitable formulary kits.

**[00206]** In additional or alternative embodiments, the composition may be in the form of a tablet, capsule, pill, powder, sustained release formulation, solution, suspension, or emulsion.

**[00207]** Solid dosage forms for oral administration include, for example but not limited to capsules, tablets, pills, powders and granules.

**[00208]** In such solid dosage forms, the compositions as disclosed herein may be mixed with at least one inert, pharmaceutically acceptable excipient or carrier, such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol and silicic acid; b) binders such as carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose and acacia; c) humectants such as glycerol; d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates and sodium carbonate; e) solution retarding agents

such as paraffin; f) absorption accelerators such as quaternary ammonium compounds; g) wetting agents such as cetyl alcohol and glycerol monostearate; h) absorbents such as kaolin and bentonite clay and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

**[00209]** Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The active components can also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients. In the preparation of pharmaceutical formulations as disclosed herein in the form of dosage units for oral administration the compound selected can be mixed with solid, powdered ingredients, such as lactose, saccharose, sorbitol, mannitol, starch, arnylopectin, cellulose derivatives, gelatin, or another suitable ingredient, as well as with disintegrating agents and lubricating agents such as magnesium stearate, calcium stearate, sodium stearyl fumarate and polyethylene glycol waxes. The mixture is then processed into granules or pressed into tablets.

**[00210]** The composition may be in unit dosage forms suitable for single administration of precise dosages. In further or additional embodiments the amount of compound is in the range of about 0.001 to about 1000 mg/kg body weight/day. In further or additional embodiments the amount of compound is in the range of about 0.5 to about 50 mg/kg/day. In further or additional embodiments the amount of compound is about 0.001 to about 7 g/day. In further or additional embodiments the amount of compound is about 0.002 to about 6 g/day. In further or additional embodiments the amount of compound is about 0.005 to about 5 g/day. In further or additional embodiments the amount of compound is about 0.01 to about 5 g/day. In further or additional embodiments the amount of compound is about 0.02 to about 5 g/day. In further or additional embodiments the amount of compound is about 0.05 to about 2.5 g/day. In further or additional embodiments the amount of compound is about 0.1 to about 1 g/day. In some embodiments, dosage levels below the lower limit of the aforesaid range may be more than adequate. In other embodiments, dosage levels above the upper limit of the aforesaid range may be required.

**[00211]** In one aspect the daily dose of (R)-3-amino-1-(3-(cyclohexylmethoxy)phenyl)propan-1-ol is about 4 mg to about 100 mg. In another aspect the daily dose of (R)-3-amino-1-(3-(cyclohexylmethoxy)phenyl)propan-1-ol is about 2 mg; about 5 mg; about 7 mg; about 10 mg; about 15 mg; about 20 mg; about 40 mg; about 60 mg; about 75 mg; or about 100 mg.

**[00212]** In some embodiments, a composition for oral delivery contains at least about 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, 99, 99.5, 99.9, or 99.99% of a compound described herein. In other embodiments, a composition for the oral delivery contains no more than about 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, 99, 99.5, or 100% of a compound described herein. In some embodiments, a composition contains about 1-100%, about 10-100%, about 20-100%, about 50-100%, about 80-100%, about 90-100%, about 95-100%, or about 99-100% of a compound described herein. In some embodiments, a

composition contains about 1-90%, about 10-90%, about 20-90%, about 50-90%, or about 80-90% of a compound described herein. In some embodiments, a composition contains about 1-75%, about 10-75%, about 20-75%, or about 50-75% of a compound described herein. In some embodiments, a composition contains about 1-50%, about 10-50%, about 20-50%, about 30-50%, or about 40-50 % of a compound described herein. In some embodiments, a composition contains about 1-40%, about 10-40%, about 20-40%, or about 30-40% of a compound described herein. In some embodiments, a composition contains about 1-30%, about 10-30%, or about 20-30% of a compound described herein. In some embodiments, a composition contains about 1-20%, or about 10-20% of a compound described herein. In some embodiments, a composition contains about 1-10% of a compound described herein.

### Methods of Treatment

[00213] Provided herein is a method for treating diabetic retinopathy in a patient (alleviating one or more symptoms, or stasis of one or more symptoms) by administering to the patient a therapeutically effective amount of a composition provided herein. The treatment can result in improving the patient's condition and can be assessed by determining if one or more of the following factors has occurred: decreased macular edema, or increased visual acuity. The compounds described herein can also be used in medicaments for the treatment of diabetic retinopathy.

[00214] A "patient" is a mammal who exhibits one or more clinical manifestations and/or symptoms of a disease or disorder described herein. Non-limiting examples of patients include, but are not limited to, a human or a non-human animal such as a primate, rodent, cow, horse, pig, sheep, *etc.* In certain situations, the patient may be asymptomatic and yet still have clinical manifestations of the disease or disorder. In one embodiment, a patient to be treated is a human.

[00215] The compositions provided herein can be administered once or multiple times depending on the health of the patient, the progression of the disease or condition, and the efficacy of the treatment. Adjustments to therapy and treatments can be made throughout the course of treatment.

[00216] Signs and symptoms of diabetic retinopathy include, but are not limited to, one or more of the following: changes in the blood vessels; retinal swelling (macular edema); pale deposits on the retina; damaged nerve tissue; visual appearance of leaking blood vessels; loss of central or peripheral vision; temporary or permanent vision loss; spotty, blurry, hazy or double vision; eye pain; floaters ; impaired color vision; vision loss; a dark or blind spot in the central vision; venous dilation and intraretinal micro vascular abnormalities; neuropathy; fluctuating and progressive deterioration of vision; macular edema; macular ischemia; traction retinal detachment; endothelial cell proliferation; photopsias; rubeosis or nvi; micro aneurysms; hard exudates; haemorrhages; and cotton wool spots; are the symptoms for diabetic retinopathy.

[00217] In one embodiment, treatment of DR with a compound described herein blocks formation of abnormal blood vessels, slows leakage from blood vessels, reduces retinal swelling, prevents retinal detachment, prevents or slows blindness, and/or reduces vision loss.

[00218] The compound to be administered in such methods is administered by any suitable means such as those described herein and known in the art.

[00219] For the prevention or treatment of disease, the appropriate dosage of compound will depend, in part, on the patient to be treated, the severity and course of the disease, whether the compound is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the compound, and the discretion of the attending physician. The compound is suitably administered to the patient at one time or over a series of treatments.

[00220] The compositions can be administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the patient's immune system to utilize the active ingredient. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. Suitable regimes for initial administration and booster shots are also variable. Depending on the type and severity of the disease, about 0.1  $\mu\text{g}/\text{kg}$  to about 150  $\text{mg}/\text{kg}$  of compound is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. Other initial dosages include, but are not limited to, about 0.25  $\mu\text{g}/\text{kg}$ , about 0.5  $\mu\text{g}/\text{kg}$ , about 1  $\mu\text{g}/\text{kg}$ , about 10  $\mu\text{g}/\text{kg}$ , about 50  $\mu\text{g}/\text{kg}$ , about 100  $\mu\text{g}/\text{kg}$ , about 250  $\mu\text{g}/\text{kg}$ , about 500  $\mu\text{g}/\text{kg}$ , about 750  $\mu\text{g}/\text{kg}$ , about 1  $\text{mg}/\text{kg}$ , about 5  $\text{mg}/\text{kg}$ , about 10  $\text{mg}/\text{kg}$ , about 15  $\text{mg}/\text{kg}$ , about 20  $\text{mg}/\text{kg}$ , about 25  $\text{mg}/\text{kg}$ , about 50  $\text{mg}/\text{kg}$ , about 75  $\text{mg}/\text{kg}$ , about 100  $\text{mg}/\text{kg}$ , about 125  $\text{mg}/\text{kg}$ , about 150  $\text{mg}/\text{kg}$  or more. Thereafter, a typical daily dosage may range from about 0.1  $\mu\text{g}/\text{kg}$  to about 150  $\text{mg}/\text{kg}$  or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. Dosages may be given once daily, every over day, every week, every month, or every other month. Additionally, the dose(s) of a compound can be administered twice a week, weekly, every two weeks, every three weeks, every 4 weeks, every 6 weeks, every 8 weeks, every 12 weeks, or any combination of weeks therein. Dosing cycles are also contemplated such as, for example, administering compounds once or twice a week for 4 weeks, followed by two weeks without therapy. Additional dosing cycles including, for example, different combinations of the doses and weekly cycles described herein are also contemplated. One or more symptoms may be assessed during treatment and dosages adjusted accordingly. Dosages may be administered orally and/or intravirally.

[00221] A composition can be administered alone or in combination with a second treatment either simultaneously or sequentially dependent upon the condition to be treated. When two or more compositions, or a composition and a treatment, are administered, the compositions or composition/treatment can be administered in combination (either sequentially or simultaneously). A composition can be administered in a single dose or multiple doses.

[00222] The term "unit dose" when used in reference to a composition refers to physically discrete units suitable as unitary dosage for humans, each unit containing a predetermined quantity of active material

calculated to produce the desired therapeutic effect in association with the required diluent; *i.e.*, carrier, or vehicle.

[00223] Depending on the type and severity of the disease, about 0.1  $\mu\text{g}/\text{kg}$  to about 150  $\text{mg}/\text{kg}$  of compound is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. Other initial dosages include, but are not limited to, about 0.25  $\mu\text{g}/\text{kg}$ , about 0.5  $\mu\text{g}/\text{kg}$ , about 1  $\mu\text{g}/\text{kg}$ , about 10  $\mu\text{g}/\text{kg}$ , about 50  $\mu\text{g}/\text{kg}$ , about 100  $\mu\text{g}/\text{kg}$ , about 250  $\mu\text{g}/\text{kg}$ , about 500  $\mu\text{g}/\text{kg}$ , about 750  $\mu\text{g}/\text{kg}$ , about 1  $\text{mg}/\text{kg}$ , about 5  $\text{mg}/\text{kg}$ , about 10  $\text{mg}/\text{kg}$ , about 15  $\text{mg}/\text{kg}$ , about 20  $\text{mg}/\text{kg}$ , about 25  $\text{mg}/\text{kg}$ , about 50  $\text{mg}/\text{kg}$ , about 75  $\text{mg}/\text{kg}$ , about 100  $\text{mg}/\text{kg}$ , about 125  $\text{mg}/\text{kg}$ , about 150  $\text{mg}/\text{kg}$  or more. Thereafter, a typical daily dosage may range from about 0.1  $\mu\text{g}/\text{kg}$  to about 150  $\text{mg}/\text{kg}$  or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful.

[00224] In one embodiment, treatment of a patient having age-related macular degeneration, choroidal neovascularization and/or diabetic retinopathy as described herein includes improvement of at least one of the symptoms described herein. Improvement includes, for example, a 2%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% improvement in one or more signs or symptoms described herein. Compositions can be administered to a patient in a therapeutically effective amount which is effective for producing some desired therapeutic effect, at a reasonable benefit/risk ratio applicable to any medical treatment. For the administration of the present compositions to human patients, the compositions can be formulated by methodology known by one of ordinary skill in the art.

[00225] As used herein, the term “treatment” refers to both therapeutic treatment and prophylactic measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented from worsening. In one embodiment, treatment of a patient having diabetic retinopathy as described herein means that one or more signs or symptoms does not worsen or progress. In another embodiment, treatment of a patient having age-related macular degeneration and/or choroidal neovascularization as described herein means that one or more signs or symptoms does not worsen or progress. As used herein, “prevention” refers to prophylaxis, prevention of onset of symptoms, prevention of progression of one or more signs or symptoms of diabetic retinopathy, age-related macular degeneration and/or choroidal neovascularization. As used herein, “inhibition,” “treatment” and “treating” are used to refer to, for example, stasis of symptoms, prolongation of survival, partial or full amelioration of symptoms.

[00226] “Administering” is defined herein as a means providing the composition to the patient in a manner that results in the composition being inside the patient’s body. Such an administration can be by any route including, without limitation, modes of administration described herein or conventionally known in the art. “Concurrent administration” means administration within a relatively short time period from each other; such time period can be less than 2 weeks, less than 7 days, less than 1 day and could even be administered simultaneously.

[00227] Actual dosage levels of the active ingredients in the compositions can be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of factors including the activity of the particular compound employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular composition employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[00228] In one embodiment, the compound may be administered in a single dose, once daily. In other embodiments, the compound may be administered in multiple doses, more than once per day. In other embodiments, the compound may be administered twice daily. In other embodiments, the compound may be administered three times per day. In other embodiments, the compound may be administered four times per day. In other embodiments, the compound may be administered more than four times per day.

[00229] A response is achieved when the patient experiences partial or total alleviation, or reduction of signs or symptoms of illness, and specifically includes, without limitation, prolongation of survival. The expected progression-free survival times can be measured in months to years, depending on prognostic factors including the number of relapses, stage of disease, and other factors. Prolonging survival includes without limitation times of at least 1 month (mo), about at least 2 months (mos.), about at least 3 mos., about at least 4 mos., about at least 6 mos., about at least 1 year, about at least 2 years, about at least 3 years, or more. Overall survival can also be measured in months to years. The patient's symptoms can remain static or can decrease.

[00230] A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount ( $ED_{50}$ ) of the composition required. For example, the physician or veterinarian could start doses of the compounds employed in the composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. Alternatively, a dose can remain constant.

[00231] Toxicity and therapeutic efficacy of such ingredient can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the  $LD_{50}$  (the dose lethal to 50% of the population) and the  $ED_{50}$  (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio  $LD_{50}/ED_{50}$ . While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to healthy cells and, thereby, reduce side effects.

[00232] Also provided herein are methods of treating retinopathy of prematurity (ROP) in a patient in need thereof by administering a composition containing a compound described herein.

[00233] Provided herein is a method of treating or preventing retinopathy of prematurity, comprising administering to a patient in need thereof a composition comprising a visual cycle modulator (VCM) compound such as those described herein.

[00234] In one embodiment, the compound alters the visual cycle. Patients to be treated with such methods are premature infants.

[00235] In another embodiment, the patient is additionally treated with supplemental oxygen.

[00236] In another embodiment, the treatment is administered locally to the eye or systemically.

[00237] Provided herein is the use of a visual cycle modulator as described herein in the formulation of a medicament for the treatment of retinopathy of prematurity. Treatments described herein can be administered and monitored by a medical practitioner. Administration routes, dosages and specific measures of efficacy can be selected by the administering practitioner, and may depend upon factors such as the severity of disease, age, weight and gender of the patient, as well as other factors, such as other medical problems of the patient.

[00238] Efficacy for any given composition may also be determined using an experimental animal model, *e.g.*, the rat model of ROP described herein. When using an experimental animal model, efficacy of treatment may be assessed when a reduction in a marker or symptom of ROP is observed.

[00239] The amount and frequency of administration will also depend, in part, on the composition itself, its stability and specific activity, as well as the route of administration. Greater amounts of a composition will generally have to be administered for systemic, compared to topically/locally administered compositions.

[00240] The eye provides a tissue or structure well suited for topical administration of many drugs.

Intraocular injection and oral administration can also be effective. Doses will depend on route of administration, and will vary from, *e.g.*, about 0.1 mg/kg body weight to about 10 mg/kg body weight for by systemic administration, to 0.01 mg to 10 mg by topical or intraocular injection routes. Other dosages are also contemplated herein.

[00241] A “therapeutically effective amount” of a composition to be administered will be governed by such considerations, and is the minimum amount necessary to prevent, ameliorate, or treat a disease or disorder. The composition need not be, but may be optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of compound present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages. Generally, alleviation or treatment of a disease or disorder involves the lessening of one or more symptoms or medical problems associated with the disease or disorder.

[00242] In general, a compound is determined to be “therapeutically effective” in the methods described herein if (a) measurable symptom(s) of, for example, vascular abnormalities, are reduced for example by at least 10% compared to the measurement prior to treatment onset, (b) the progression of the disease is halted (*e.g.*, patients do not worsen or the vasculature stops growing pathologically, or (c) symptoms are

reduced or even ameliorated, for example, by measuring a reduction in vessel number or tortuosity. Efficacy of treatment can be judged by an ordinarily practitioner or as described herein and known in the art.

**[00243]** The compositions as disclosed herein can also be administered in prophylactically or therapeutically effective amounts. A prophylactically or therapeutically effective amount means that amount necessary, at least partly, to attain the desired effect, or to delay the onset of, inhibit the progression of, or halt altogether, the onset or progression of the particular disease or disorder being treated. Such amounts will depend, of course, on the particular condition being treated, the severity of the condition and individual patient parameters including age, physical condition, size, weight and concurrent treatment. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is preferred generally that a maximum dose be used, that is, the highest safe dose according to sound medical judgment. It will be understood by those of ordinary skill in the art, however, that a lower dose or tolerable dose can be administered for medical reasons, psychological reasons or for virtually any other reasons.

**[00244]** As used herein, “improving rod-mediated retinal function” refers to an increase in rod-mediated retinal function of at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, at least 1-fold, at least 2-fold, at least 5-fold, at least 10-fold, at least 50-fold, at least 100-fold, at least 1000-fold or higher.

**[00245]** “Rod-mediated retinal function” refers to a function of rod cells in a functioning retina and can include such clinical end-points as degree of peripheral vision, low-level light vision, scotopic/“night vision”, and sensitivity to peripheral movement. Rod-mediated retinal function can be assessed *in vivo* by, for example, electroretinography measurement of rod activation of photo-transduction or deactivation of photo-transduction; recovery of the dark current following photobleaching; measurement of the ERG a-wave or b-wave; speed of recovery to photo-transduction; or rod-mediated response amplitudes. Methods for measuring rod-mediated retinal function are known in the art and/or explained herein in more detail.

**[00246]** Efficacy of treatment can be monitored by the administering clinician. Where the disease or disorder is retinopathy of prematurity, the International Classification of Retinopathy or Prematurity (ICROP) can be applied. The ICROP uses a range of parameters to classify the disease. These parameters include location of the disease into zones (zones 1, 2 and 3), the circumferential extent of the disease based on clock hours 1-12, severity of the disease (stages 1-5), and the presence or absence of “Plus Disease.”

**[00247]** The zones are centered on the optic nerve. Zone 1 is the posterior zone of the retina, defined as the circle with a radius extending from the optic nerve to double the distance to the macula. Zone 2 is an annulus with the inner border defined by zone 1 and the outer border defined by the radius defined as the distance from the optic nerve to the nasal ora serrata. Zone 3 is the residual temporal crescent of the retina.

[00248] The circumferential extent of the disease is described in segments as if the top of the eye were 12 on the face of a clock. For example one might report that there is stage 1 disease for 3 clock hours from 4 to 7 o'clock.

[00249] The Stages describe the ophthalmoscopic findings at the junction between the vascularized and avascular retina. Stage 1 is a faint demarcation line. Stage 2 is an elevated ridge. Stage 3 is extraretinal fibrovascular tissue. Stage 4 is sub-total retinal detachment. Stage 5 is total retinal detachment.

[00250] In addition, "Plus disease" may be present at any stage. "Plus disease" describes a significant level of vascular dilation and tortuosity observed at the posterior retinal vessels. This reflects the increase of blood flow through the retina.

[00251] Any improvement on the ICROP relative to pre-treatment classification is considered to be effective treatment. Similarly, where prevention of disease is the goal, treatment is considered effective if one or more signs or symptoms of ROP is(are) less severe in a treated individual relative to the expected course of disease in a similar individual not receiving such treatment. The disease has been known and characterized to an extent that skilled clinicians can often predict the extent of disease that would occur in the absence of treatment, based, for example, on knowledge of earlier patients. The failure to develop or experience a worsening of one or more symptoms of ROP, or, for that matter any other retinal disease or disorder involving abnormal vascularization, can be considered effective prevention of disease in an individual otherwise expected to develop or experience worsening of such disease. Similarly, any improvement relative to expected disease state in the absence of treatment can be considered effective treatment.

[00252] As an alternative to the ICROP scale, other clinically accepted markers of retinal disease known to those of skill in the art can also be measured to monitor or determine the efficacy of treatment or prevention of retinal diseases or disorders as described herein. Generally a difference of at least 10% in a marker of retinal disease is considered significant.

[00253] Provided herein are methods for reducing or inhibiting vascularization in the eye (e.g., neovascularization) of a patient. Also provided herein is a method for treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein the ophthalmic disease or disorder associated with neovascularization is retinal neovascularization. Another embodiment provides a method for treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein the ophthalmic disease or disorder associated with neovascularization is choroidal neovascularization. Another embodiment provides a method for treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein the ophthalmic disease or disorder associated with neovascularization is selected from sickle cell retinopathy, Eales disease, ocular ischemic syndrome, carotid cavernous fistula, familial exudative vitreoretinopathy, hyperviscosity syndrome, idiopathic occlusive arteriolitis, radiation retinopathy, retinal vein occlusion, retinal artery occlusion, retinal embolism, birdshot retinochoroidopathy, retinal vasculitis, sarcoidosis, toxoplasmosis, uveitis, choroidal melanoma, chronic retinal detachment, incontinentia pigmenti, and retinitis pigmentosa. Another embodiment provides a method for treating an ophthalmic disease or disorder

associated with neovascularization in the eye of a patient wherein the ophthalmic disease or disorder associated with neovascularization is wet age-related macular degeneration. Another embodiment provides a method for treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient wherein the ophthalmic disease or disorder associated with neovascularization is neovascular age-related macular degeneration.

**[00254]** Provided herein is a method for treating neovascular age-related macular degeneration (e.g., wet age-related macular degeneration (AMD)) or choroidal neovascularization (CNV) in a patient by administering to the patient a therapeutically effective amount of a composition provided herein. The compounds described herein can also be used in medicaments for the treatment of macular degeneration (e.g., age-related macular degeneration (AMD)) or choroidal neovascularization (CNV). As provided herein all references to age-related macular degeneration refer to the neovascular or wet stage of the disease.

**[00255]** Provided herein is a method for treating age-related macular degeneration (AMD) in a patient by administering to the patient a therapeutically effective amount of a composition provided herein. The treatment can result in improving the patient's condition and can be assessed by determining if one or more of the following factors has occurred: Drusen; pigmentary alterations; exudative changes (e.g., hemorrhages in the eye, hard exudates, subretinal/sub-RPE/intraretinal fluid); atrophy (incipient and geographic); visual acuity drastically decreasing (two levels or more; ex: 20/20 to 20/80); preferential hyperacuity perimetry changes (for wet AMD); blurred vision (those with non-exudative macular degeneration may be asymptomatic or notice a gradual loss of central vision, whereas those with exudative macular degeneration often notice a rapid onset of vision loss); central scotomas (shadows or missing areas of vision); distorted vision (*i.e.*, metamorphopsia; a grid of straight lines appears wavy and parts of the grid may appear blank. Patients often first notice this when looking at mini-blinds in their home); trouble discerning colors (specifically dark ones from dark ones and light ones from light ones); slow recovery of visual function after exposure to bright light; and a loss in contrast sensitivity. Described herein are methods of treating or preventing AMD via the administration of the compounds described herein. The compounds described herein can also be used in medicaments for the treatment of AMD. In one embodiment, one or more signs or symptoms of AMD are improved following administration of one of the compounds described herein to a patient. Improvement also encompasses stasis of one or more symptoms such that they do not worsen.

**[00256]** "Treatment" of diseases involving CNV refers to diseases involving CNV, where a symptom caused by an above disease is suppressed or ameliorated. The treatment of diseases involving CNV also refers to suppressing CNV progression and functional impairment of neural retina caused by hemorrhage or leakage of plasma components from abnormal newly generated vessels.

**[00257]** As used herein, "suppressing CNV" refers to suppressing inflammation in the retina (suppressing the growth of inflammatory cells in the retina) and suppressing the production of angiogenic factors by inflammatory cells, in addition to suppressing neovascularization. An inflammation reaction in the retina may be induced by an injury, or by accumulation of metabolic decomposition products, such as drusen.

[00258] CNV can be confirmed to be suppressed by detecting the size (volume) of neovascularization using fluorescein fundus angiography or the like. When the volume of neovascularization is reduced after administration of an agent of the present disclosure, CNV is regarded as suppressed. Methods for detecting CNV are not limited to the methods described above, and CNV can be detected by known methods, and also by the methods described in the Examples herein.

[00259] As a disease involving CNV progresses, vision is impaired due to image distortion, central scotoma, and such. In such cases of visual impairment, when visual acuity is improved upon administration of a compound described herein, the compound is regarded as useful for patients with such a disease involving CNV. Provided herein is a method for treating choroidal neovascularization. The treatment can result in improving the patient's condition and can be assessed by determining if visual acuity has increased. Described herein are methods of treating or preventing choroidal neovascularization via the administration of the compounds described herein.

[00260] Choroidal neovascularization (CNV) commonly occurs in macular degeneration in addition to other ocular disorders and is associated with proliferation of choroidal endothelial cells, overproduction of extracellular matrix, and formation of a fibrovascular subretinal membrane. Retinal pigment epithelium cell proliferation and production of angiogenic factors appears to effect choroidal neovascularization. Choroidal neovascularization (CNV), the development of abnormal blood vessels beneath the retinal pigment epithelium (RPE) layer of the retina. These vessels break through the Bruch's membrane, disrupting the retinal pigmented epithelium, bleed, and eventually cause macular scarring which results in profound loss of central vision (disciform scarring).

[00261] In one embodiment, treatment of CNV with a compound described herein decreases, slows or inhibits development of abnormal blood vessels beneath the retinal pigment epithelium layer of the retina, slows or inhibits damage of the Bruch's membrane, and slows or inhibits disruption of the retinal pigmented epithelium and slows or inhibits macular scarring.

[00262] Retinal neovascularization develops in numerous retinopathies associated with retinal ischemia, such as sickle cell retinopathy, Eales disease, ocular ischemic syndrome, carotid cavernous fistula, familial exudative vitreoretinopathy, a hyperviscosity syndrome, idiopathic occlusive arteriolitis, radiation retinopathy, retinal vein occlusion, retinal artery occlusion, or retinal embolism. Retinal neovascularization can also occur with inflammatory diseases (such as birdshot retinochoroidopathy, retinal vasculitis, sarcoidosis, toxoplasmosis, or uveitis), or other conditions such as choroidal melanoma, chronic retinal detachment, incontinentia pigmenti, and rarely in retinitis pigmentosa.

[00263] A factor common to almost all retinal neovascularization is retinal ischemia, which is thought to release diffusible angiogenic factors (such as VEGF). The neovascularization begins within the retina and then breaches the retinal internal limiting membrane. The new vessels grow on the inner retina and the posterior surface of the vitreous after it has detached (vitreous detachment). Neovascularization may erupt from the surface of the optic disk or the retina. Retinal neovascularization commonly progresses to vitreoretinal neovascularization. Iris neovascularization and neovascular glaucoma often follow retinal neovascularization.

[00264] The efficacy of the treatment of the measured by various endpoints commonly used in evaluating intraocular neovascular diseases. For example, vision loss can be assessed. Vision loss can be evaluated by, but not limited to, *e.g.*, measuring by the mean change in best correction visual acuity (BCVA) from baseline to a desired time point (*e.g.*, where the BCVA is based on Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart and assessment at a test distance of 4 meters), measuring the proportion of subjects who lose fewer than 15 letters in visual acuity at a desired time point compared to baseline, measuring the proportion of subjects who gain greater than or equal to 15 letters in visual acuity at a desired time point compared to baseline, measuring the proportion of subjects with a visual-acuity Snellen equivalent of 20/2000 or worse at a desired time point, measuring the NEI Visual Functioning Questionnaire, measuring the size of CNV and amount of leakage of CNV at a desired time point, *e.g.*, by fluorescein angiography, etc. Ocular assessments can be done, *e.g.*, which include, but are not limited to, *e.g.*, performing eye exam, measuring intraocular pressure, assessing visual acuity, measuring slitlamp pressure, assessing intraocular inflammation, *etc.*

[00265] Provided herein is a method for protecting an eye during medical procedures requiring exposure of the eye to bright light, to laser light, resulting in prolonged and/or excessive dilation of the pupil, or otherwise sensitizing the eye to light, the method comprising administration of a composition comprising a compound described herein to a patient in need thereof.

[00266] In one embodiment, the medical procedure is refractive eye surgery, corneal surgery, cataract surgery, glaucoma surgery, canaloplasty, vitreo-retinal surgery, pan retinal photocoagulation, eye muscle surgery, oculoplastic surgery, laser therapy, or focal or grid laser photocoagulation. In one embodiment, the medical procedure is refractive eye surgery. In one embodiment, the medical procedure is corneal surgery. In one embodiment, the medical procedure is cataract surgery. In one embodiment, the medical procedure is glaucoma surgery. In one embodiment, the medical procedure is canaloplasty. In one embodiment, the medical procedure is vitreo-retinal surgery. In one embodiment, the medical procedure is pan retinal photocoagulation. In one embodiment, the medical procedure is eye muscle surgery. In one embodiment, the medical procedure is oculoplastic surgery. In one embodiment, the medical procedure is laser therapy. In one embodiment, the medical procedure is focal or grid laser photocoagulation.

[00267] In one embodiment, the composition is administered to the patient orally before and after the medical procedure.

[00268] In one embodiment, the composition is administered orally prior to the medical procedure. In one embodiment, the composition is administered about 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h, 4 h, 6 h, 12 h, or 24 h prior to the procedure.

[00269] In one embodiment, the composition is administered after the medical procedure. In one embodiment, the composition is administered 1 h, 3 h, 6 h, 12 h, 24 h, or 48 h after the medical procedure. In one embodiment, the composition is administered 24 h after the medical procedure. In one embodiment, the composition is administered 48 h after the medical procedure. In one embodiment, the composition is administered 24 h and 48 h after the medical procedure.

[00270] In one embodiment, the composition is administered as a single dose of compound. In one embodiment, the composition comprises about 2 mg, 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, or about 100 mg.

[00271] The compound to be administered in such methods is administered by any suitable means such as those described herein and known in the art.

[00272] For the prevention or treatment of disease, the appropriate dosage of compound will depend, in part, on the patient to be treated, the severity and course of the disease, whether the compound is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the compound, and the discretion of the attending physician. The compound is suitably administered to the patient at one time or over a series of treatments.

[00273] The compositions can be administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be administered depends on the subject to be treated, capacity of the patient's immune system to utilize the active ingredient. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. Suitable regimes for initial administration and booster shots are also variable.

[00274] A composition can be administered alone or in combination with a second treatment either simultaneously or sequentially dependent upon the condition to be treated. When two or more compositions, or a composition and a treatment, are administered, the compositions or composition/treatment can be administered in combination (either sequentially or simultaneously). A composition can be administered in a single dose or multiple doses.

[00275] Compounds described herein can be, as needed, administered in combination with one or more standard therapeutic treatments known in the art and as described, for example, in more detail below.

### **Combination Therapy**

[00276] Diabetic retinopathy is a consequence of the underlying diabetic condition and additional means to lower the risk of developing it or to slow its progression is to: maintain optimal blood sugar levels; have regular, thorough eye exams; follow a healthy eating plan: eat different kinds of foods, and eat the right amount of carbohydrates with each meal; exercise regularly; take medicine exactly as prescribed; eat a low-fat and low-salt diet to keep your cholesterol and blood pressure at normal levels; do not smoke; keep blood pressure and cholesterol level under control; and carefully monitor blood pressure during pregnancy.

[00277] It would be understood that any of the methods described herein could be combined with one or more additional therapies including, but not limited to, laser therapy (e.g., focal or grid laser photocoagulation or focal laser treatment or scatter (pan-retinal) laser photocoagulation or scatter laser treatment), cryotherapy, fluorescein angiography, vitrectomy, corticosteroids (e.g., intravitreal triamcinolone acetonide), Anti-vascular endothelial growth factor (VEGF) treatment (e.g., Pegaptanib (*Macugen*; *Pfizer, Inc.*, *New York, USA*), Ranibizumab (*Lucentis*; *Genentech, Inc.*, *South San Francisco, California, USA*), Bevacizumab (*Avastin*; *Genentech, Inc.*), and VEGF Trap-Eye (*Regeneron Pharmaceuticals, Inc.*, *Tarrytown, New York, USA*)), vitrectomy for persistent diffuse diabetic macular edema, pharmacologic vitreolysis in the management of diabetic retinopathy, fibrates, renin-

angiotensin system (ras) blockers, peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists, Anti- Protein Kinase C (Ruboxistaurin), Islet cell transplantation; Therapeutic Oligonucleotides, Growth hormone and insulin growth factor(IGF), and control of systemic factors.

[00278] The terms "co-administration", "administered in combination with" and their grammatical equivalents or the like, as used herein, are meant to encompass administration of the selected compounds to a single patient, and are intended to include treatment regimens in which the compounds are administered by the same or different route of administration or at the same or different times. In some embodiments, the compounds described herein will be co-administered with other agents. These terms encompass administration of two or more compounds to a patient so that both compounds are present in the patient at the same time. These terms also encompass administration of one compounds and a treatment (e.g., laser therapy) to a patient so that both compounds are present in the patient at the same time. They include simultaneous administration in separate compositions, administration at different times in separate compositions, and/or administration in a composition in which both compounds are present. Thus, in some embodiments, the compounds and the other agent(s)/treatments are administered in a single composition or at a single time. In some embodiments, compounds and the other agent(s) are admixed in a single composition.

### **Laser therapy**

[00279] Laser photocoagulation has been used for the treatment of non-proliferative diabetic retinopathy, macular edema, and proliferative diabetic retinopathy since the 1960s.

[00280] Laser treatment generally targets the damaged eye tissue. Some lasers treat leaking blood vessels directly by "spot welding" and sealing the area of leakage (photocoagulation). Other lasers eliminate abnormal blood vessels that form from neovascularization. Lasers may also be used to destroy the peripheral parts of the normal retina that are not involved in seeing. This is done to help maintain vision in the central portion of the retina.

[00281] The two types of laser treatments commonly used to treat significant diabetic eye disease are:

#### **Focal or grid laser photocoagulation or focal laser treatment**

[00282] This type of laser energy is aimed directly at the affected area or applied in a contained, grid-like pattern to destroy damaged eye tissue and clear away scars that contribute to blind spots and vision loss. This method of laser treatment generally targets specific, individual blood vessels.

[00283] This is the main retinopathy laser treatment method for maculopathy from diabetic macular edema. The retinal laser seals retinal blood vessels that are leaking fluid and blood. This reduces further fluid and blood leakage, and reduces the swelling of the macula. The retinal laser may also somehow stimulate the retinal cells to 'pump' away any excess fluid at the macula. The laser is only directed at certain parts of the macula; the rest of the peripheral retina is untouched.

[00284] The aim of retinal laser treatment is not to improve the vision but to prevent it from getting worse.

#### **Scatter (pan-retinal) laser photocoagulation or scatter laser treatment**

[00285] Pan-retinal photocoagulation is the first line of treatment for proliferative diabetic retinopathy. It applies about 1,200 to 1,800 tiny spots of laser energy to the outermost (peripheral) regions of the retina,

leaving the inner portion untouched. This laser treatment can shrink the abnormal blood vessels. This treatment involves lasering large areas of the retina with the aim of coagulating or burning the ischemic retinal cells in the retinal periphery.

[00286] After pan retinal laser, the ischemic cells throughout the retinal periphery become replaced by scar tissue. This reduces the production of chemicals that stimulate the growth of the abnormal new blood vessels. Scatter laser treatment is usually done in two or more sessions.

[00287] Laser surgery is often helpful in treating diabetic retinopathy. To reduce macular edema, laser light is focused on the damaged retina to seal leaking retinal vessels. For abnormal blood vessel growth (neovascularization), the laser treatments are delivered over the peripheral retina. The small laser scars that result will reduce abnormal blood vessel growth and help bond the retina to the back of the eye, thus preventing retinal detachment. Laser surgery can greatly reduce the chance of severe visual impairment.

### **Cryotherapy**

[00288] Cryotherapy (freezing) may be helpful in treating diabetic retinopathy. If the vitreous is clouded by blood, laser surgery cannot be used until the blood settles or clears. In some of these cases retinal cryotherapy may help shrink the abnormal blood vessels and bond the retina to the back of the eye.

### **Fluorescein angiography**

[00289] Fluorescein angiography has been useful as a research tool in understanding the clinic pathologic changes in the retinal circulation of eyes with diabetic retinopathy. It has also helped to classify diabetic retinopathy and to predict progression from baseline fluorescein angiography characteristics, particularly patterns of capillary nonperfusion.

[00290] It will identify sources of perimacular leakage and guide laser treatment of macular edema. Fluorescein angiography may not be needed in the treatment of Proliferative diabetic Retinopathy, but can be useful to assess signs of retinal ischemia. In some cases Fluorescein angiography can identify new vessels that are not otherwise seen.

[00291] In patients with impaired glucose tolerance, Fluorescein angiography may detect incipient retinal microvascular changes, indicating early break-down of the blood-retinal barrier before diabetes becomes manifest. These and other studies leave no doubt that fluorescein angiography may detect definite early retinal vascular changes in diabetic subjects without clinical retinopathy.

[00292] However, routine use of Fluorescein angiography in managing diabetic retinopathy at present should be guided by clinical experiences as little evidence is available to provide firm guidelines.

### **Vitrectomy**

[00293] Vitrectomy, the surgical removal of the vitreous gel from the middle of the eye, is often used for patients with more advanced retinal disease. The procedure is intended to prevent the complete detachment of the retina. This procedure is commonly used to treat non-clearing vitreous hemorrhage, vitreomacular traction, epiretinal membranes, and retinal detachment.

[00294] During vitrectomy surgery, an operating microscope and small surgical instruments are used to remove blood and scar tissue that accompany abnormal vessels in the eye. Removing the vitreous hemorrhage allows light rays to focus on the retina again.

[00295] Vitrectomy often prevents further vitreous hemorrhage by removing the abnormal vessels that caused the bleeding. Removal of the scar tissue helps the retina return to its normal location. Vitrectomy may be followed or accompanied by laser treatment.

[00296] Vitrectomy can reduce visual loss if performed early in people with vitreous haemorrhage, especially if they have severe proliferative retinopathy.

[00297] Conventional laser treatment may fail in eyes with vitreous hemorrhage or in eyes with tractional retinal detachments and active progressive PDR. Early vitrectomy has been shown to improve visual recovery in patients with proliferative retinopathy and severe vitreous hemorrhage.

### **Refractive Eye Surgery**

[00298] Refractive eye surgery involves various methods of surgical remodeling of the cornea or cataract (e.g. radial keratotomy uses spoke-shaped incisions made with a diamond knife). In some instances, excimer lasers are used to reshape the curvature of the cornea. Successful refractive eye surgery can reduce or cure common vision disorders such as myopia, hyperopia and astigmatism, as well as degenerative disorders like keratoconus. Other types of refractive eye surgeries include keratomileusis (a disc of cornea is shaved off, quickly frozen, lathe-ground, then returned to its original power), automated lamellar keratoplasty (ALK), laser assisted in-situ keratomileusis (LASIK), intraLASIK, laser assisted sub-epithelial keratomileusis (LASEK aka Epi-LASIK), photorefractive keratectomy, laser thermal keratoplasty, conductive keratoplasty, limbal relaxing incisions, astigmatic keratotomy, radial keratotomy, mini asymmetric radial keratotomy, hexagonal keratotomy, epikeratophakia, intracorneal ring or ring segment implant (Intacs), contact lens implant, presbyopia reversal, anterior ciliary sclerotomy, laser reversal of presbyopia, scleral expansion bands, and Karmra inlay.

### **Corneal Surgery**

[00299] Examples of corneal surgery include but are not limited to corneal transplant surgery, penetrating keratoplasty, keratoprostheses, phototherapeutic keratectomy, pterygium excision, corneal tattooing, and osteo-odonto-keratoprostheses (OOKP). In some instances, corneal surgeries do not require a laser. In other instances, corneal surgeries use a laser (e.g., phototherapeutic keratectomy, which removes superficial corneal opacities and surface irregularities). In some instances, patients are given dark eyeglasses to protect their eyes from bright lights after these procedures.

### **Cataract and Glaucoma Surgery**

[00300] Cataract surgery involves surgical removal of the lens and replacement with a plastic intraocular lens. Typically, a light is used to aid the surgeon.

[00301] Glaucoma surgery facilitates the escape of excess aqueous humor from the eye to lower intraocular pressure. In some instances, these medical procedures use a laser (e.g., laser trabeculoplasty applies a laser beam to burn areas of the trabecular meshwork, located near the base of the iris, to increase fluid outflow; laser peripheral iridotomy applies a laser beam to selectively burn a hole through the iris near its base; etc.). Canaloplasty is an advanced, nonpenetrating procedure designed to enhance drainage through the eye's natural drainage system utilizing microcatheter technology in a simple and

minimally invasive procedure. Other medical procedures used for the treatment of glaucoma include lasers, non-penetrating surgery, guarded filtration surgery, and seton valve implants.

### **Corticosteroids (Intravitreal triamcinolone acetonide)**

**[00302]** Corticosteroid reduces vascular permeability and reduces the breakdown of the blood retinal barrier. It inhibits VEGF gene transcription and translation and leukocyte adhesion to vascular walls. They especially address the complications related to increased vascular permeability.

**[00303]** Intra vitreal triamcinolone acetonide (IVTA) (4 mg), helped to reduce the risk of progression of diabetic retinopathy. However, the study concluded that use of IVTA to reduce the likelihood of progression of retinopathy is not warranted at this time because of the increased risk of glaucoma and cataract associated with IVTA and because PDR already can be treated successfully and safely with panretinal photocoagulation.

**[00304]** Several small randomized clinical trials demonstrated that the combination of laser photocoagulation (panretinal and macular) with IVTA was associated with improved best-corrected visual acuity and decreased central macular thickness and total macular volume when compared with laser photocoagulation alone for the treatment of PDR and macular edema. On the other hand, a recent study demonstrated no beneficial effect of combined IVTA plus panretinal photocoagulation and macular photocoagulation in eyes with coexisting high-risk proliferative diabetic retinopathy (PDR) and clinically significant macular edema as compared with panretinal photocoagulation and macular photocoagulation as standard treatment in those patients.

### **Anti-vascular endothelial growth factor (VEGF) treatment**

**[00305]** Currently, there are four anti-VEGF agents that are used for the management of diabetic retinopathy, including Pegaptanib (*Macugen; Pfizer, Inc., New York, USA*), Ranibizumab (*Lucentis; Genentech, Inc., South San Francisco, California, USA*), Bevacizumab (*Avastin; Genentech, Inc.*), and VEGF Trap-Eye (*Regeneron Pharmaceuticals, Inc., Tarrytown, New York, USA*).

**[00306]** Pegaptanib is a pegylated RNA aptamer directed against the VEGF-A 165 isoform. A phase II clinical trial of intravitreal pegaptanib in patients with DME with 36 weeks of follow-up demonstrated better visual acuity outcomes, reduced central retinal thickness, and reduced need for additional photocoagulation therapy. A retrospective analysis of the same study on patients with retinal neovascularization at the baseline showed regression of neovascularization after intravitreal pegaptanib administration. Recently in a retrospective study it was demonstrated that repeated intravitreal pegaptanib produced significant improvement in best-corrected visual acuity and reduction in mean central macular thickness in patients with diabetic macular edema.

**[00307]** Ranibizumab is a recombinant humanized monoclonal antibody fragment with specificity for all isoforms of human VEGF-A. Pilot studies of intravitreal ranibizumab demonstrated reduced foveal thickness and maintained or improved visual acuity in patients with DME. Recently, *Nguyen et al. (2009)* demonstrated that during a span of 6 months, repeated intravitreal injections of ranibizumab produced a significantly better visual outcome than focal/grid laser treatment in patients with DME. Diabetic Retinopathy Clinical Research Network (2010a) evaluated intra-vitreal 0.5 mg ranibizumab or 4 mg triamcinolone combined with focal/grid laser compared with focal/grid laser alone for treatment of diabetic

macular edema. *Nguyen et al. (2010)*, in a randomized study, showed that intraocular injection of ranibizumab provided benefit for diabetic macular edema for at least 2 years, and when combined with focal or grid laser treatments, the amount of residual edema was reduced, as were the frequency of injections needed to control edema.

[00308] VEGF Trap is a 115 kDa recombinant fusion protein consisting of the VEGF binding domains of human VEGF receptors 1 and 2 fused to the Fc domain of human IgG1. One pilot study showed that a single intravitreal injection of VEGF Trap-Eye was well tolerated and was effective in patients with diabetic macular edema.

[00309] Bevacizumab is a full length recombinant humanized antibody active against all isoforms of VEGF-A. It is FDA-approved as an adjunctive systemic treatment for metastatic colorectal cancer. Several studies reported the use of the off-label intra vitreal bevacizumab (IVB) to treat diabetic macular edema (DME), complications of proliferative diabetic retinopathy (PDR), and iris neovascularization. Several studies demonstrated that IVB injection resulted in marked regression of retinal and iris neovascularization, and rapid resolution of vitreous hemorrhage in patients with Proliferative diabetic retinopathy (PDR). In addition, IVB injection was demonstrated to be an effective adjunctive treatment to PRP in the treatment of high-risk Proliferative diabetic retinopathy (PDR) and neovascular glaucoma. The short-term results suggest that IVB has the potential not only to prevent the increase in retinal thickness, but also reduce the retinal thickness of eyes with diabetic macular edema (DME) after cataract surgery.

#### **Vitrectomy for persistent diffuse Diabetic Macular Edema**

[00310] Vitrectomy with removal of the premacular posterior hyaloid for persistent diffuse macular edema (DME) has gained rapid widespread acceptance. The large number of series evaluating the efficacy of vitrectomy (with or without internal limiting membrane peeling) has yielded conflicting results. In a trial it was observed that vitrectomy with internal limiting membrane peeling was superior to observation in eyes with persistent diffuse diabetic macular edema (DME) that previously failed to respond to conventional laser treatment and positively influenced distance and reading visual acuity as well as the morphology of the edema. Other studies suggested that vitrectomy with and without internal limiting membrane peeling may provide anatomic and visual benefit in eyes with diffuse nontractional unresponsive diabetic macular edema (DME) refractory to laser photocoagulation.

[00311] Other studies showed that the benefits of vitrectomy for diabetic macular edema (DME) in terms of visual acuity and macular thickness were limited to patients who exhibited signs of macular traction, either clinically and/or on optical coherence tomography.

#### **Pharmacologic Vitreolysis in the management of Diabetic Retinopathy**

[00312] During a demonstration it was observed that intravitreal injection of microplasmin with induction of the combination of posterior vitreous detachment (PWD) and vitreous liquefaction increased intravitreal oxygen tension. On the other hand, hyaluronidase induced vitreous liquefaction without posterior vitreous detachment (PWD) induction failed to increase intravitreal oxygen tension. Moreover, when microplasmin treated animals were exposed to 100% oxygen, there was an accelerated increase in oxygen levels in the midvitreous cavity compared to control or hyaluronidase treated eyes. These findings suggest that the

beneficial effects of surgical vitrectomy in increasing oxygen tension in the vitreous cavity may be reproduced with enzymatic induction of PVD and vitreous liquefaction without the time, risks, and expense of surgery. In 2009, it was demonstrated that intravitreal injection of autologous plasmin enzyme without the performance of vitrectomy induced complete PVD and effectively reduced macular thickening due to refractory diffuse diabetic macular edema and improved visual acuity. Therefore, a traumatic pharmacologic separation of the posterior vitreous cortex with clean cleavage between the internal limiting membrane and the posterior hyaloids without performing a vitrectomy can reduce the risk of intraoperative iatrogenic damage such as retinal tears, and damage to the nerve fibers, and the postoperative sequelae.

### **Fibrates**

[00313] Fibrates are widely prescribed lipid-lowering drugs in the treatment of dyslipidemia. Their main clinical effects, mediated by peroxisome proliferative activated receptor alpha activation, are a moderate reduction in total cholesterol and low-density lipoprotein cholesterol levels, a marked reduction in triglycerides and an increase in high-density lipoprotein cholesterol. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study demonstrated that long-term lipid-lowering therapy with fenofibrate reduced the progression of diabetic retinopathy and the need for laser treatment in patients with type 2 diabetes, although the mechanism of this effect does not seem to be related to plasma concentration of lipids. Recently, ACCORD Study Group (2010) demonstrated that fenofibrate for intensive dyslipidemia therapy reduced the rate of progression of diabetic retinopathy in persons with type 2 diabetes.

### **Renin-angiotensin system (RAS) blockers**

[00314] Several studies suggested that RAS blockers might reduce the burden of diabetic retinopathy. The findings of the Eurodiab Controlled trial of Lisinopril in Insulin-dependent Diabetes (EUCLID) suggested that blockade of the renin-angiotensin system with the angiotensin-converting enzyme inhibitor lisinopril could reduce both incidence and progression of retinopathy in type 1 diabetes.

### **Peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists**

[00315] The PPAR $\gamma$  agonist rosiglitazone inhibited both the retinal leukostasis and retinal leakage observed in the experimental diabetic rats. In addition, the decreased expression of the endogenous PPAR $\gamma$  in mice leads to the aggravation of retinal leukostasis and retinal leakage in diabetic mice. Rosiglitazone maleate (Avandia; GlaxoSmithKline, North Carolina, USA) is an orally administered medication used to improve glycemic control in patients with diabetes mellitus. This medication activates the PPAR $\gamma$  and leads to insulin sensitization in adipose and other tissues, with potential anti-angiogenic activity.

### **Anti- Protein Kinase C (Roboxistaurin)**

[00316] PKC mediates several ocular complications of diabetes. It is activated by VEGF and is a potential target for therapy of diabetic retinopathy.

[00317] Roboxistaurin (RBX), an oral PKC $\beta$  inhibitor is a selective inhibitor with adequate bioavailability to permit oral administration once daily. In the Protein Kinase C  $\beta$  inhibitor-Diabetic Retinopathy Study 2 (PKC-DRS2), oral administration of RBX (32 mg per day) reduced sustained

moderate visual loss, need for laser treatment for macular edema, and macular edema progression, while increasing occurrence of visual improvement in patients with non-proliferative retinopathy.

### **Islet cell transplantation**

**[00318]** Recent studies demonstrated that improved islet transplant outcomes could be observed with enhanced islet isolation, glucocorticoid-free immunosuppression, and provision of an adequate islet mass of more than 10,000 islet equivalents per kg of body weight. These improvements have resulted in benefits to type 1 diabetic subjects, including long-term C-peptide secretion, improved glycemic control, and reduced hypoglycemic episodes.

### **Therapeutic Oligonucleotides**

**[00319]** Oligonucleotides represent one of the new treatment entities targeting specific links in the disease process. There are two main categories of oligonucleotide therapeutic agents: antisense oligonucleotides, including short interfering RNA (siRNA), and oligonucleotide aptamers.

**[00320]** Antisense oligonucleotides are novel therapeutics designed to bind to specific messenger RNA (mRNA) that result in the degradation of the message encoding the targeted protein, thus affecting a decrease in the production of a particular protein associated with the targeted disease. Antisense oligonucleotide delivery via an intravitreous injection is a reasonable strategy in the treatment of retinal diseases. Alternative options for the drug delivery of antisense and other oligonucleotides have been under investigation, including periorbital administration, iontophoresis, and sustained release formulations.

### **Growth hormone and insulin growth factor (IGF)**

**[00321]** Growth hormone and Insulin growth factor (IGF) modulate the function of retinal endothelial precursor cells and drive retinal angiogenesis in response to hypoxia; IGF 1 can also disrupt the blood retina barrier and increase retinal vascular permeability.

### **Intravitreal hyaluronidase**

**[00322]** Intravitreal ovine hyaluronidase injection is effective in clearing vitreous hemorrhage. Several human case series demonstrated that intravitreal injection of autologous plasmin enzyme was a safe and effective adjunct to vitreous surgery for the treatment of diabetic macular edema and proliferative diabetic retinopathy.

### **Control of systemic factors:**

**[00323]** Primary prevention of diabetic retinopathy involves strict glycemic, lipid and blood pressure control. Some of the systemic factors that should be controlled for prevention of diabetic retinopathy are detailed below.

### **Glycemic control**

**[00324]** Hyperglycemia instigates the cascade of events that eventually leads to the development of diabetic retinopathy. Thus, one treatment that may be used to slow down the progression of diabetic retinopathy is glycemic control. Glycemic control may reduce the risk of development and progression of diabetic retinopathy in both type 1 and type 2 diabetes.

**Blood pressure control**

[00325] Hypertension exacerbates diabetic retinopathy through increased blood flow and mechanical damage (stretching) of vascular endothelial cells, stimulating release of VEGF. Tight blood pressure control may reduce the risks of retinopathy progression by about a third, visual loss by half, and the need for laser treatment by a third in people with type 2 diabetes. Blood pressure control may also reduce the incidence and progression of diabetic retinopathy.

**Serum lipid control**

[00326] Dyslipidaemia has a role in the pathogenesis of Diabetic Retinopathy. The severity of retinopathy was associated with increasing triglycerides and inversely associated with HDL cholesterol. Hydroxy methyl glutaryl coenzyme A (HMG CoA) inhibitors may be useful in the management of Diabetic Retinopathy (DR) and diabetic macular oedema (DMO) in patients with Dyslipidaemia.

**EXAMPLES**

[00327] The application may be better understood by reference to the following non-limiting examples. The following examples are presented in order to more fully illustrate representative embodiments and should in no way be construed, however, as limiting the broad scope of the application.

[00328] The term ACU-4429 refers to the compound (R)-3-amino-1-(3-(cyclohexylmethoxy)phenyl)propan-1-ol. The term ACU-4935 refers to the compound (R)-3-amino-1-(3-(2-propylpentyloxy)phenyl)propan-1-ol.

**Example 1: Accepted animal models of Diabetic Retinopathy**

[00329] Mice, rats, hamsters, dogs, cats, and monkeys are some of the common animal models that are used for studying Diabetic Retinopathy.

[00330] Animal experiments have been pivotal in the understanding of the pathogenesis of retinopathy since systematic structural, functional and biochemical studies cannot be undertaken in human subjects. Animal experiments are of immense importance in an attempt to develop adjuvant treatment strategies. Characteristic retinal lesions in diabetes have successfully been reproduced in experimental diabetic or galactose-fed animals.

[00331] Data obtained from 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 ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration arrange that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition) as determined in cell culture. Levels in plasma can be measured, for example, by high performance liquid chromatography. Such information can be used to more accurately determine useful doses in humans.

[00332] Some of the common animal models for studying Diabetes Retinopathy along with the source and relevant text are detailed below:

*Mice*

[00333] Protocols which may be used to test compounds for efficacy of treatment in mice include those described in, for example, Diabetic Retinopathy by Elia Duh, Springer, Humana Press, 2009; Kern *et al.* (*Arch Ophthalmol.* 1996;114(8):986-990); Feit-Leichman *et al.* (*Investigative Ophthalmology & Visual Science*, 46(11): 4281-4287, November 2005).

*Rats*

[00334] Protocols which may be used to test compounds for efficacy of treatment in rats include those described in, for example, Diabetic Retinopathy by Elia Duh, Springer, Humana Press, 2009; Sima *et al.* (*Current Eye Research*, 1985, Vol. 4(10) Pages 1087-1092); Kato *et al.* (*Journal of Diabetes and Its Complications*, Volume 17(6): 374-379, November 2003); Sima *et al.* (*Metabolism*, 32(7, Suppl. 1): 136-140, July 1983); Lu *et al.* (*Journal of Ophthalmology*, 47(1): 28-35, 2003); and Deng *et al.* (*International Journal of Diabetes*, vol. 6 (issue 1), 1998).

*Hamsters and other rodents*

[00335] Protocols which may be used to test compounds for efficacy of treatment in hamsters and other rodents include those described in, for example, Diabetic Retinopathy by Elia Duh, Springer, Humana Press, 2009.

*Dogs*

[00336] Protocols which may be used to test compounds for efficacy of treatment in dogs include those described in, for example, Diabetic Retinopathy by Elia Duh, Springer, Humana Press, 2009; Engerman *et al.* (*Arch Ophthalmol.* 1995; 113(3):355-358); and Kador *et al.* (*Arch Ophthalmol.* 1990; 108(9):1301-1309).

*Cats*

[00337] Protocols which may be used to test compounds for efficacy of treatment in cats include those described in, for example, Diabetic Retinopathy by Elia Duh, Springer, Humana Press, 2009; Mansour *et al.* (*Investigative Ophthalmology & Visual Science*, Vol. 31, No. 3, March 1990); and Henson and O'Brien (ILAR Journal Volume 47(3): 234-242).

*Monkeys / primates*

[00338] Protocols which may be used to test compounds for efficacy of treatment in monkeys and primates include those described in, for example, Kim *et al.* (*Invest Ophthalmol Vis Sci.* 2004;45:4543-4553); Akimba: A Novel Murine Model for Diabetic Retinopathy ([www.Bio-link.com](http://www.Bio-link.com)); and Diabetic Retinopathy by Elia Duh, Springer, Humana Press, 2009.

**Example 2: Use of compounds for the treatment of Diabetic Retinopathy**

[00339] A single-center, open-label, dose-escalating pilot study is initiated to evaluate the biologic activity of oral administration of compounds described herein in patients with center-involving clinically significant diabetic macular edema (DME) and to report any associated adverse events. Patients with

DME involving the center of the macula and best-corrected visual acuity (BCVA) in the study eye between 20/63 and 20/400 are enrolled.

[00340] Eligible patients are randomly assigned in a 1:1 ratio to receive daily oral doses of compound (2 mg, 5 mg, 7 mg, 10 mg or 20 mg) administered until month 24. Primary end points are the frequency and severity of ocular and systemic adverse events. Secondary end points are 1) best corrected visual assessment as assessed with the Early Treatment Diabetic Retinopathy Study (ETDRS) chart, with the use of standardized refraction and testing protocol at a starting test distance of 2 m and 2) measurement of retinal thickness by optical coherence tomography. The evaluating physician is unaware of the patient's treatment assignment; the physician who administers the dose is aware of the patient's treatment assignment regarding test or sham treatment but is unaware of the dose of compound. Other personnel at each study site, patients, and personnel at the central reading center are unaware of the patient's treatment assignment.

[00341] Efficacy analyses are performed on an intention-to-treat basis among all patients with the use of a last-observation-carried-forward method for missing data. For all pair-wise comparisons, the statistical model is adjusted for baseline score for visual acuity (<55 letters vs. ≥55 letters). Between-group comparisons for dichotomous end points are performed with the use of the Cochran chi-square test. Change from baseline visual acuity is analyzed with the use of analysis-of-variance models. For end points for lesion characteristics, analysis-of-covariance models adjusting for the baseline value are used. The Hochberg–Bonferroni multiple-comparison procedure is used to adjust for the two pair-wise treatment comparisons for the primary end point. Safety analyses include all treated patients.

[00342] Compounds are expected to be well-tolerated therapy for patients with DME. The compounds will have the potential to maintain or improve best corrected visual acuity and reduce retinal thickness in patients with center-involved clinically significant DME.

#### **Example 3: Manganese-enhanced magnetic resonance imaging (MEMRI) Protocol**

[00343] Rats are to be maintained in regular laboratory lighting (12 hours light, 12 hours dark) prior to start of experimental period – light exposure, bleaching, dark adaption will vary by cohort (see below).

[00344] Animals are to be dosed by oral gavage according to group assignment below. Rats are to be weighed each week of the experimental period.

[00345] Dilate pupils by applying 1 drop of tropicamide (0.5%) 10-30 minutes before photobleaching. Photobleach animals for 10 minutes by exposure to 5000lux 4 hours before MRI imaging.

[00346] Inject rats immediately after bleach, 4 hours prior to the start of imaging session. MEMRI signal reflects state of expression of activity-dependent channels during experimental period.

[00347] Inject MnCl<sub>2</sub> is intraperitoneally in lower right abdomen of awake rat.

[00348] MnCl<sub>2</sub> is injected at 60mg/kg using a 20 mg/mL stock solution.

[00349] Mark each rat injected and record injection, time of bleach, start and end of imaging as well as light conditions in notebook

[00350] Keep rats in dark (IOP room) during the 4 hours between injection and transport to the imaging center for practice experiments for all groups except group 4. Keep animals from group 4 exposed to light during 4 hours between MnCl<sub>2</sub> injection and MRI imaging. Otherwise follow light-dark-bleaching cycle described for each cohort.

[00351] Transport rats to imaging center via IACUC-approved route, following closely light-dark cycle for each cohort.

[00352] Image either both eyes of each rat, or unilateral as mandated by particular experiment.

[00353] MRI parameters include:

[00354] A snapshot FLASH inversion recovery (IR) imaging sequence is used to acquire a single imaging slice bisecting the retina in the axial and sagittal planes, using a 12mm inner diameter linear surface coil. Imaging parameters are TR/TE = 1000/2.7 ms, with a 125 ms inversion time (TI), sweep width = 73.5 kHz, number of acquisitions = 120; slice thickness = 0.7mm, field of view = 12mm x 12mm, with 256x256 data matrix, resulting in an in-plane resolution of 47 microns. The approximate scan time per animal is ~16 minutes.

[00355] The total time required to image one eye (including setup and scout imaging) is approximately 1 hour. If animal moves, re-image.

[00356] (T1 mapping): Determined as optimal during protocol development

A snapshot FLASH inversion recovery (IR) imaging sequence is used to acquire a single imaging slice bisecting the retina in the axial and sagittal planes, using a 12mm inner diameter linear surface coil. Imaging parameters are TR/TE = 2000/2.7 ms, sweep width = 73.5 kHz, number of acquisitions = 32; slice thickness = 0.7mm, field of view = 12mm x 12mm, with 192x192 data matrix (zero padded to 256x256), resulting in a nominal in-plane resolution of 47 microns. The signal acquired at six inversion times [TI = 50, 150, 300, 400, 900, 1800ms] were used to obtain a T1 map.

[00357] Wait until animal has woken up from anesthesia before transporting. Use heat lamp after imaging to help maintain body temperature while animal is waking up from anesthesia

[00358] Cohorts

Group	Drug Treatment	Light Treatment After Bleach	Number of Animals
1	ACU-4429 (1 mg/kg/day)	Dark Adaptation	5
2	ACU-4429 (10 mg/kg/day)	Dark Adaptation	5
3	ACU-4429 Vehicle	Dark Adaptation	5
4	ACU-4429 Vehicle	Light Adaptation	5
5	Retinyl Acetamide (200mg/kg)	Dark Adaptation	5
6	Retinyl Acetamide Vehicle	Dark Adaptation	5
<b>Total</b>			30

*Study Design*

[00359] Timeline for Groups 1-3 are illustrated in Figure 1.

[00360] Timeline for Group 4 is illustrated in Figure 2.

[00361] Timeline for Groups 5-6 are illustrated in Figure 3.

*Single Dose Study*

[00362] The purpose of this study is to determine whether a single (high) dose of ACU-4429 reduces the return of retinal cationic activity ( $Mn^{2+}$  uptake) following dark adaptation post-bleaching. Groups 1-4 (ACU-4429 *vs.* vehicle) will be dosed and kept in room light for 2 hours. Groups 5 and 6 (retinyl acetamide *vs.* vehicle) will be dosed 18 hours before bleach. The animals will have pupil dilation and be exposed to a moderate bleaching white light (5,000 lux of diffuse white fluorescent light) for 10 minutes. Immediately after bleach, the animals will be intraperitoneally (IP) injected with  $Mn^{2+}$ , followed by 4 hrs of dark adaptation (animals will be kept dark-adapted while in the imaging queue). Animals in Groups 3 will be left in ambient room light to serve as light control (the expectation is that the retinas treated with Retinyl acetamide and ACU-4429 to behave as if they were light adapted). MRI imaging (30 minutes-1 hr per animal) will be conducted at 4 hours after  $Mn^{2+}$  injection (i.p.) and will be performed in the same light conditions animals were housed in prior to the imaging. Dosing of the animals will be staggered to insure that the time from dose to imaging is the same for all animals

*Multi-dose Study*

[00363] The purpose of this study is to test whether repeat ACU-4429 (10 mg/kg/day) treatment in normal cyclic light over time reduces the return of retinal cationic activity ( $Mn^{2+}$  uptake) following dark adaptation. Three groups: Group 1: ACU-4429 at 5 mg/kg bid (10 mg/kg/day); Group 2: vehicle (dark adapted); Group 3: vehicle (room light). All dosing will be done at lights on and at lights off for 6 days under conditions of normal cyclic light exposure (12 hours of about 100 lux of diffuse white fluorescent light). Immediately following the morning dose of Day 7, a drop of atropine sulfate (1%) will be applied to both eyes of all animals to dilate the pupils. Six hours after administration of the last dose and after at least 6 hours in normal light, Group 1 (ACU-4429) and Group 2 (Dark adapted) will be IP injected with  $Mn^{2+}$ , followed by 4 hrs of dark adaptation (animals will be kept dark-adapted while in the imaging queue) and imaged in the dark (30 minutes-1 hr per animal). Group 3 (room light) will be IP injected with  $Mn^{2+}$  6 hours after last dose and remain in normal room light 4 more hours until imaging. Imaging of Group 3 will occur under normal light.

**Example 4: Reduction of Oxygen-Induced Retinopathy in Rats**

[00364] **Purpose:** A test compound is assessed in rats with oxygen-induced retinopathy (OIR), a common model of human retinopathy of prematurity (ROP). Both OIR and ROP are characterized by abnormal retinal vasculature and by lasting dysfunction of the neural retina.

[00365] **Methods:** OIR is induced in four litters of Sprague-Dawley pups (N=24) by exposure to alternating periods of 50% and 10% oxygen from the day of birth (P0) to P14. The light cycle is 12 hr light (10-30 lux) and 12 hr dark; the light-to-dark transition coincides with each oxygen alternation. For

15 days beginning P7, within one hour of this transition, the first and fourth litters are orally administered 6 mg/kg of a clinical development candidate; the second and third litters receive only vehicle. At P20-22, when marked retinal vascular abnormality is typically observed, electroretinograms are recorded and receptor and post-receptor function are evaluated. Treatment effects are evaluated by ANOVA.

**[00366] Assessment:** Maximal rod response and the amplification constant of phototransduction changed by treatment with the clinical development candidate are assessed. Additionally, the time-constant of deactivation of phototransduction is assessed by a double-flash protocol. Post-receptor sensitivity (log s) and maximal scotopic b-wave amplitude are also assessed. Alteration of the photoreceptor response after treatment with the clinical development candidate and responses originating in the inner retina may be assessed. The inner retina is supplied by the retinal vasculature; quantitative image analysis of fundus photographs is used to determine the degree of vascular abnormality associated with OIR following such treatment. It is anticipated that the degree of vascular abnormality will be reduced in animals treated with the clinical development candidate.

**Example 5: Visual Cycle Modulation and Rod Function in a Rat Model of ROP.**

**[00367]** Rat models of ROP provide a convenient *in vivo* system in which the relation of the photoreceptors to the retinal vasculature can be studied and manipulated.

**[00368]** Both OIR and ROP are characterized by lasting dysfunction of the neural retina and by abnormal retinal vasculature. The systemic effects of a clinical development candidate, a visual cycle modulator (VCM), are studied on rats with oxygen-induced retinopathy (OIR). .

**[00369]** Retinopathy is induced in Sprague-Dawley pups (N=46) by exposing them to alternating 24 hour periods of 50±1% and 10±1% oxygen from the day of birth to postnatal day (P) 14. The light cycle is controlled at 12 hours 10-30 lux and 12 hours dark, except during test days when constant darkness is maintained. The light-to-dark transition is timed to coincide with each oxygen alternation.

**[00370]** For two weeks, beginning on P7, during this transition, the first and fourth litters are orally administered 6 mg/kg of the clinical development candidate; the second and third litters are administered an equivalent volume of vehicle (20% dimethyl sulfoxide, DMSO) alone. The administration schedule is designed to continue over the age range that begins with the onset of rapid increase in the rhodopsin content of the retina and lasts until rhodopsin content exceeds 50% of its adult amount (Fulton and Baker, *Invest Ophthalmol Vis Sci* (1984) 25:647).

**[00371]** The treated rats are held in room air (20.8% oxygen) for approximately 20 minutes between each oxygen alternation from P7-14. The rats are assessed following a longitudinal design with tests at P20-22, P30-32, and P60-62. These dates are selected because they capture the height of vascular abnormality, a period of marked recovery, and an adult age, respectively. At each test age, the function of the neural retina and the morphology of the retinal vasculature are assessed using non-invasive techniques.

**[00372]** Shortly (0-2 days) after the final dose, the effects of the compounds are assessed on the neural retina by electroretinography (ERG). The timing and intensity of the stimuli, which is designed to assess rod photoreceptor and rod-mediated post-receptor neural function, are under computer control. Two sets

of experiments are performed. In the first, rod and rod-mediated neural function in the dark-adapted retina are assessed. In the second, the recovery of the rod photoreceptor from a bright, rhodopsin-bleaching stimulus is assessed. Each set of experiments is performed on approximately half of the patients from each litter.

[00373] To assess whether VCM treatment affected the retinal vasculature, wide-field images of the ocular fundus are obtained that show the major vessels of the retina following each ERG session. As shown in Figure 19, the images are composited to display a complete view of the posterior pole, the region within the circle bounded by the vortex veins and concentric to the optic nerve head, and the retinal region that in human patients is most important to the diagnosis of high-risk ROP. The arterioles are analyzed with RISA custom image analysis software (Gelman, *Invest Ophthalmol Vis Sci* (2005) 46: 4734).

**Example 6: Animal models of Laser-Induced Choroidal Neovascularization and Macular Degeneration**

*Murine Model of Choroidal Neovascularization*

[00374] The effect of the VCM compounds described herein can be assessed in a murine model of choroidal neovascularization.

[00375] Briefly, 4 to 5 week old C57BL/6 mice are anesthetized ketamine hydrochloride:xylazine (100 mg/kg:10mg/kg) and the pupils dilated with 1% tropicamide (Alcon Laboratories, Inc Fort Worth, TX). Three burns of a 532-nm diode laser photocoagulation (75- $\mu$ m spot size, 01-second duration, 120 mW) are delivered to each retina using the slit lamp delivery system of a photocoagulator (OcuLight;Iridex, Mountain View, CA) and a handheld cover slip as a contact lens. Burns are performed in the 9, 12 and 3 o'clock positions of the posterior pole of the retina. Production of a bubble at the time of lasering, which indicates rupture of Bruch's membrane, is an important factor in obtaining CNV; thus only burns in which a bubble is produced are included in the study.

[00376] Four independent experiments are performed to investigate the effect of a clinical development candidate when orally administered on day 0 after rupture of Bruch's membrane. Mice in Group 1-4 are orally administered a daily dose of 0.3, 1, 3, and 10 mg/kg of the clinical development candidate, respectively. Group 4 receive vehicle only.

[00377] After 14 days, mice are anesthetized and perfused with fluorescein-labeled dextran ( $2 \times 10^6$  average molecular weight, Sigma-Aldrich) and choroidal flat mounts are prepared. Briefly, the eyes are removed, fixed for 1 hour in 10% phosphate-buffered formalin, and the cornea and lens are removed. The entire retina is carefully dissected from the eyecup, radial cuts are made from the edge of the eyecup to the equator in all four quadrants, and the retina is flat-mounted in aqueous mounting medium (Aquamount; BDH, Poole, UK). Flat mounts are examined by fluorescence microscopy (Axioskop; Carl Zeiss Meditec, Thornwood, NY), and the images are digitized with a three charge-coupled device (CCD) color video camera (1K-TU40A, Toshiba, Tokyo, Japan). A frame grabber image-analysis software is

used to measure the area of each CNV lesion. Statistical comparisons are made using ANOVA with Dunnett's correction for multiple comparisons.

*Murine Model of Suppression of Choroidal Neovascularization*

**[00378]** Though animals do not develop age related macular degeneration (AMD) *per se*, choroidal neovascularization resembling that seen in AMD can be produced by using a laser to produce focal disruptions in Bruch's membrane and the overlying retinal pigment epithelium (RPE). This injury stimulates the abnormal growth of underlying choroidal capillaries into the RPE layer and subretinal space. Disruption of Bruch's membrane is common to all forms of choroidal neovascularization (CNV), including that which characterizes the wet form of AMD.

**[00379]** In the laser-induced model of choroidal neovascularization, groups of 9 or 10 mice are treated with oral administration of (1) a clinical development candidate, or (2) sham treatment one day prior to laser injury and on days 2, 5, 8, and 11 after laser. At 14 days after laser injury, the mice are injected intravenously with fluorescein-labeled dextran (50 mg), euthanized, and eyes are rapidly dissected for choroidal flat mounts or frozen in optimum cutting temperature embedding compound and sectioned for evaluation of the lesions.

**[00380]** CNV lesions are visualized by fluorescein angiography and graded according to standard procedures.

**Example 7: Efficacy Study in the Chronic Light- Induced Choroidal Neovascularization**

**[00381] Purpose:** The purpose of this study was to test the efficacy of 3 months (90 days) once daily oral treatment with a clinical development candidate at 0.3 and 3 mg/kg/day for protection against 3000 Lux light damage *in vivo* using Wistar rats. Long term light damage (3 months) in rats has been shown to result in photoreceptor degeneration and choroidal neovascularization (CNV). Efficacy of an exemplary clinical development candidate in protecting against light induced ONL loss and CNV was evaluated.

**[00382] Materials and Methods:** On the day prior to the start of dosing and once weekly for 13 weeks, the clinical development candidate was weighed into new empty glass scintillation vials. The clinical development candidate was dissolved in deionized water to a concentration necessary to achieve desired dose at the desired dose volume (0.5 mL/animal). The dosing solutions were stored at 4° C and used for dose administration once per day for one week. The vehicle used for dosing control groups was deionized water. Sixteen Female Wistar rats (Charles River Laboratories) were used for this study. The animals were approximately 12 weeks at the initiation of dosing with an average body weight of 220 grams.

**[00383] Assay:** Animals were dosed once daily in the morning (within 1 hour of light onset) orally, by gavage, with the assigned vehicle control or test articles using a 1 mL syringe fitted with a 20 gauge oral gavage needle. The animals were housed in cyclic light so that there was 12 hours of 3000 lux white light at the center of the cages alternating with 12 hours darkness. Upon the completion of the study animals were euthanized with carbon dioxide followed by creating pneumothorax. Immediately following the cervical dislocation both eyes of the animal were removed for analysis. The analyses consisted of staining of sections and flatmount analysis. The eye cups were fixed in 4% PFA for 1 hour

at room temperature. One eye cup was processed for paraffin embedding, sectioned and stained with H&E or isolectin B4. The other eye was fixed for flat mounting. Flatmount eyes were dissected into the retina and choroid/sclera complex. Both the retina, choroid/sclera complex were stained with isolectin B4.

**[00384] Study Design**

Treatment Designations and Animal Assignments Group	Treatment	Dose (mg/kg)	3000 Lux exposure	No. of animals per group	Total
NC(1,2)	Vehicle	NA	No	2	4
3,2	Vehicle	NA	Yes	2	4
5,6	ACU-4429	3	Yes	2	4
7,8	ACU-4429	0.3	Yes	2	4

NC = Normal light Control

**[00385] Data analysis:** Sections of the eye were examined by microscope after H&E staining and ONL area near the optic nerve was photographed at 40×10 magnifications for outer nuclear cell counts. The microscope photographs were printed on an 8" x 11" paper. The numbers of ONL nuclei intersected by two vertical lines evenly dispersed on the picture were counted and average cell numbers represent the ONL thickness for that eye. Parafin sections were stained with isolectin B4 to determine if choroidal neovascularization was present. Isolectin B4 stains blood vessels. (See Figure 16.) To quantify choroidal neovascularization the number of vessels crossing from the choroid and through the retina were counted per section and analyzed in Excel. The vessels were counted in 10-33 sections and data were reported as an average per animal. Since the flatmount data was inconclusive, it was excluded from this report.

**[00386] Results**

*ONL raw count*

Conditions	DC				LC				4429, 3mg/kg				4429, 0.3mg/kg			
Animal #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
count 1	8	7	11	10	1	2	0	1	2	1	1	1	0	0	1	1
count 2	8	6	9	12	1	1	0	1	3	2	1	1	1	1	1	2
count 3	10	9	10	9	1	0	1	1	1	2	2	1	0	1	1	2
count4	10	9	10	9	1	0	1	2	3	2	2	1	0	1	1	1
Average	9.0	7.8	10.0	10.0	1.0	0.8	0.5	1.3	2.3	1.8	1.5	1.0	0.3	0.8	1.0	1.5
Group average	9.2				0.9				1.6				0.9			

*One-way ANOVA*

Tukey's Multiple Comparison Test	Mean Diff.	q	P value

LC (Vehicle) vs DC	-8.325	25.25	P < 0.01
LC (Vehicle) vs 4429, 3mg/kg	-0.750	2.275	P > 0.05
LC (Vehicle) vs 4429, 0.3mg/kg	0.00	0	P > 0.05
4429, 0.3mg/kg vs 3mg/kg	-0.750	2.275	P > 0.05

[00387] Figure 22 illustrates the number of rows of nuclei in the outer nuclear layer in H&E section from animals treated with ambient light and 3000 lux per vehicle or the clinical development candidate. Data are mean  $\pm$  SEM.

### *Raw vessel count*

Conditions	DC				LC				3 mg/kg				0.3 mg/kg			
counts	0						0		0			1				
	0								0			0				
	0								0			0				
	0								0			0				
	0								0							
Average	0.03	0	0	0	0.30	0.38	0.65	0.41	0.19	0.16	0.16	0.14	0.03	0.15	0	0.24
Group average	<b>0.008</b>				<b>0.437</b>				<b>0.162</b>				<b>0.104</b>			

*One-way ANOVA*

Tukey's Multiple Comparison Test	Mean Diff.	q	P value
LC (Vehicle) vs. DC	0.4296	9.046	P <0.01
LC (Vehicle) vs. 4429, 3mg/kg	0.2755	5.801	P <0.01
LC (Vehicle) vs. 4429, 0.3mg/kg	0.3328	7.008	P <0.01
4429, 0.3 mg/kg vs. 3mg/kg	-0.0573	1.207	P>0.05

[00388] Figure 23 illustrates the number of vessels crossing layers/sections.

**Conclusions:** The clinical development candidate protects the retina from light induced ONL thinning. The treatment with the clinical development candidate provided significant protection against choroidal neovascularization.

**Example 8: Phase I Dose-Ranging Study of ACU-4429, a Novel Visual Cycle Modulator, in Healthy Volunteers**

[00389] Visual Cycle Modulation (VCM) refers to the biological conversion of a photon into electrical signal in the retina. (See, e.g., Figures 4A and 4B)

[00390] The retina contains light-receptor cells known as “rods” (responsible for night vision) and “cones” (responsible for day vision). Rod cells are much more numerous and active than cones. Rod over-activity creates the build-up of toxins in the eye, whereas cones provide the vast majority of our visual information – including color. The VCM essentially “slows down” the activity of the rods and reduces the metabolic load on the cones.

[00391] Isomerase/RPE65 represents one target for inhibition as it is specific to the visual cycle. Rod cells are the major source of A2E (90% of photoreceptor cells)

[00392] A2E Toxicities:

- Free radical generation upon light exposure;

- Detergent-like properties can damage RPE cell membrane;
- Inhibits RPE lysosomes (leads to drusen formation); and
- Activation of complement factors.

[00393] ACU-4429 was designed to prevent or inhibit the generation of toxic by-products of the visual cycle, which can lead to degenerative eye conditions. It is administered to patients as an oral, daily pill rather than by injection into the eye. Preclinical data indicate that ACU-4429 slows the rod visual cycle.

*Phase 1 data:*

[00394] Safety and tolerability was observed in healthy volunteers aged 55-80. A dose-dependent modulation of visual cycle was observed by electroretinography (ERG).

*Clinical Safety and Tolerability*

[00395] 125 healthy subjects were dosed with ACU-4429. It was well tolerated in these healthy subjects with no AEs of concern to DMC. Headaches were seen in some subjects, but were transient and could be unrelated to drug. Mild and transient visual AEs were observed. ACU-4429 produced a very good pharmacological response even at lower doses. No changes in cone ERGs were observed.

[00396] Overall, ACU-4429 has oral bioavailability. There was a linear correlation between dose and AUC and  $C_{max}$  and a steady state is reached after the first dose. A dose dependent decrease in ERG b-wave amplitude was observed.

[00397] AUC increased approximately proportionally with dose, therefore systemic exposure can be easily adjusted in the clinic with increase or decrease of oral dose of ACU-4429. Maximal plasma concentration ( $C_{max}$ ) also increased linearly with dose. ACU-4429 was readily absorbed from the GI tract. (See Figure 7.) ACU-4429 Phase 1a Rod ERG Suppression (24h) is illustrated in Figure 6.

Dose	Suppression
20 mg	29% $\pm$ 35%
40 mg	86% $\pm$ 10%
60 mg	93% $\pm$ 4%
75 mg	98% $\pm$ 1%

*Phase 1b Study Design*

<b>Study Design</b>	Single-center, randomized, double-masked, placebo-controlled, multi-dose escalating study
<b>Objective</b>	Assess safety, tolerability, and pharmacokinetics (PK)

<b>Dose</b>	Five cohorts, randomized 6:2 5, 10, 20, 30, 40 mg 14 days per cohort
<b>Endpoints</b>	Safety, tolerability, and PK
<b>Major Inclusion Criteria</b>	Healthy volunteers of both genders, aged 55 - 80, weight $\geq 50$ and $\leq 110$ kg
<b>Major Exclusion Criteria</b>	<ul style="list-style-type: none"> <li>Ocular conditions (cataracts, glaucoma, uveitis, diabetic retinopathy, and active conjunctivitis)</li> <li>Change in prescription chronic medications within 28 days</li> <li>Treatment in the past year with a retinoid compound</li> <li>Treatment within the last week with Viagra<sup>®</sup>, Cialis<sup>®</sup>, Levitra<sup>®</sup></li> <li>Concomitant treatment with hypnotics, anti-depressants and psychoactive substances; digitalis glycosides (digoxin, ouabain, digitoxin); L-DOPA; chloroquine or hydroxychloroquine; systemic corticosteroids; topical anti-glaucoma medications; medications for treatment of “wet” AMD</li> </ul>

*Phase 1b – Demographics*

	<b>ACU-4429 N=30</b>	<b>Placebo N=10</b>
Age, mean (SD)	39.8 (8.48)	37.7 (8.55)
Male, n (%)	22 (73.3%)	8 (80%)
Race, n (%)		
White	25 (83.3%)	5 (50.0%)
Black or African American	5 (6.7%)	3 (30%)
Asian	0	1 (10%)
Other	0	1 (10%)

*Phase 1b – Summary Adverse Events*

Cohort	Number of subjects with visual AEs	Number of visual AEs
5 mg	0	0
10 mg	2	21*
20 mg	6	29
30 mg	6	26
40 mg	6	33

\*1 subject had 19 visual AEs; all visual adverse events were mild.

*Phase 1b PK Data*

[00398]  $C_{max}$  was approximately 4 hours after 1<sup>st</sup> and last dose; PK parameters similar to Phase 1a study; and levels reached a steady state after 1<sup>st</sup> dose. (See Figure 7).

**Example 9: Experiment to test if ACU-4935 reduced VEGF up-regulation caused by hypoxic conditions**

[00399] Figure 8 depicts a protocol used to test if ACU-4935 reduced VEGF up-regulation caused by hypoxic conditions. Briefly, animals were adapted for dark for 16 hours, then dosed with ACU-4935. Animals were photobleached for 10 minutes with 50000 Lux 2 hours after being dosed, followed by a 2 hour recovery in the dark. Hypoxia was induced with 6% O<sub>2</sub> for 6 hours. A portion of the animals were euthanized and samples were collected at time 0. Another portion of the animals were returned to the dark for 2 more hours prior to being euthanized and sample collection.

[00400] Samples were tested for VEGF protein (Figure 9) and mRNA expression (Figure 10). Slight differences were observed in VEGF protein expression following treatment with ACU-4935. VEGF mRNA levels were decreased at time 0 and slightly increased 2 hours post-hypoxia following treatment with ACU-4935 compared to the vehicle control.

**Example 10: Ocular Distribution of [14C]-ACU-4429 in Beagle Dogs**

[00401] ACU-4429 (C<sub>16</sub>H<sub>25</sub>NO<sub>2</sub>·HCl) is an oral visual cycle modulator which has been shown to reduce the activity of the rod visual system, thereby alleviating the metabolic load on the retina.

[00402] The following experiment was conducted to examine the pharmacokinetic profile, ocular distribution, and excretion of ACU-4429 and its metabolites in male beagle dogs after single and repeated oral doses of 0.3 mg/kg of [<sup>14</sup>C]-ACU-4429 (40 μCi/kg).

[00403] [<sup>14</sup>C]-ACU-4429 (0.3 mg/kg, 40 μCi/kg) as a powder in capsule was administered as a single oral dose or repeated doses (once daily for 7 days) to a total of 36 male beagle dogs that were not fasted.

Mass balance was assessed through 168 hours after a single dose or through 336 hours after the first daily dose; urine and feces were analyzed for radioactivity and metabolic profiling. Blood was collected at 0.25, 1, 2, 4, 8, 12, 48, 72, 96, 168 and 192 hours following the final dose; blood and plasma were analyzed for radioactivity and plasma for metabolic profiling. Eye tissues (choroid, iris-capillary body, and RPE) were collected at 4, 8, 12, 24, 48, 72, and 168 hours after the final dose (3 animals/time point) and analyzed for radioactivity (right eyes) or metabolic profiling (left eyes).

[00404] In beagle dogs, orally administered [<sup>14</sup>C]-ACU-4429 was readily absorbed ( $T_{max} = 4$  hours) and eliminated from plasma; the majority of radioactivity was not preferentially associated with RBCs. Radioactivity was rapidly eliminated through urine and feces (46% and 44%, respectively), and clearance from plasma was essentially complete by 48 hours post-dose. Other data indicated ACU-4429 parent molecule was preferentially distributed to melanin-containing ocular tissues, including the proposed site of VCM action, the RPE, in spite of rapid systemic clearance (See, Figures 11 and 12).

In eye tissues, ACU-4429-C<sub>max</sub> was 278-fold higher than in plasma (930 vs. 3.34 ng-eq/g) after 7 consecutive days of oral dosing (Figure 11).

#### References

[00405]<sup>1</sup>Kubota *et al.*, *Retina*, 2012, 32(1): 183-188.

[00406]<sup>2</sup>Sparrow *et al.*, *Vision Res.*, 2003, 43(28): 2983-2990; Travis *et al.*, *Ann. Rev. Pharmacol. Toxicol.*, 2007: 47: 469-512.

#### **Example 11: VCMs as Inhibitors of Retinal Neovascularization**

[00407] Under dark conditions, ion channels in the retina are open, allowing excess ions to flow into retinal cells. The retina requires energy and oxygen to pump out the excess flow of ions. Under normal healthy conditions, the blood supply to the retina is just barely sufficient to support this process, which produces more heat and consumes more oxygen than any function in other cells. If the blood supply is compromised, as often occurs in patients with diabetes, hypoxia can develop in the retina. The retina creates new, small, leaky vessels to compensate, leading to the proliferative diabetic retinopathy.

[00408] Visual cycle modulators (VCMs), such as ACU-4420 and ACU-4935, inhibit the visual cycle isomerase<sup>2</sup>, thereby mimicking a state of constitutive phototransduction and decreasing the dark current (see Figure 14). Without being bound by theory, it is believed that decreasing the dark current will reduce metabolic strain and associated oxygen requirements in the retina, which should reduce hypoxia, production of hypoxic inducible factor 1 (HIF-1 $\alpha$ ) and vascular endothelial growth factor (VEGF), and result in inhibition of new vessel growth.

[00409] This study evaluated the effects of the VCMs ACU-4429 and ACU-4935 on retinal neovascularization in a mouse model of oxygen-induced retinopathy (OIR).<sup>3-5</sup>

[00410] 129 SvE mouse pups (PO) were treated as diagrammed in Figure 15. ACU-4429 (0.03 to 10 mg/kg), ACU-4935 (0.3 mg/kg/day), positive controls (10 mg/kg/day Ruboxistaurin) or vehicle was administered intraperitoneally twice for 4 days.

#### **Parameters for ACU-4429 and ACU-4935**

VCM	Chemical formula	IC <sub>50</sub> ( <i>in vitro</i> isomerase activity)	ED <sub>50</sub> ( <i>in vivo</i> isomerase assay single dose, mice)
ACU-4429	C <sub>16</sub> H <sub>25</sub> NO <sub>2</sub> ·HCl	4.4 nM	0.18 mg/kg
ACU-4935	C <sub>17</sub> H <sub>29</sub> NO <sub>2</sub>	5.2 nM	0.0004 mg/kg

[00411] Pups were euthanized on P17, when neovascularization was maximal and eyes were removed for analysis. When retinoids were to be extracted, mice were moved to a dark room on P16 and euthanized under a red light.

[00412] Areas of retinal neovascularization were visualized with isolectin staining of flatmount preparations and quantified with the lasso tool in Adobe Photoshop; total area of neovascularization indicated the sum of individual areas across the retina, and % neovascularization was relative to the total area of the retina<sup>4</sup>.

Retinoids were extracted from right eyes under red light and analyzed for 11-*cis*-ROL-oxime content to indicate 11-*cis*-ROL concentrations and as an indicator of cycle isomerase activity.

[00413] Statistical analyses were performed using GraphPad Prism software.

[00414] In mice with OIR, treatment with either ACU-4420 or ACU-4935 significantly reduced retinal neovascular area compared to treatment with vehicle. Retinal neovascular area was reduced by 32% with ACU-4429 (3 mg/kg/day), 23% with ACU-4935 (0.3 mg/kg/day), and 29% with Ruboxistaurin (10 mg/kg/day, positive control); the mean reduction was significantly (p<0.05) greater than with vehicle with both of the VCMs and did not differ significantly (p<0.05) from Ruboxistaurin.

[00415] ACU-4429 inhibited neovascularization and production of 11-*cis*-RAL in a dose dependent manner with ED<sub>50</sub> values of 0.46 mg/kg and 0.88 mg/kg, respectively.

#### References

- [00416] 1. Arden *et al.*, *Br. J. Ophthalmol.*, 2005; 89(6): 764-769.
- [00417] 2. Kubota *et al.*, *Retina*, 2012; 32(1): 183-188.
- [00418] 3. Chan *et al.*, *Lab. Invest.*, 2005; 85(6): 721-733.
- [00419] 4. Connor *et al.*, *Nat. Protoc.*, 2009; 4(100): 1565-1573.
- [00420] 5. Yoshida *et al.*, *FASEB J.*, 2010; 24(6): 1759-1767.

#### Example 12: Electroretinography Materials and Methods

##### *Calibration of light flashes*

[00421] ERG stimuli are delivered using an Espion e<sup>2</sup> with ColorDome Ganzfeld stimulator (Diagnosys LLC, Lowell, MA). The rate of photoisomerization per rod (R\*) for the green LED flash is calculated by measuring the flux density incident upon an integrating radiometer (IL1700; International Light, Newburyport, MA) positioned at the location of the rat's cornea, and following the procedures detailed by Lyubarsky and Pugh (1996). The LED is treated as monochromatic with l equal to 530 nm. The intensity of the flash is given by

$$i(\lambda) = Q(\lambda) \cdot T(\lambda) \cdot \frac{a_{\text{pupil}}}{a_{\text{retina}}} \cdot a_{\text{rod}}(\lambda) \quad (1)$$

where  $i(\lambda)$  is  $R^*$ ,  $Q(\lambda)$  is the calculated photon density at the cornea,  $T(\lambda)$  is the transmissivity of the ocular-media and pre-receptor retina (~80% at 530 nm; Alpern et al., 1987), and  $a_{\text{pupil}}$ ,  $a_{\text{retina}}$  and  $a_{\text{rod}}(\lambda)$  are respective estimates of the area of the dilated pupil  $\sim \text{mm}^2$ ; Dodt and Echte, 1961), the area of the retinal surface ( $\sim 50 \text{ mm}^2$ ; Hughes, 1979), and the end-on light collecting area of the rod photoreceptor ( $\sim 1.5 \text{ mm}^2$  at 530 nm).  $a_{\text{rod}}(\lambda)$  takes into account the length of the outer segment, the absorption spectrum of the rod, and the optical density of the photopigment, as well as the radius of the photoreceptor (Baylor et al., 1979). Since several of these parameter values are unknown for the rat rod that is affected by OIR, stimuli are expressed as the expected values in adult control rats.  $Q(\lambda)$  is found by

$$Q(\lambda) = \lambda \cdot \frac{P\lambda}{h \cdot c} \quad (2)$$

where  $P\lambda$  is the radiant flux (W),  $h$  is Plank's constant and  $c$  is the speed of light (Wyszecki and Stiles, 1982). To evaluate the intensity of 'white' xenon-arc flashes, an intensity series with interspersed green and white flashes is recorded and the equivalent light is estimated based on the shift of the stimulus/response curves for the scotopic  $b$ -wave.

#### *Calibration of the bleaching light*

**[00422]** The bleach is produced using an Ektographic III B slide projector (Eastman Kodak, Rochester, NY) with an EXR 300 W halogen lamp (color temperature 3350°). To diffuse the light, a hemisected Ping-Pong ball is placed over the eye. The projector is positioned on a platform so that its lens is approximately 6 cm from the surface of the ball. The power of the light is measured using the radiometer, with the integration feature turned off, positioned under the Ping-Pong ball at the location of the rats' head. The calculation of the number of photons incident upon the photodetector (quanta  $\text{cm}^{-2} \text{ s}^{-1}$ ) is calculated using eq. (2) and assuming  $\lambda = 500 \text{ nm}$ . The strength of the bleach is estimated by

$$R_0(t) = \exp\left(-\frac{Q(\lambda) \cdot t}{Q_e}\right) \quad (3)$$

where  $1 - R_0$  is the fraction of rhodopsin bleached at the termination of the light exposure,  $t$  is the duration (60 s) of the exposure, and  $Q_e$  (quanta  $\text{cm}^{-2}$ ), the inverse of photosensitivity, is the energy needed to leave  $1/e$  of rhodopsin unbleached (Perlman, 1978). Earlier measurements indicate that the value of  $Q_e$  in Sprague Dawley rats is approximately  $15.8 \log \text{quanta cm}^{-2}$  (Fulton and Baker, 1984). Thus, the light, which produces approximately  $15.9 \log \text{quanta cm}^{-2}$ , bleached ~60% of the rhodopsin in the retina.

#### *Preparations*

**[00423]** Dark-adapted subjects are anesthetized with a loading dose of approximately  $75 \text{ mg kg}^{-1}$  ketamine and  $8 \text{ mg kg}^{-1}$  xylazine, injected intraperitoneally. This is followed by a booster dose (50% of loading dose)

administered intramuscularly. The pupils are dilated with a combination of 1% phenylephrine hydrochloride and 0.2% cyclopentolate hydrochloride (Cyclomydril; Alcon, Fort Worth, TX). The corneas are anesthetized with one drop of 0.5% proparacaine hydrochloride. A Burian-Allen bipolar electrode (Hansen Laboratories, Coralville, IA) is placed on the cornea and the ground electrode is placed on the tail. The red light is extinguished, and the animals remain in total darkness for an additional 10 min to allow them to return to a fully dark-adapted state before experimentation commences.

*The activation of phototransduction*

[00424] At the first test date, animals are assigned half-hazard such that half of each litter (rounded up if odd in number) participates in studies of the activation and deactivation of phototransduction, and of post-receptor retinal function; the remainder participate in the bleaching experiments. Characteristics of the rod photoresponse are estimated from the ERG by fitting the parameters of the Hood and Birch (1992) formulation of the Lamb and Pugh (1992; Pugh and Lamb, 1993) model of the biochemical processes involved in the activation of phototransduction to the *a*-waves elicited by the five brightest flashes:

$$P_3(i, t) = Rm_{P3} \cdot (1 - \exp(-1/2 \cdot i \cdot S \cdot (t - t_d)^2))$$

for  $t_d < t < 20$  ms. (4)

[00425] In this model,  $i$  is the intensity of the flash ( $R^*$ ) and  $t$  is elapsed time (s). The values of the free parameters in the model,  $Rm_{P3}$ ,  $S$ , and  $t_d$ , are optimized using a routine (fmins; MATLAB R11, The Mathworks, Natick, MA) that minimizes the sum of squared deviates.  $Rm_{P3}$  is the amplitude ( $\mu$ V) of the saturated rod response; it is proportional to the magnitude of the dark current and depends upon the number of channels available for closure by light in the ROS (Lamb and Pugh, 1992; Pugh and Lamb, 1993), which, under normal conditions, in turn depends directly upon the length of the ROS (Reiser et al., 1996).  $S$  is a sensitivity ( $R^{*-1} s^{-2}$ ) parameter that, if stimulus intensity is correctly specified, is related to the amplification constant,  $A$ , which summarizes the kinetics of the series of processes initiated by the photoisomerization of rhodopsin and resulting in closure of the channels in the plasma membrane of the photoreceptor.  $t_d$  is a brief delay (s). Fitting of the model is restricted to the leading edge of the *a*-wave.

*Deactivation of phototransduction*

[00426] In the same rats, using a double-flash paradigm, the time-course of the rod response to a 'green' ( $\lambda_{max} \approx 530$  nm) conditioning flash (CF) producing approximately 150  $R^*$  is derived. This green flash, while eliciting an *a*-wave of less than half of the saturated rod response, is nevertheless sufficient to fully suppress the dark current. First, the response to the CF is recorded alone. Then, the amplitude of the response to an intense, rod-saturating (approximately 10,000  $R^*$ ) 'white' xenon-arc probe flash is determined. The amplitude of the PF response,  $a_{max}$  ( $\mu$ V), which is measured at 8 ms after presentation (just before the trough of the *a*-wave), is taken as proportional to the maximal rod dark current. Next, the CF and PF are presented together, separated by 10 predetermined inter-stimulus intervals (10 ms, 20 ms, 50 ms, 0.1 s, 0.15 s, 0.2 s, 0.4 s, 0.7 s, 1 s, and 1.4 s). In double-flash conditions, the response to the CF recorded alone served as the baseline for measuring the amplitude of the response to the PF at each inter-

stimulus time  $t$ ,  $a_{\text{sat},t}$ . The proportion of the dark current suppressed by the CF at elapsed time  $t$ ,  $\text{SF}_t$ , is, therefore, given by

$$\text{SF}_t = 1 - \frac{a_{\text{sat},t}}{a_{\text{max}}} \quad (5)$$

[00427] To derive a value for the time-course of deactivation, the trough of the rod response is determined and a line is fit through the recovery phase. The latency to 50% recovery, (ms), is noted.

#### *Post-receptor function*

[00428] Rod-mediated, post-receptor function is evaluated, in the same animals, from the ERG *b*-wave. A series of 13 'green' flashes producing from approximately 0.075 to 300 R\* is used to elicit *b*-wave responses. To the amplitudes ( $\mu\text{V}$ ) of such responses, the parameters of the Naka-Rushton function,

$$\frac{V(i)}{V_m} = \frac{i}{i + \sigma} \quad (6)$$

are optimized. In this equation,  $V(i)$  is the amplitude of the response to a flash of  $i$  intensity (R\*),  $V_m$  is the saturated amplitude of the *b*-wave, and  $\sigma$  is the intensity that evokes a *b*-wave with amplitude of half  $V_m$ . The function is fit only up to those intensities at which *a*-wave intrusion is first observed. If  $i$  is correctly specified,  $\log \sigma$  is a measure of post-receptor sensitivity.

#### *Recovery from a bleach*

[00429] In the second set of experiments, performed on cohorts, the recovery of the dark current from the bleach is assessed. The rod-saturating PF (10,000 R\*), presented to the dark-adapted eye, is used to determine the magnitude of the dark current. Following the bleaching exposure, the response to the PF is monitored at 2 min intervals for approximately 40 min. At each time, the fraction of the dark current recovered ( $1 - \text{SF}_t$ ) is calculated. The time to 50% recovery of the saturating rod photoresponse,  $t_{50}$ , is found by optimizing the parameters of the function

$$t(P) = -t_0 \cdot \ln \left( \frac{P - P_0}{B} \right) \quad (7)$$

and then solving the equation for  $P = 50\%$ . In this equation,  $t(P)$  is the time required for the *a*-wave to reach  $P$  percent of its dark-adapted value,  $t_0$  is the time constant of regeneration,  $P_0$  is the normalized amplitude of the dark-adapted *a*-wave (100%), and  $B$  is a scalar. Often,  $t_{50}$  is longer than the recording session and is therefore extrapolated.

#### *Stimulus delivery*

[00430] The timing and intensity of the ERG stimuli are under computer control. The inter-stimulus interval and number of sweeps averaged for the intensity series used to assess receptor and post-receptor response sensitivities and amplitudes are detailed below. For deactivation experiments, the response to

the conditioning flash is averaged eight times, the response to the probe flash is averaged four times and, in double-flash conditions, all traces are averages of two sweeps, recorded 1 min apart. In the bleaching experiment, the probe flash is delivered singly every 2 min.

*ERG intensity series.*

Light source	Intensity <sup>a</sup> (R*)	Sweeps (minimum)	I.S.I. (s)
‘Green’ LED	0.075	32	0.35
	0.15	24	0.40
	0.30	24	0.45
	0.60	18	0.50
	1.0	18	0.60
	2.5	14	0.75
	5.0	14	1.0
	9.5	11	1.5
	20	11	2.0
	40	8	2.5
	75	8	4.0
	150	6	5.5
	300	6	8.0
Xenon-arc	1000	5	18
	2500	4	27
	5000	4	40
	10,000	3	60
	20,000	3	90

<sup>a</sup> The efficiency (R\* cd<sup>-1</sup> s<sup>-1</sup> m<sup>2</sup>) of the ‘green’ LED and xenon-arc flashes are respectively calculated at ~150 and ~75.

#### *Analysis of retinal vessels*

**[00431]** Vascular tortuosity is evaluated in both eyes of subjects using a noninvasive technique, a necessity in this longitudinal study. The OIR model employed in this study is characterized by a 100% incidence of NV; it is also characterized by tortuous retinal vessels. In patients, the posterior pole is the region most important to the diagnosis of high-risk ROP.

Correspondingly, following each ERG session, wide-field images of the ocular fundus showing the major vessels of the retina are obtained and composited to display a complete view of the posterior pole, defined here as the region within the circle bounded by the vortex veins and concentric to the optic nerve head; the vortex veins define the equator. The arterioles are identified and their tortuosity measured using RISA software, as previously described (Akula et al., 2007; Akula et al., 2008; Gelman et al., 2005; Hansen et al., 2008; Martinez-Perez et al., 2002, 2007). Briefly, each vessel is cropped from the main image and

segmented individually. If necessary, the segmented image is manually edited to remove extraneous features such as the background choroidal vasculature. RISA constructed a skeleton and marked terminal and bifurcation points. The user then selected the vessel segments for analysis and RISA automatically calculated the integrated curvature,  $IC$ , for the selected segments of each vessel.  $IC$  captures any departure from linear course and is the sum of angles along the vessel, normalized by the vessel length (radians pixel<sup>-1</sup>). Thus, a theoretical straight vessel has  $IC = 0$ . High values of  $IC$  capture well vessels that a clinician would be likely to designate as tortuous. Arteriolar tortuosity, TA (radians pixel<sup>-1</sup>), is calculated for each subject as the mean integrated curvature of all measurable arterioles in both eyes (median 10).

#### **Example 13: Human Clinical Trial for Retinopathy of Prematurity**

**[00432] Purpose:** The main purpose of this study is to evaluate the safety of a clinical trial candidate when orally administered to newborns with ROP. Further objective of this study is to evaluate the efficacy of the clinical trial candidate to reduce the progression of ROP through serial ophthalmologic examinations planned at different intervals according to the severity of ROP, in comparison with what is observed in a control group receiving conventional treatment (treatment adopted by the ETROP Cooperative Group).

**[00433] Methods:** An interventional pilot randomized controlled trial is conducted to evaluate the safety and efficacy of the clinical trial candidate when used in addition to the conventional approach (treatment adopted by the ETROP Cooperative Group) versus the conventional approach alone to treat preterm newborns (gestational age less than 32 weeks) with a stage 2 ROP (zone II-III without plus).

**[00434]** Patients are excluded if any of the following exclusion criteria is met at enrollment in the study: (1) more than 10 episodes of bradycardia of prematurity/day (HR< 90 bpm); (2) atrio-ventricular (A-V) block (2nd or 3rd degree); (3) significant congenital heart anomaly (not including patent ductus arteriosus, patent foramen ovale or small ventricular septal defect); (4) heart failure; (5) hypotension (mean blood pressure <45 mmHg); (6) hypoglycemia (<50mg/dL); and (7) platelet count <100000/mm<sup>3</sup>.

**[00435]** In order to compare the proportions of newborns that progresses to more-severe ROP in treated group and control group, the estimated sample size was calculated, considering normal distribution, an alpha error of 0.05 and a power of 80 percent. The sample size for each group is 22 participants. The incidence of progression from stage 2 ROP to higher stages increases with the decreasing of the gestational age. To ensure a homogeneous distribution of the gestational age in both groups (treated and controls), the recruited newborns will be randomized and stratified according to their gestational age in three different groups: group 1 (23-25 weeks), group 2 (26-28 weeks), and group 3 (29-32 weeks).

**[00436]** At the beginning of the study, patients in each gestational group are further divided into two groups, one receiving the clinical development candidate orally in suspension form at the dose of 0.5 mg/kg/6 hours, and the other receiving placebo in suspension form. In both treated and placebo groups, the convention treatment adopted by the ETROP Cooperative Group continues. Both the treated and placebo groups are subject to ophthalmological examinations at 40 weeks of gestational age. The

ophthalmologists are blindfolded as to which patients receive the clinical development candidate and which patients receive placebo.

**[00437] Assessment:** to evaluate the safety of the clinical development candidate, cardiac and respiratory parameters (heart frequency, blood pressure, oxygen saturation, respiratory support), are continuously monitored. Blood samplings are performed as soon as the stage 2 ROP will be diagnosed, to check renal, liver and metabolic balance. Kruskal-Wallis test is used to assess possible differences between newborns receiving the clinical development candidate and newborns receiving placebo. The safety is also evaluated by means of relative risk (RR). RR is calculated as the ratio between the probability of side effects in the treated group with respect to the control group. RR is also calculated as the ratio between the probability that ROP progresses to more-severe ROP in treated group with respect to the control group. In this case, values of RR lower than 1 are associated to the efficacy of the treatment. If necessary, RR for each gestational age group is obtained.

**[00438]** For efficacy, all newborns (treated and control groups) are evaluated at 40 weeks of gestational age by using a recently published battery of behavioral tests designed to assess various aspects of visual function (Ricci et al, *Early Hum Dev*. 2008 Feb;84(2):107-13), which includes items that assess ocular movements (spontaneous behavior and in response to a target), the ability to fix and follow a black/white target (horizontally, vertically, and in an arc), the reaction to a colored target, the ability to discriminate between black and white stripes of increasing spatial frequency, and the ability to keep attention on a target that is moved slowly away from the infant. Visual function is evaluated again at 1, 4 1/2, 12, 18 and 24 months corrected age (Ricci et al. *J Pediatr*. 2010 Apr;156(4):550-5) with particular regards to visual acuity (binocular and monocular), measured by means of well known instruments based on preferential force choice (Teller acuity cards), stereopsis and ocular motricity.

**Example 14: Human Clinical Trial for Choroidal Neovascularization**

**[00439] Purpose:** The main objective of this study is to evaluate the safety of a clinical development candidate when orally administered to patients with choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD). Further objective of this study is to evaluate the efficacy of the clinical development candidate for the treatment of choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD), in comparison with what is observed in a control group receiving placebo treatment.

**[00440] Methods:** An interventional pilot randomized controlled trial is conducted to compare the safety and efficacy of the clinical development candidate versus placebo for patients with choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD). Patients are eligible if (1) they are male or female of 50 years of age or greater; (2) they are diagnosed with primary or recurrent subfoveal CNV secondary to AMD, including those with predominantly classic, minimally classic or active occult lesions with no classic component; (3) they have a BCVA score between 73 and 24 letters (approximately 20/40 to 20/320 Snellen equivalent), inclusively, in the study eye; (4) total area of CNV (including both classic and occult components) encompassed within the lesion is at least 50% of the total lesion area; and (5) total lesion area is no more than 12 disc areas.

[00441] Patients are ineligible if one of the following conditions are met: (1) patients who have in the fellow eye a Snellen equivalent below 20/200; (2) presence of angiod streaks, presumed ocular histoplasmosis syndrome, myopia (exceeding -8 diopters), or CNV secondary to causes other than AMD in the study eye; (3) subfoveal fibrosis or atrophy in the study eye; (4) vitreous hemorrhage, retinal tear or history of rhegmatogenous retinal detachment or macular hole (Stage 3 or 4) in the study eye; (5) active, or history of, ocular inflammation or infection in the study eye within the last 30 days prior to screening; (6) uncontrolled glaucoma in the study eye; (7) treatment in the study eye with verteporfin, external-beam radiation therapy, subfoveal focal laser photocoagulation, vitrectomy, submacular surgery, or transpupillary thermotherapy within 30 days prior to screening; (8) previous treatment with anti-angiogenic drugs (pegaptanib, ranibizumab, bevacizumab, anecortave acetate, corticosteroids, protein kinase C inhibitors, squalamine, siRNA, VEGF-Trap etc.) for neovascular AMD in the study eye; (9) history of intraocular surgery in the study eye including pars plana vitrectomy, except for uncomplicated cataract surgery more than 60 days prior to screening; History of YAG laser posterior capsulotomy in the study eye within 30 days prior to screening.

[00442] At the beginning of the study, patients are divided into six groups. The clinical development candidate is administered orally in tablet form at the dose of 2, 5, 7, 10, and 20 mg/day, respectively, to the first five groups of patients for 3 months. Placebo is administered orally in tablet form to the sixth group of patients during the same time period. Both the treated and placebo groups will be subject to ophthalmological examinations at the end of each month. The ophthalmologists are blindfolded as to which patients receive the clinical development candidate and which patients receive placebo.

[00443] **Assessment:** To evaluate the safety of the clinical development candidate, cardiac and respiratory parameters (heart frequency, blood pressure, oxygen saturation, respiratory support) are monitored after oral administration of the clinical development candidate. Blood samplings are also performed to check renal, liver and metabolic balance. The safety of the clinical development candidate is further evaluated by means of relative risk (RR). RR will be calculated as the ratio between the probability of side effects in the treated group with respect to the control group. RR is also calculated as the ratio between the probability that DR progresses to more-severe DR in treated group with respect to the control group. In this case, values of RR lower than 1 will be associated to the efficacy of the treatment.

[00444] To evaluate the efficacy of the clinical development candidate, outcome measures include the incidence of ocular and nonocular adverse events, the percentage of patients gaining  $\geq 15$  letters of visual acuity (VA) at 3 months from baseline, the percentage of patients losing  $\geq 15$  letters of VA at 3 months from baseline, and mean change in VA and central retinal thickness (CRT) at 3 months from baseline.

**Example 15: Human Clinical Trial for Retinal Neovascularization Associated with Uveitis**

[00445] **Purpose:** The main objective of this study is to evaluate the safety of a clinical development candidate when orally administered to patients with retinal neovascularization (RNV) associated with uveitis. Further objective of this study is to evaluate the efficacy of the clinical development candidate

for the treatment of with retinal neovascularization (RNV) associated with uveitis, in comparison with what is observed in a control group receiving placebo treatment.

**[00446] Methods:** An interventional pilot randomized controlled trial is conducted to compare the safety and efficacy of the clinical development candidate versus placebo for patients with retinal neovascularization (RNV) associated with uveitis. Patients are eligible if (1) they are male and female patients with non-infectious intermediate or posterior uveitis or panuveitis in at least one eye, age 18 to 70 years of age inclusive, who are otherwise in good health; (2) macular edema with average central retinal thickness  $\geq 250 \mu\text{m}$ ; (3) a vitreous haze score  $\geq 1$ , but  $\leq 3$  (based on the National Eye Institute grading system); (4) Best Corrected Visual Acuity no worse than 20/400 and no better than 20/40; and (5) Daily prednisone dose  $< 1 \text{ mg/kg}$ .

**[00447]** Patients are not eligible if one of the following conditions is met: (1) patients with choroidal neovascularization; (2) patients with Serpiginous choroidopathy, Acute multifocal placoid pigment epitheliopathy, or White dot retino-choroidopathies (e.g., multiple evanescent white dot syndrome (MEWDS) or multifocal choroiditis); (3) macular edema associated with other ocular disease (e.g., diabetic retinopathy); (4) patients who had a prior vitrectomy; (5) any eye condition that may affect the evaluation of visual acuity and retinal thickness; (6) concurrent use of certain immunosuppressive agents (specific washout periods for different agents are defined in the protocol); (7) use of systemic medications known to be toxic to the lens, retina, or optic nerve (e.g. deferoxamine, chloroquine, and ethambutol) currently or in the past 6 months; and (8) other protocol-defined inclusion/exclusion criteria may apply.

**[00448]** At the beginning of the study, patients are divided into six groups. The clinical development candidate is administered orally in tablet form at the dose of 2, 5, 7, 10, and 20 mg/day, respectively, to the first five groups of patients for 3 months. Placebo is administered orally in tablet form to the sixth group of patients during the same time period. Both the treated and placebo groups will be subject to ophthalmological examinations at the end of each month. The ophthalmologists are blindfolded as to which patients receive the clinical development candidate and which patients receive placebo.

**[00449] Assessment:** To evaluate the safety of the clinical development candidate, cardiac and respiratory parameters (heart frequency, blood pressure, oxygen saturation, respiratory support) are monitored after oral administration of the clinical development candidate. Blood samplings are also performed to check renal, liver and metabolic balance. The safety of the clinical development candidate is further evaluated by means of relative risk (RR). RR will be calculated as the ratio between the probability of side effects in the treated group with respect to the control group. RR is also calculated as the ratio between the probability that DR progresses to more-severe DR in treated group with respect to the control group. In this case, values of RR lower than 1 will be associated to the efficacy of the treatment.

**[00450]** To evaluate the efficacy of the clinical development candidate, Best-corrected visual acuity (BCVA) and central retinal thickness (CRT) are assessed by certified examiners at scheduled monthly ophthalmological examinations. Outcome measures include the incidence of ocular and nonocular

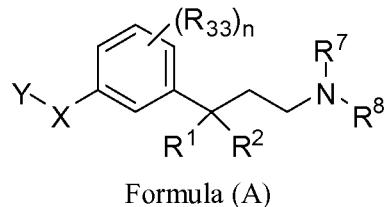
adverse events, the percentage of patients gaining  $\geq 15$  letters of visual acuity (VA) at 3 months from baseline, the percentage of patients losing  $\geq 15$  letters of VA at 3 months from baseline, and mean change in VA and central retinal thickness (CRT) at 3 months from baseline.

**[00451]** While certain embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur without departing from the scope of the embodiments. It should be understood that various alternatives to the embodiments described herein may be employed. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

## CLAIMS

## WHAT IS CLAIMED IS:

1. A method for treating an ophthalmic disease or disorder associated with diabetes in a patient comprising administering to the patient a therapeutically effective amount of a composition comprising a compound of Formula (A), or tautomer, stereoisomer, geometric isomer, N-oxide or a pharmaceutically acceptable salt thereof:



wherein,

X is selected from  $-\text{C}(\text{R}^9)=\text{C}(\text{R}^9)-$ ,  $-\text{C}\equiv\text{C}-$ ,  $-\text{C}(\text{R}^9)_2\text{O}-$ ,  $-\text{C}(\text{R}^9)_2\text{C}(\text{R}^9)_2-$ ,  $-\text{C}(\text{R}^9)_2\text{S}-$ ,  $-\text{C}(\text{R}^9)_2\text{S}(\text{O})_2-$ , or  $-\text{C}(\text{R}^9)_2\text{NR}^9-$ ;

Y is selected from:

- a) substituted or unsubstituted carbocyclyl, optionally substituted with  $\text{C}_1\text{-C}_4$  alkyl, halogen, -OH, or  $\text{C}_1\text{-C}_4$  alkoxy;
- b) substituted or unsubstituted carbocyclylalkyl, optionally substituted with  $\text{C}_1\text{-C}_4$  alkyl, halogen, -OH, or  $\text{C}_1\text{-C}_4$  alkoxy;
- c) substituted or unsubstituted aralkyl, optionally substituted with  $\text{C}_1\text{-C}_4$  alkyl, halogen, -OH, or  $\text{C}_1\text{-C}_4$  alkoxy; or
- d) substituted or unsubstituted  $\text{C}_3\text{-C}_{10}$  alkyl, optionally substituted with halogen, -OH, or  $\text{C}_1\text{-C}_4$  alkoxy;

$\text{R}^1$  is hydrogen and  $\text{R}^2$  is hydroxyl; or  $\text{R}^1$  and  $\text{R}^2$  form an oxo;

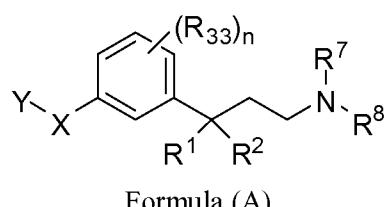
$\text{R}^7$  is hydrogen;

$\text{R}^8$  is hydrogen or  $\text{CH}_3$ ;

each  $\text{R}^9$  independently hydrogen, or substituted or unsubstituted  $\text{C}_1\text{-C}_4$  alkyl;

each  $\text{R}^{33}$  is independently selected from halogen or substituted or unsubstituted  $\text{C}_1\text{-C}_4$  alkyl, and n is 0, 1, 2, 3, or 4.

2. A method for treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient comprising administering a therapeutically effective amount of a composition comprising a compound of Formula (A), or tautomer, stereoisomer, geometric isomer, N-oxide or a pharmaceutically acceptable salt thereof:



wherein,

X is selected from  $-\text{C}(\text{R}^9)=\text{C}(\text{R}^9)-$ ,  $-\text{C}\equiv\text{C}-$ ,  $-\text{C}(\text{R}^9)_2\text{O}-$ ,  $-\text{C}(\text{R}^9)_2\text{C}(\text{R}^9)_2-$ ,  $-\text{C}(\text{R}^9)_2\text{S}-$ ,  $-\text{C}(\text{R}^9)_2\text{S}(\text{O})_2-$ , or  $-\text{C}(\text{R}^9)_2\text{NR}^9-$ ;

Y is selected from:

- a) substituted or unsubstituted carbocyclyl, optionally substituted with  $\text{C}_1\text{-C}_4$  alkyl, halogen, -OH, or  $\text{C}_1\text{-C}_4$  alkoxy;
- b) substituted or unsubstituted carbocyclylalkyl, optionally substituted with  $\text{C}_1\text{-C}_4$  alkyl, halogen, -OH, or  $\text{C}_1\text{-C}_4$  alkoxy;
- c) substituted or unsubstituted aralkyl, optionally substituted with  $\text{C}_1\text{-C}_4$  alkyl, halogen, -OH, or  $\text{C}_1\text{-C}_4$  alkoxy; or
- d) substituted or unsubstituted  $\text{C}_3\text{-C}_{10}$  alkyl, optionally substituted with halogen, -OH, or  $\text{C}_1\text{-C}_4$  alkoxy;

$\text{R}^1$  is hydrogen and  $\text{R}^2$  is hydroxyl; or  $\text{R}^1$  and  $\text{R}^2$  form an oxo;

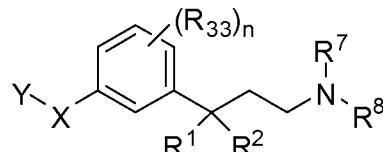
$\text{R}^7$  is hydrogen;

$\text{R}^8$  is hydrogen or  $\text{CH}_3$ ;

each  $\text{R}^9$  independently hydrogen, or substituted or unsubstituted  $\text{C}_1\text{-C}_4$  alkyl;

each  $\text{R}^{33}$  is independently selected from halogen or substituted or unsubstituted  $\text{C}_1\text{-C}_4$  alkyl, and n is 0, 1, 2, 3, or 4.

3. A method for treating or preventing retinopathy of prematurity comprising administering to the patient a therapeutically effective amount of a composition comprising a compound of Formula (A), or tautomer, stereoisomer, geometric isomer, N-oxide or a pharmaceutically acceptable salt thereof:



Formula (A)

wherein,

X is selected from  $-\text{C}(\text{R}^9)=\text{C}(\text{R}^9)-$ ,  $-\text{C}\equiv\text{C}-$ ,  $-\text{C}(\text{R}^9)_2\text{O}-$ ,  $-\text{C}(\text{R}^9)_2\text{C}(\text{R}^9)_2-$ ,  $-\text{C}(\text{R}^9)_2\text{S}-$ ,  $-\text{C}(\text{R}^9)_2\text{S}(\text{O})_2-$ , or  $-\text{C}(\text{R}^9)_2\text{NR}^9-$ ;

Y is selected from:

- a) substituted or unsubstituted carbocyclyl, optionally substituted with  $\text{C}_1\text{-C}_4$  alkyl, halogen, -OH, or  $\text{C}_1\text{-C}_4$  alkoxy;
- b) substituted or unsubstituted carbocyclylalkyl, optionally substituted with  $\text{C}_1\text{-C}_4$  alkyl, halogen, -OH, or  $\text{C}_1\text{-C}_4$  alkoxy;
- c) substituted or unsubstituted aralkyl, optionally substituted with  $\text{C}_1\text{-C}_4$  alkyl, halogen, -OH, or  $\text{C}_1\text{-C}_4$  alkoxy; or
- d) substituted or unsubstituted  $\text{C}_3\text{-C}_{10}$  alkyl, optionally substituted with halogen, -OH, or  $\text{C}_1\text{-C}_4$  alkoxy;

$\text{R}^1$  is hydrogen and  $\text{R}^2$  is hydroxyl; or  $\text{R}^1$  and  $\text{R}^2$  form an oxo;

$\text{R}^7$  is hydrogen;

$\text{R}^8$  is hydrogen or  $\text{CH}_3$ ;

each  $\text{R}^9$  independently hydrogen, or substituted or unsubstituted  $\text{C}_1\text{-C}_4$  alkyl;

each  $R^{33}$  is independently selected from halogen or substituted or unsubstituted  $C_1$ - $C_4$

alkyl, and  $n$  is 0, 1, 2, 3, or 4.

4. The method of any of claims 1-3, wherein

$n$  is 0, 1, or 2.

5. The method of claim 4, wherein

$X$  is  $-C(R^9)=C(R^9)-$ .

6. The method of claim 4, wherein

$X$  is  $-C\equiv C-$ .

7. The method of claim 4, wherein

$X$  is  $-C(R^9)_2-O-$ .

8. The method of claim 4, wherein

$X$  is  $-C(R^9)_2-C(R^9)_2-$ .

9. The method of claim 4, wherein

$X$  is  $-C(R^9)_2-S-$ .

10. The method of claim 4, wherein

$X$  is  $-C(R^9)_2-S(O)_2-$ .

11. The method of claim 4, wherein

$X$  is  $-C(R^9)_2-NR^9-$ .

12. The method of any one of claims 5, 7, 8, 9, 10, or 11, wherein  $Y$  is substituted or unsubstituted carbocyclyl, or substituted or unsubstituted  $C_3$ - $C_{10}$  alkyl.

13. The method of claim 12, wherein  $Y$  is substituted or unsubstituted carbocyclyl.

14. The method of claim 12, wherein  $Y$  is substituted or unsubstituted  $C_3$ - $C_{10}$  alkyl.

15. The method of claim 13, wherein the substituted or unsubstituted carbocyclyl is a substituted or unsubstituted 4-, 5-, 6-, or 7-membered ring.

16. The method of claim 14, wherein the substituted or unsubstituted  $C_3$ - $C_{10}$  alkyl is a substituted or unsubstituted  $C_3$ - $C_6$  alkyl.

17. The method of claim 16, wherein the substituted  $C_3$ - $C_6$  alkyl is substituted with an  $C_1$ - $C_2$  alkoxy group.

18. The method of claim 18, wherein the substituted  $C_3$ - $C_6$  alkyl is  $-CH_2CH_2CH_2OCH_3$ .

19. The method of claim 15, wherein the substituted or unsubstituted carbocyclyl is a 6-membered ring.

20. The method of claim 19, wherein the substituted or unsubstituted 6-membered ring is a substituted or unsubstituted cyclohexyl.

21. The method of claim 20, wherein  $X$  is  $-C(R^9)_2-O-$ .

22. The method of any of claims 1-3, wherein  $R^1$  is hydrogen and  $R^2$  is hydroxyl.

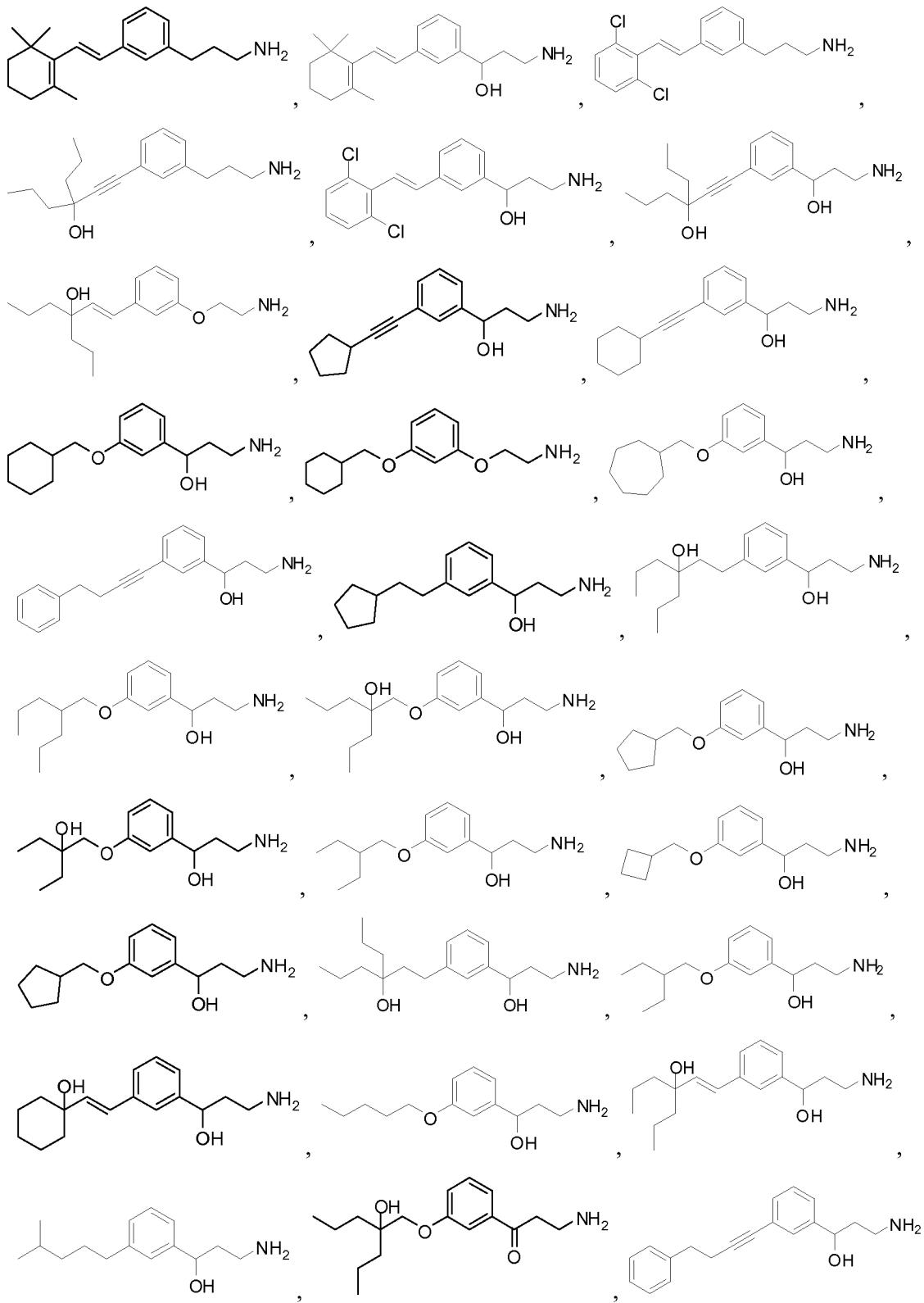
23. The method of any of claims 1-3, wherein  $R^1$  and  $R^2$  form an oxo.

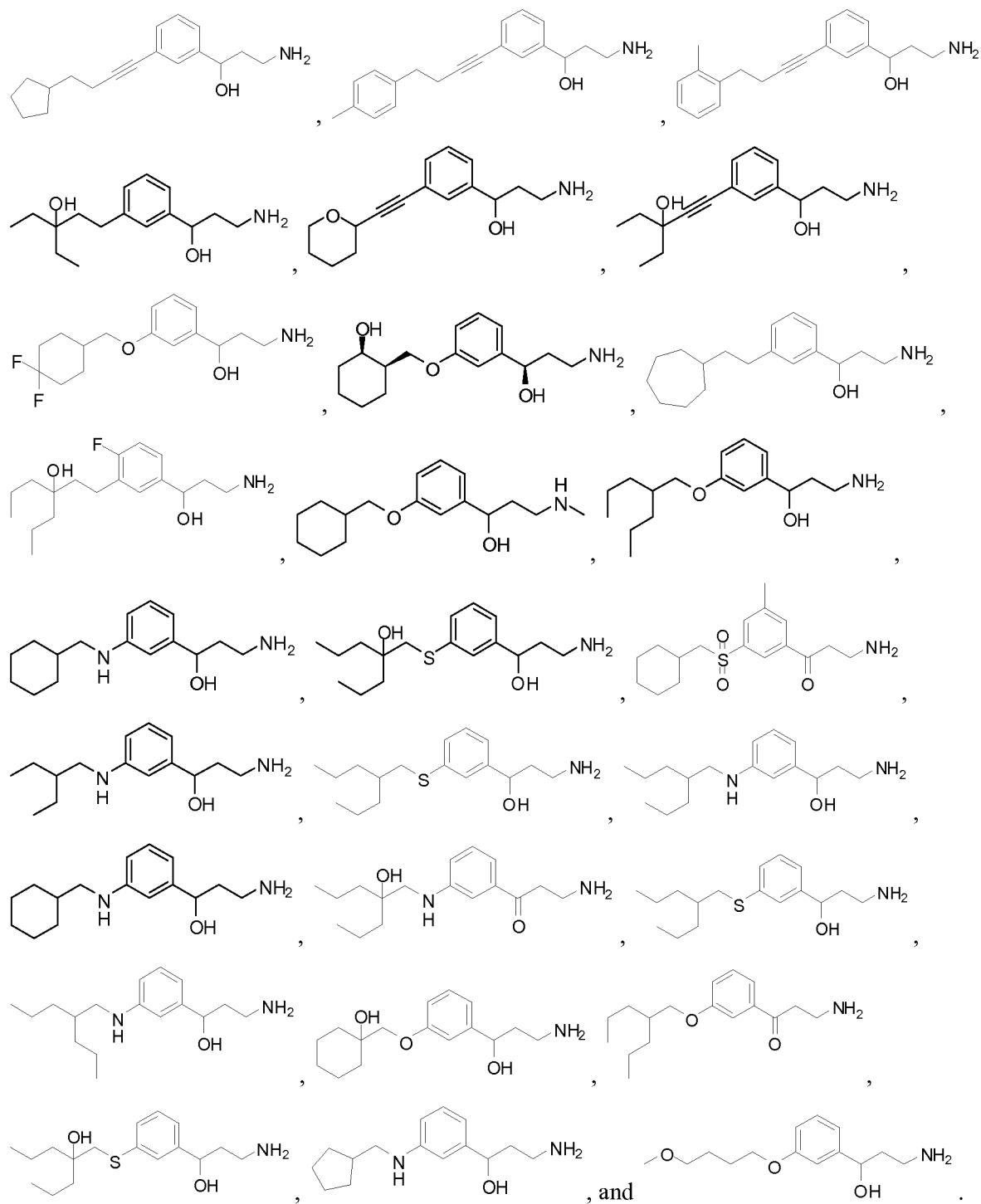
24. The method of any of claims 1-3, wherein  $R^8$  is hydrogen.

25. The method of any of claims 1-3, wherein  $R^8$  is methyl.

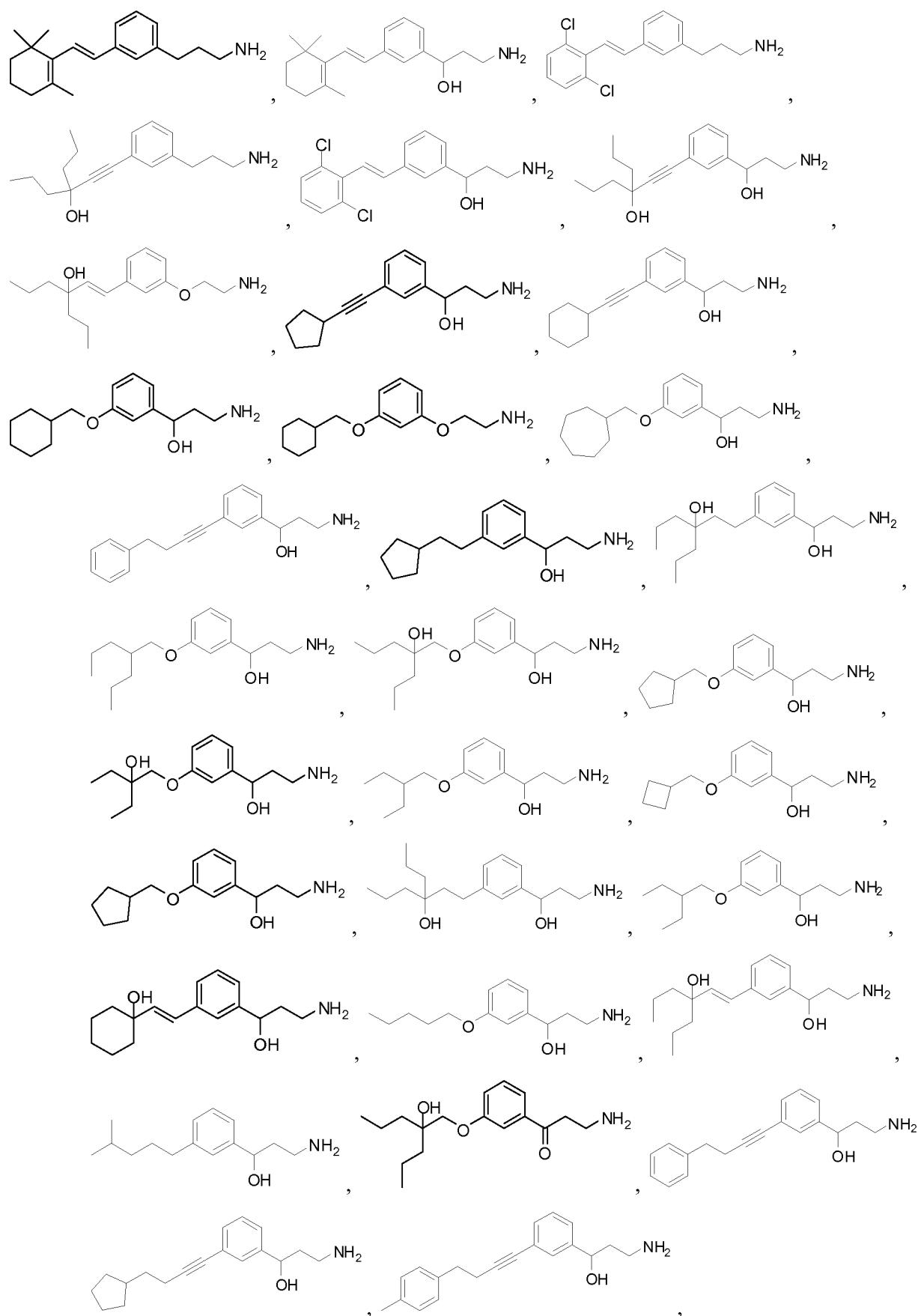
26. The method of any of claims 22-25, wherein X is  $-\text{C}(\text{R}^9)_2\text{-O}-$ .

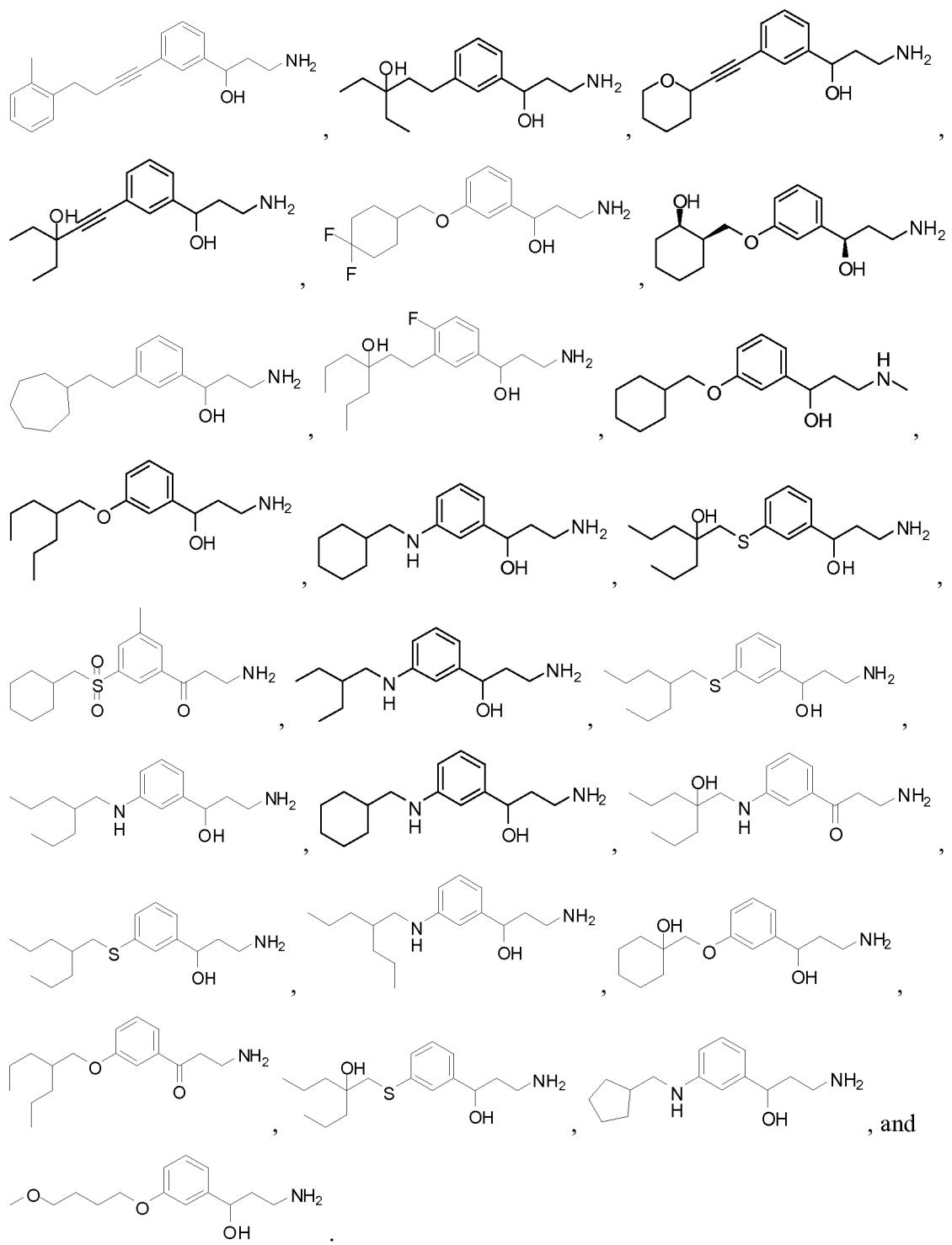
27. A method for treating an ophthalmic disease or disorder associated with diabetes in a patient comprising administering to the patient a therapeutically effective amount of a composition comprising a compound, or tautomer, stereoisomer, geometric isomer, N-oxide or a pharmaceutically acceptable salt thereof, selected from:



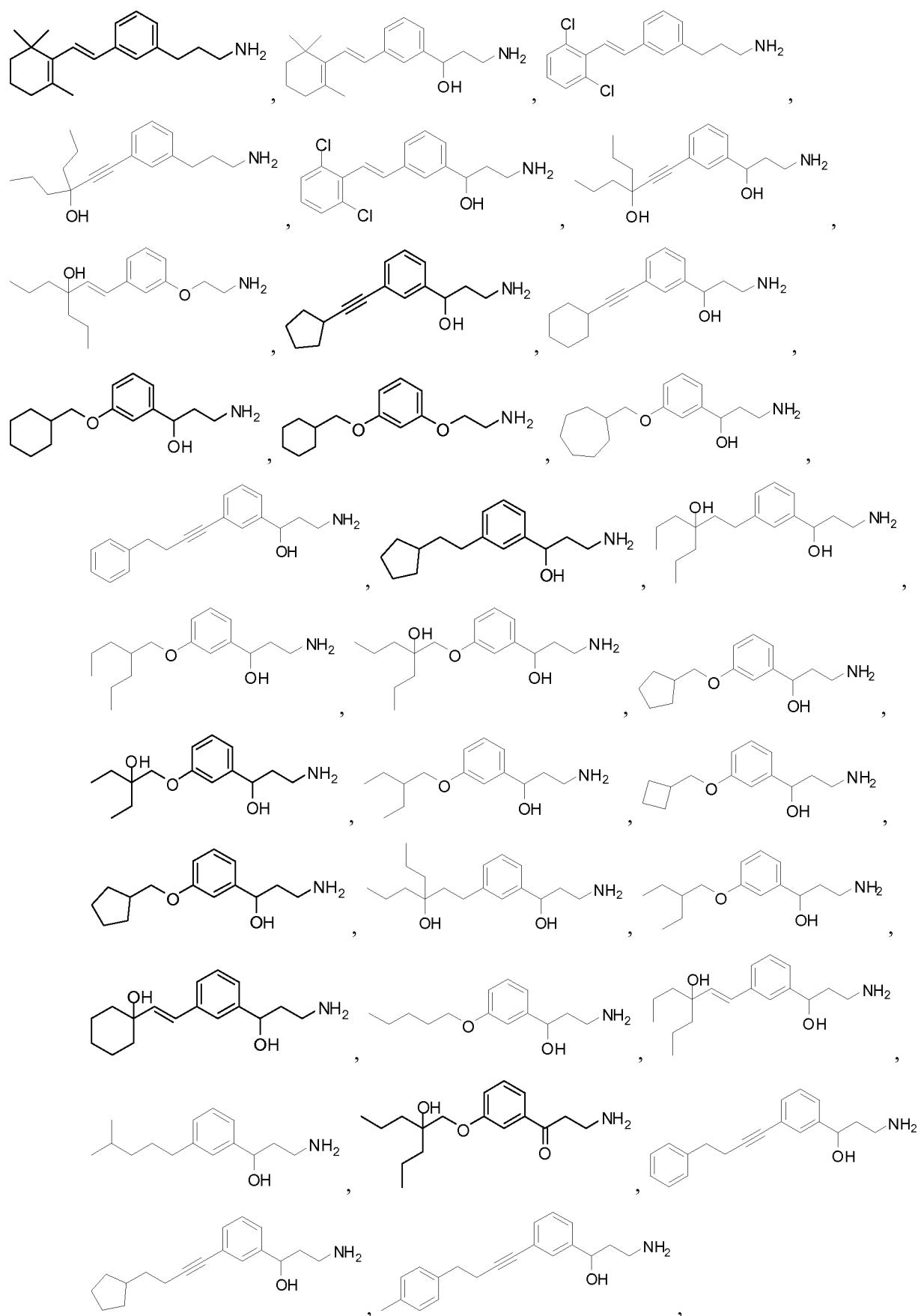


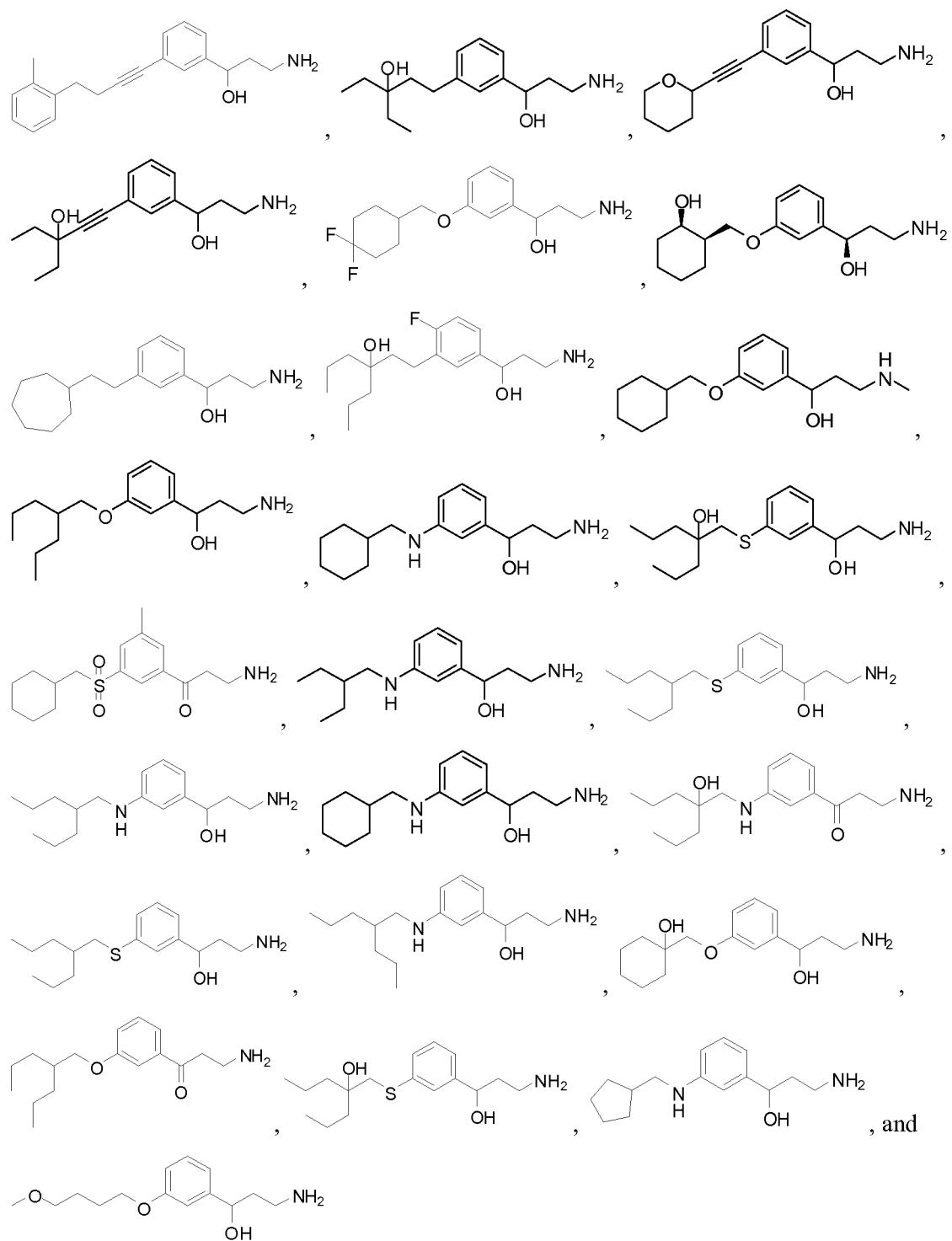
28. A method for treating an ophthalmic disease or disorder associated with neovascularization in the eye of a patient comprising administering to the patient a therapeutically effective amount of a composition comprising a compound, or tautomer, stereoisomer, geometric isomer, N-oxide or a pharmaceutically acceptable salt thereof, selected from:



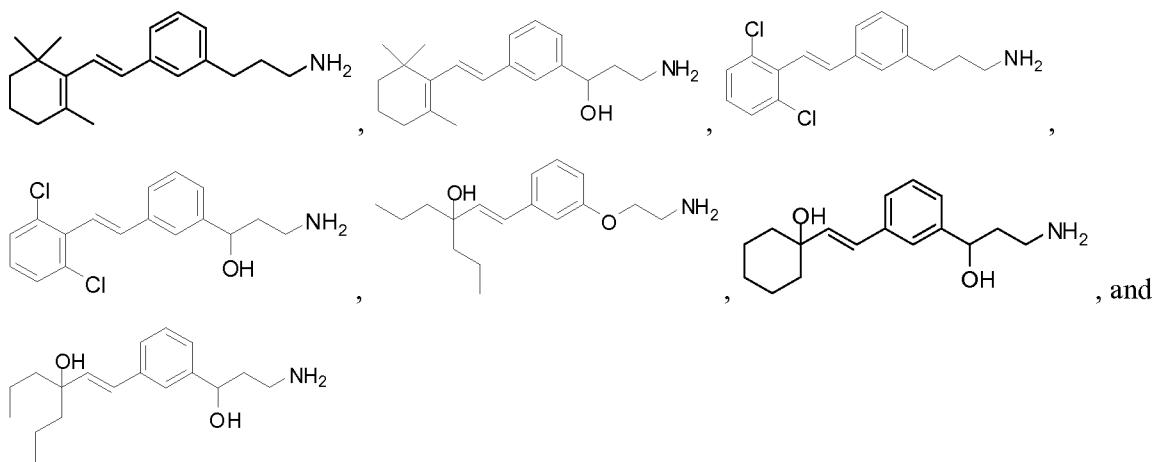


29. A method for treating or preventing retinopathy of prematurity comprising administering to the patient a therapeutically effective amount of a composition comprising a compound, or tautomer, stereoisomer, geometric isomer, N-oxide or a pharmaceutically acceptable salt thereof, selected from:

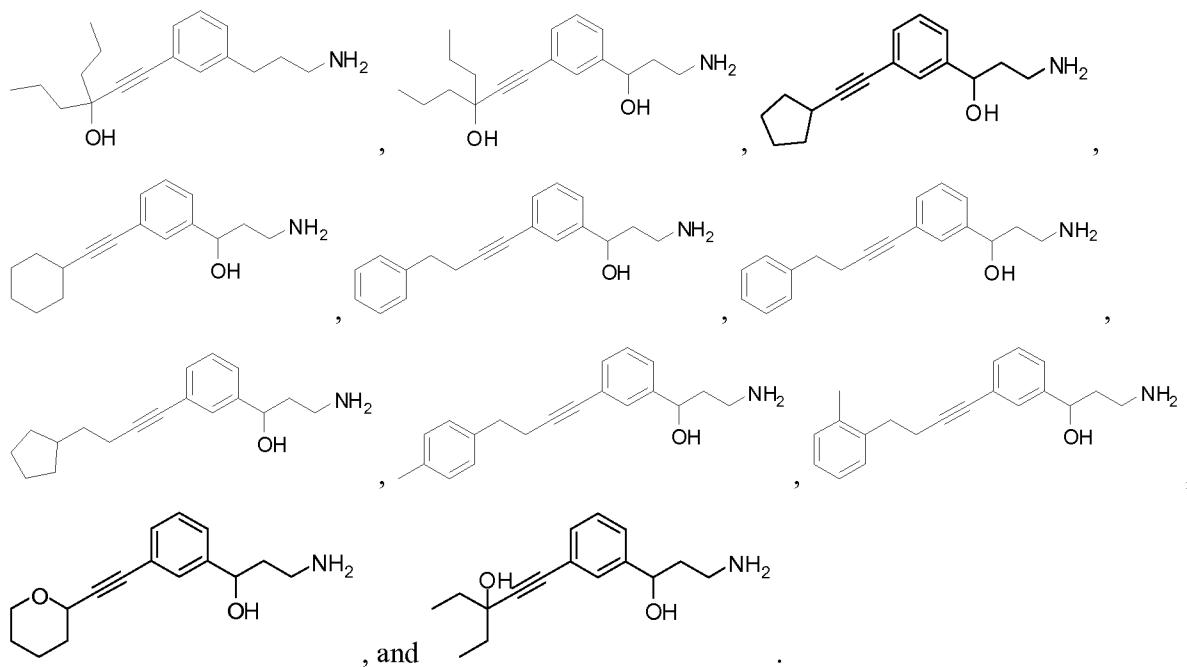




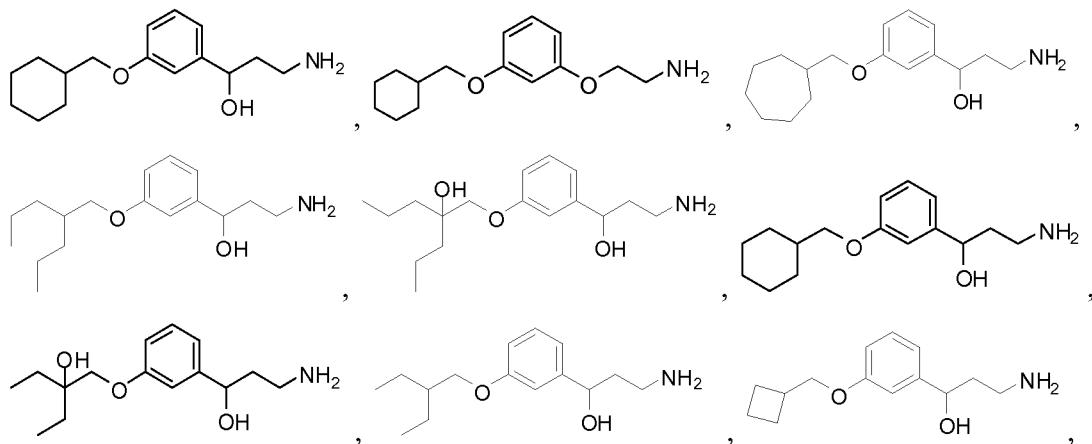
30. The method of any of claims 27-29, wherein the composition comprises a compound, or stereoisomer, geometric isomer, N-oxide or a pharmaceutically acceptable salt thereof, selected from:

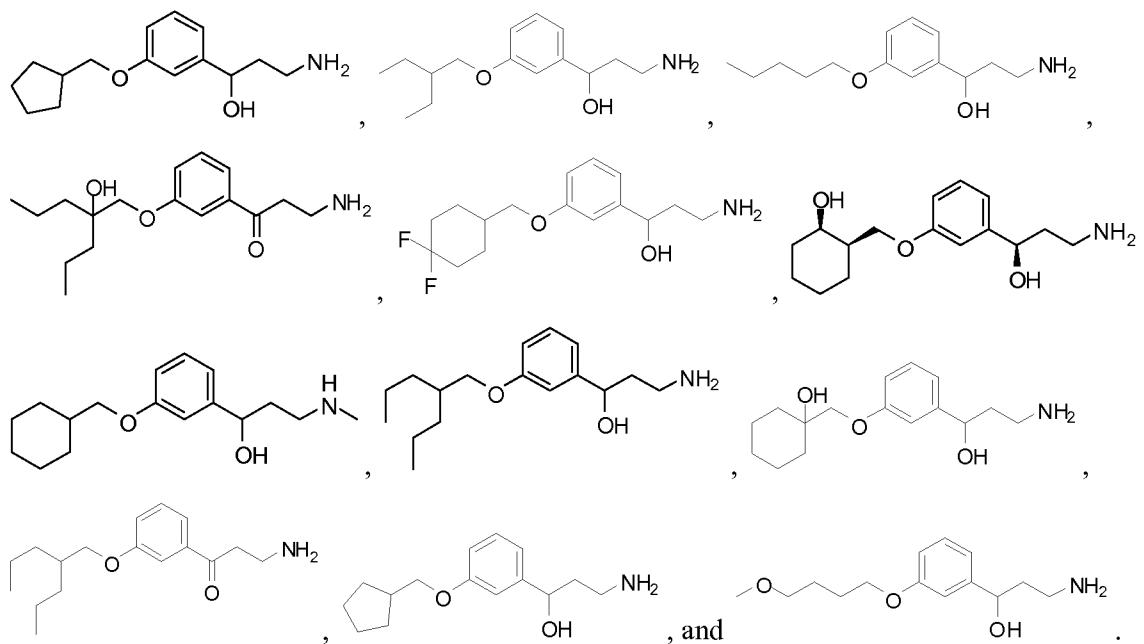


31. The method of any of claims 27-29, wherein the composition comprises a compound, or stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, selected from:

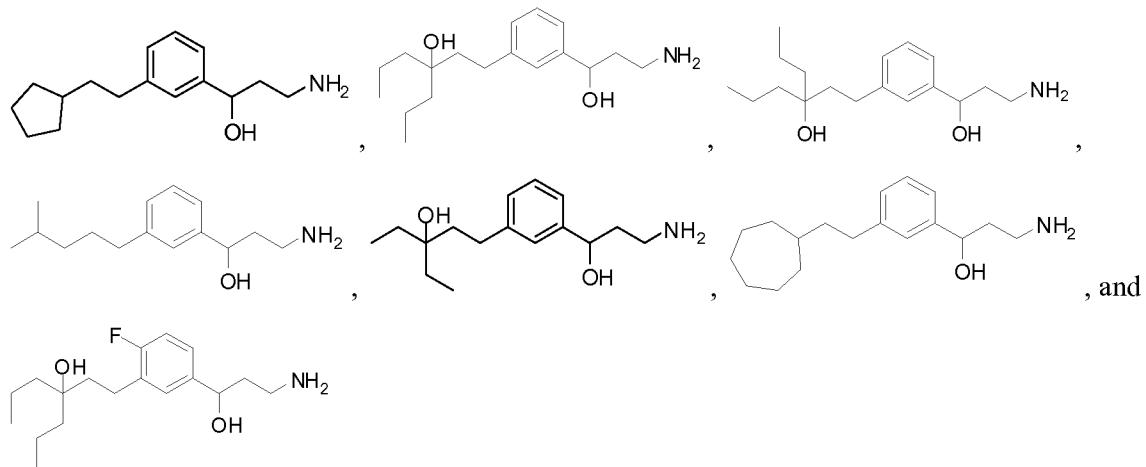


32. The method of any of claims 27-29, wherein the composition comprises a compound, or tautomer, stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, selected from:

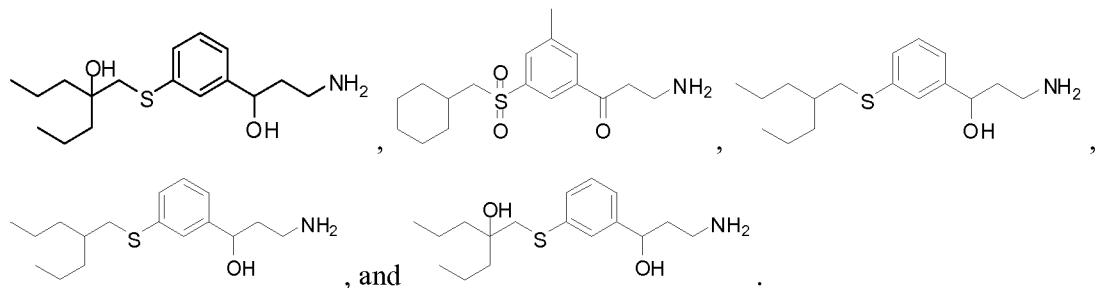




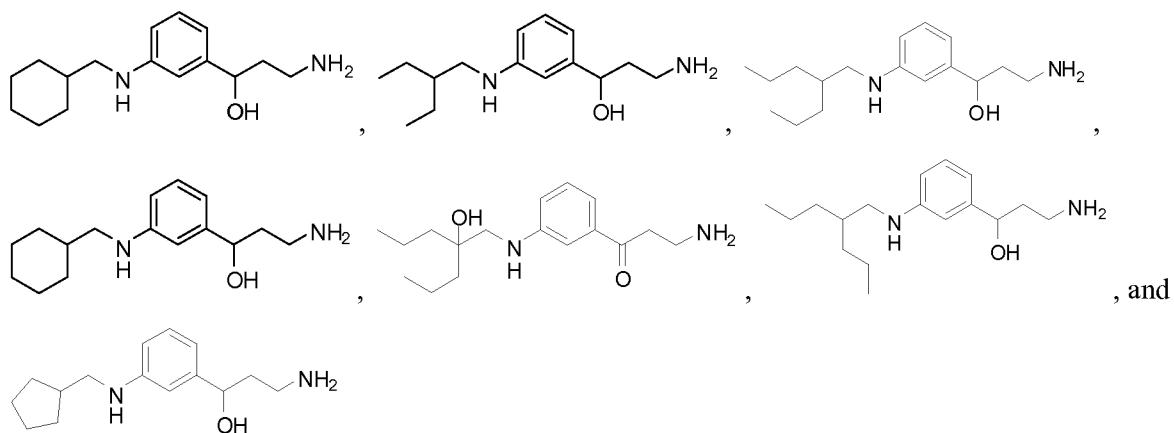
33. The method of any of claims 27-29, wherein the composition comprises a compound, or stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, selected from:



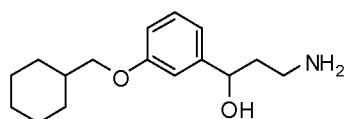
34. The method of any of claims 27-29, wherein the composition comprises a compound, or tautomer, stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, selected from:



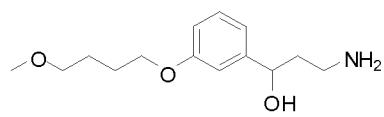
35. The method of any of claims 27-29, wherein the composition comprises a compound, or tautomer, stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, selected from:



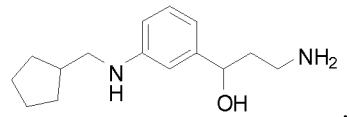
36. The method of any of claims 27-29, wherein the composition comprises a compound, or stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, having the structure:



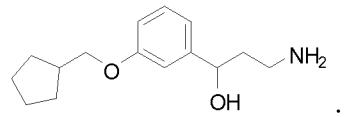
37. The method of any of claims 27-29, wherein the composition comprises a compound, stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, having the structure:



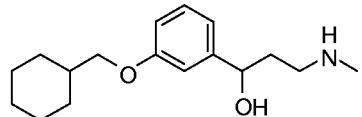
38. The method of any of claims 27-29, wherein the composition comprises a compound, or stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, having the structure:



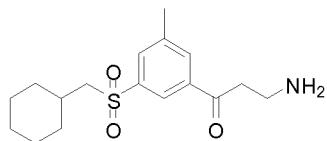
39. The method of any of claims 27-29, wherein the composition comprises a compound, or stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, having the structure:



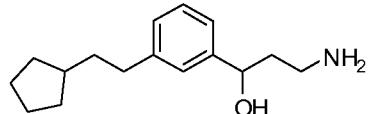
40. The method of any of claims 27-29, wherein the composition comprises a compound, or stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, having the structure:



41. The method of any of claims 27-29, wherein the composition comprises a compound, or stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, having the structure:



42. The method of any of claims 27-29, wherein the composition comprises a compound, or stereoisomer, N-oxide or a pharmaceutically acceptable salt thereof, having the structure:



43. The method of any one of claims 1-42, wherein the composition is administered to the patient orally.

44. The method of any one of claims 1-43, wherein the composition is administered once per day.

45. The method of any one of claims 1-44, wherein treatment results in improvement of central vision in the patient.

46. The method of any one of claims 1-45, further comprising administering one or more additional therapeutic regimens.

47. The method of claim 46, wherein said one or more therapeutic regimens is laser therapy, cryotherapy, fluorescein angiography, vitrectomy, corticosteroids, anti-vascular endothelial growth factor (VEGF) treatment, vitrectomy for persistent diffuse diabetic macular edema, pharmacologic vitreolysis in the management of diabetic retinopathy, fibrates, renin-angiotensin system (ras) blockers, peroxisome proliferator-activated receptor gamma agonists, Anti-Protein Kinase C (PKC), islet cell transplantation, therapeutic oligonucleotides, growth hormone and insulin growth factor (IGF), control of systemic factors or a combination thereof.

48. The method of claim 1, wherein the ophthalmic disease or disorder associated with diabetes is diabetic retinopathy.

49. The method of claim 1, wherein the ophthalmic disease or disorder associated with diabetes is non-proliferative diabetic retinopathy.

50. The method of claim 1, wherein the ophthalmic disease or disorder associated with diabetes is proliferative diabetic retinopathy.

51. The method of claim 1, wherein the ophthalmic disease or disorder associated with diabetes is diabetic maculopathy.

52. The method of claim 1, wherein the ophthalmic disease or disorder associated with diabetes is diabetic macular edema.

53. The method of claim 1, wherein the ophthalmic disease or disorder associated with diabetes is neovascular glaucoma.

54. The method of claim 1, wherein the ophthalmic disease or disorder associated with diabetes is macular ischemia.

55. The method of claim 47, wherein the one or more therapeutic regimens comprises administration of ranibizumab, bevacizumab, or pegaptanib.

56. The method of claim 2, wherein the ophthalmic disease or disorder associated with neovascularization is wet age-related macular degeneration.

57. The method of claim 2, wherein the ophthalmic disease or disorder associated with neovascularization is choroidal neovascularization.

58. The method of claim 2, wherein the ophthalmic disease or disorder associated with neovascularization is selected from: defects in Bruch's membrane, increases in amount of ocular vascular endothelial growth factor (VEGF), myopia, myopic degeneration, deterioration of central vision, metamorphopsia, color disturbances, hemorrhaging of blood vessels, or a combination thereof.

59. The method of claim 2, wherein the ophthalmic disease or disorder associated with neovascularization is retinal neovascularization.

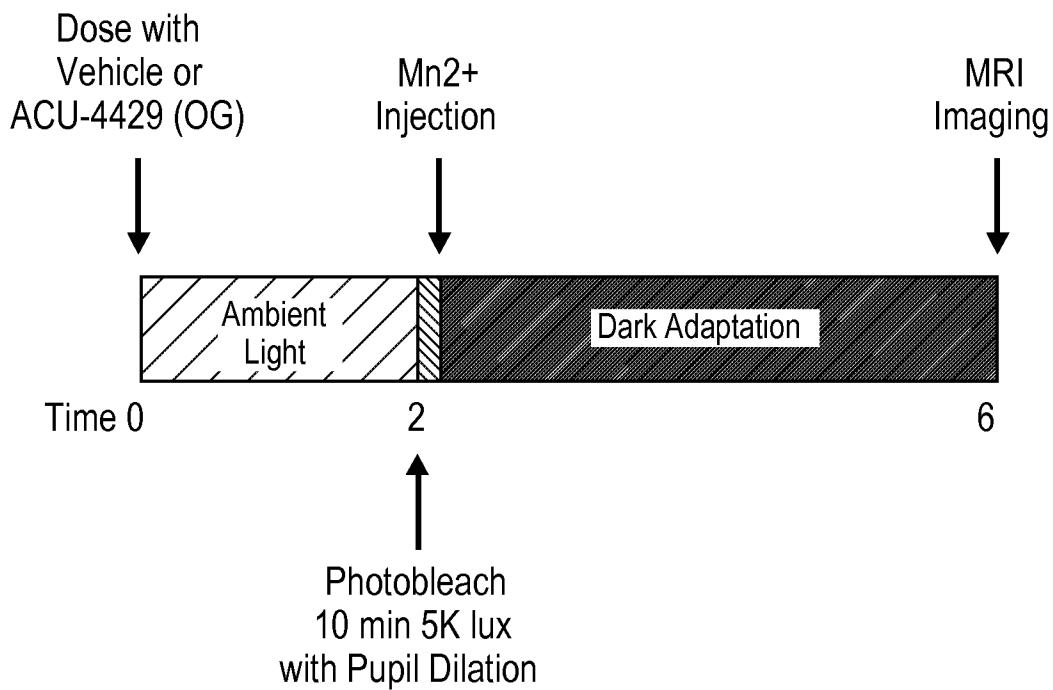
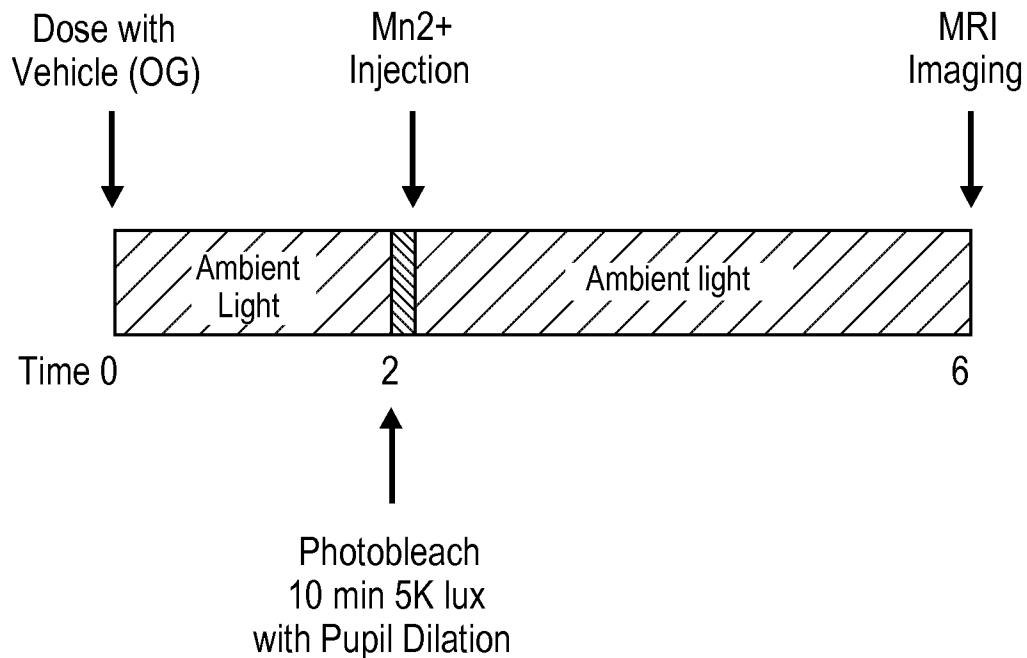
60. The method of claim 3 wherein the patient is a premature infant.

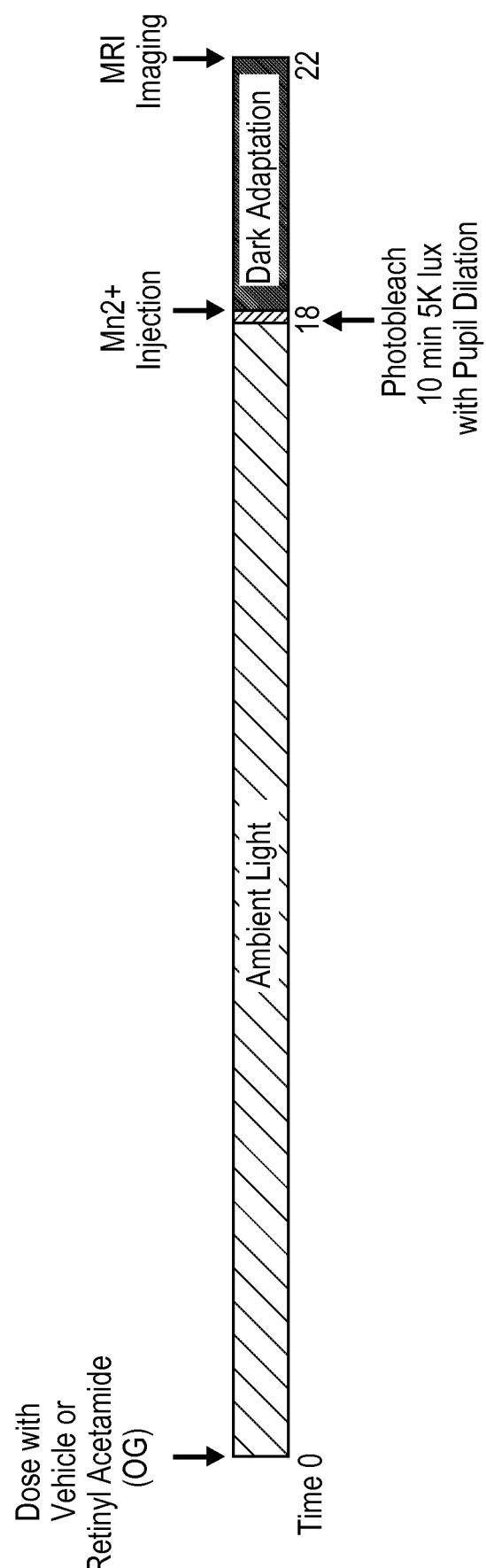
61. The method of claim 3 wherein the patient is further treated with supplemental oxygen.

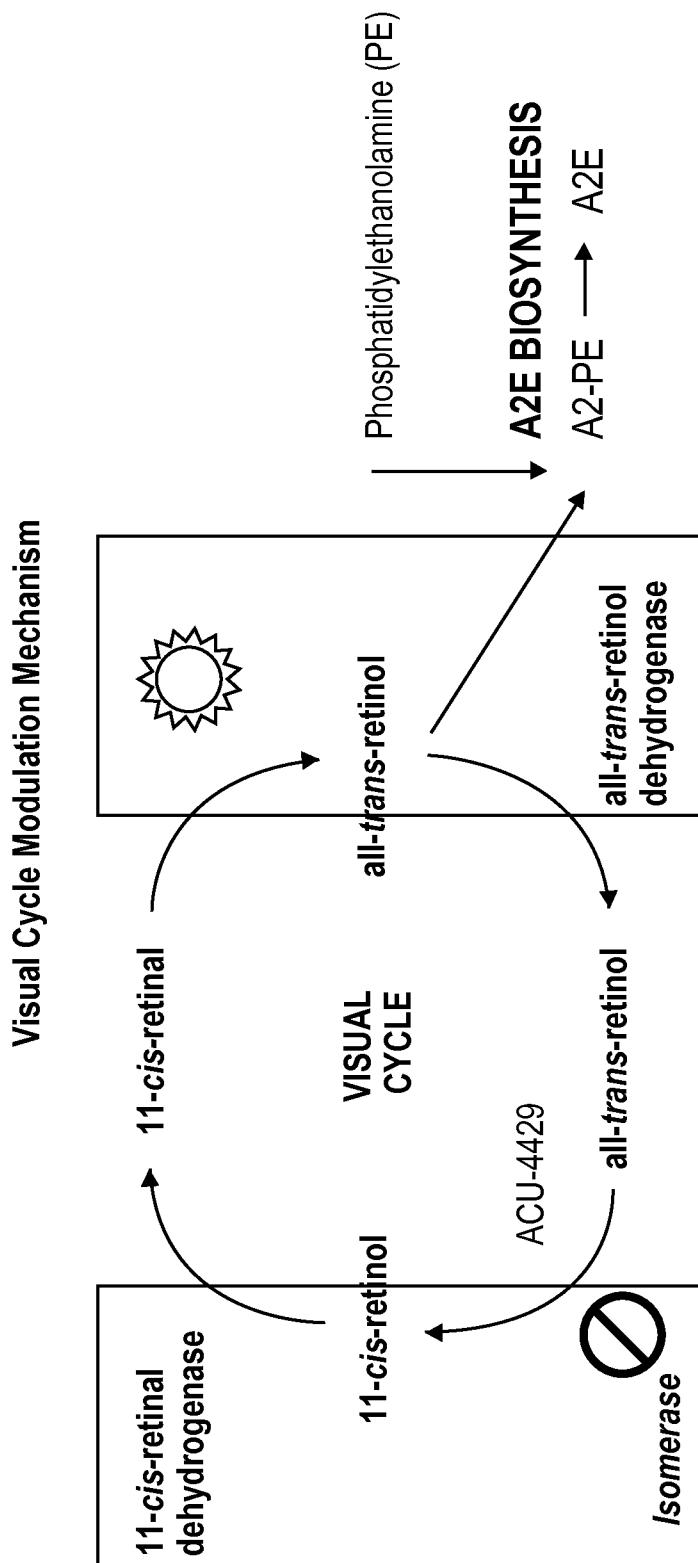
62. A method for protecting an eye during medical procedures requiring exposure of the eye to bright light, to laser light, procedures resulting in prolonged and/or excessive dilation of the pupil, or that otherwise sensitize the eye to light, the method comprising administration of a composition comprising a compound described herein to a patient in need thereof.

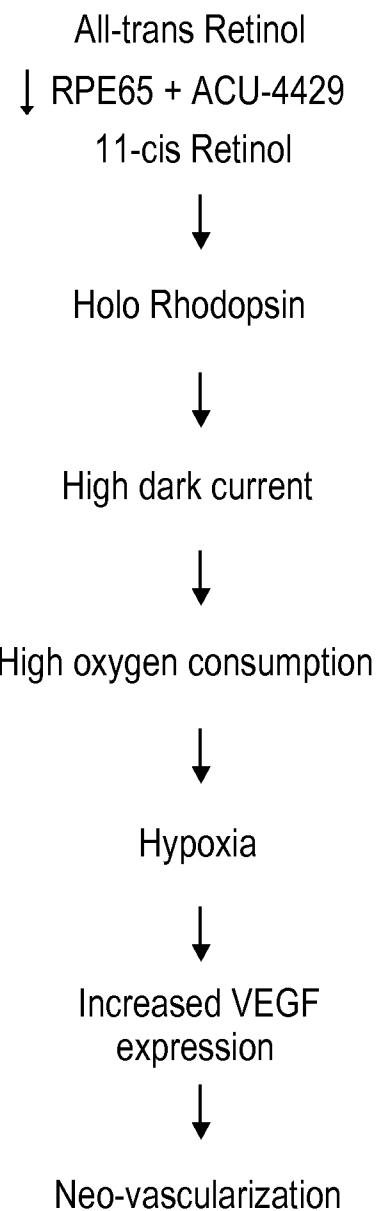
63. The method of claim 62, wherein the composition is administered orally prior to the medical procedure.

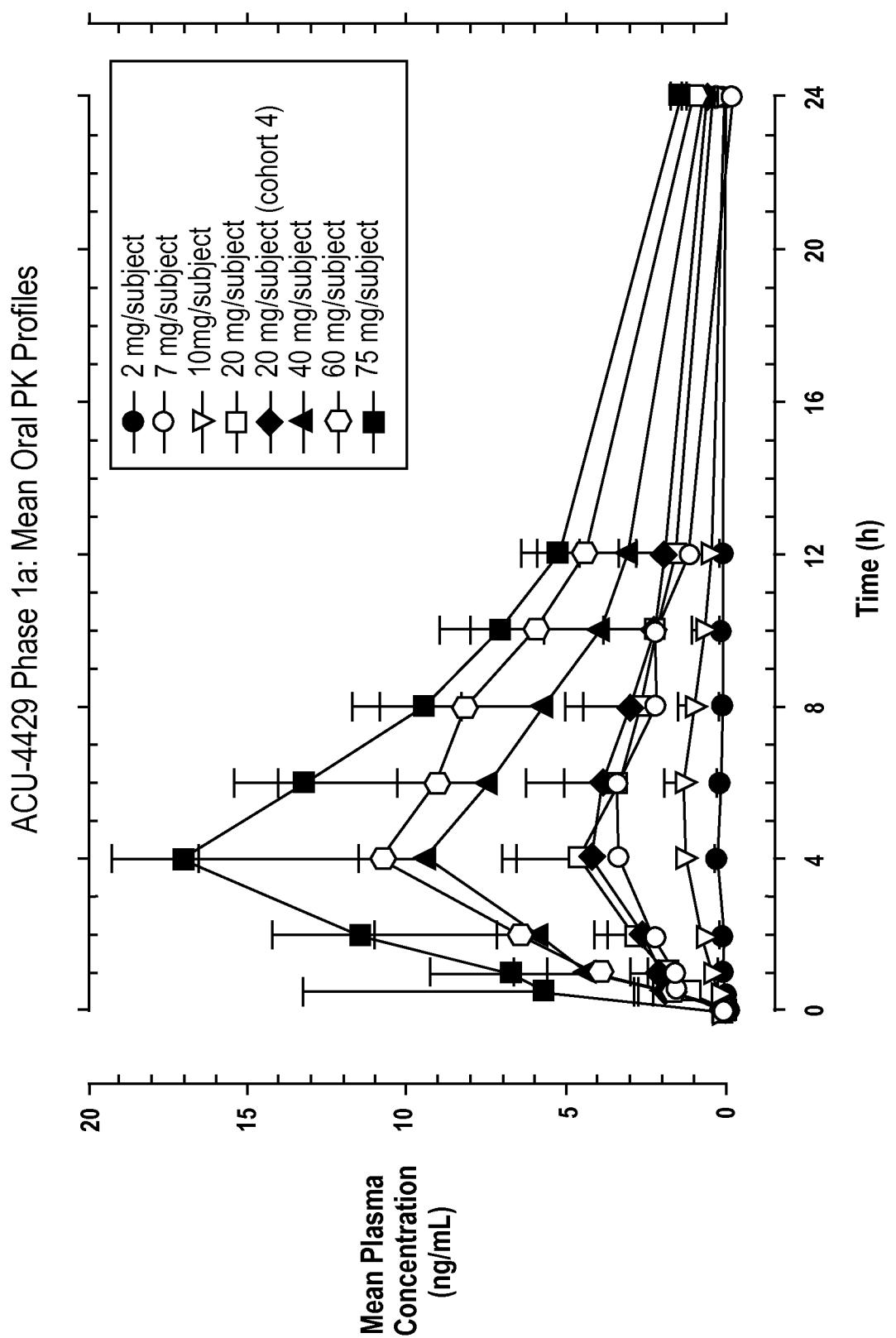
64. The method of claim 62, wherein the composition is administered orally within 24 hours before and/or within 48 hours after the medical procedure.

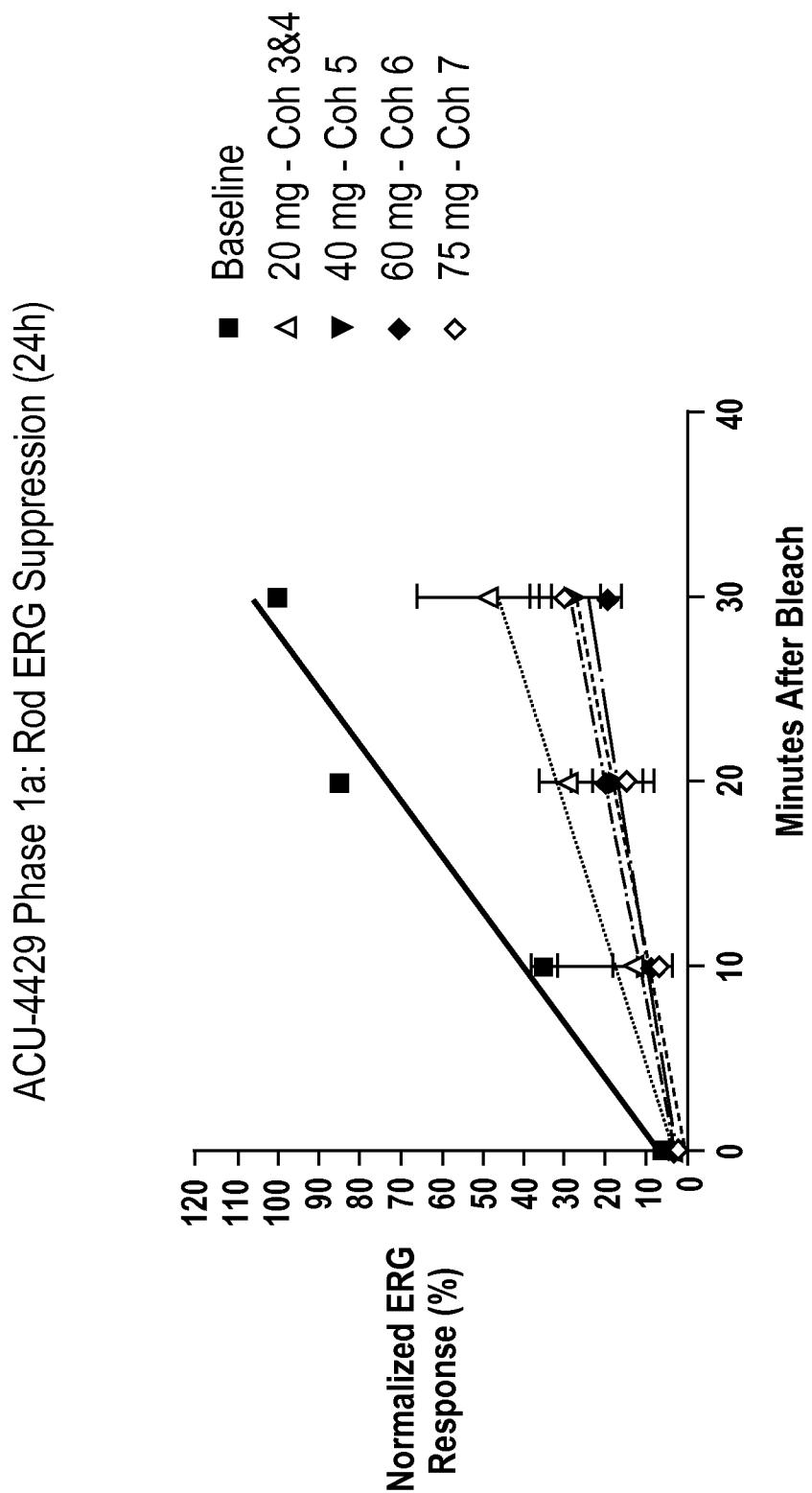
**FIG. 1****FIG. 2**

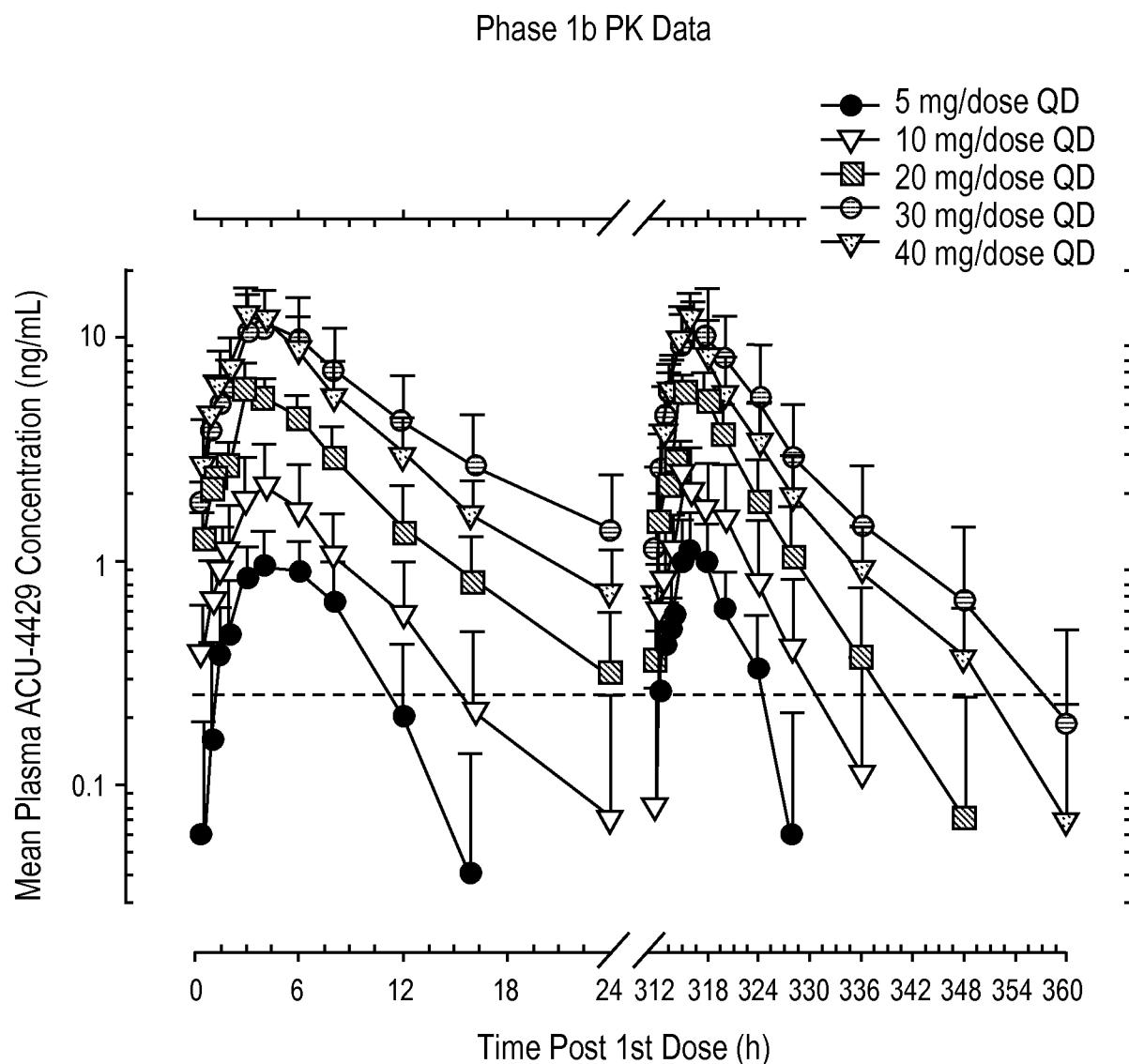
**FIG. 3**

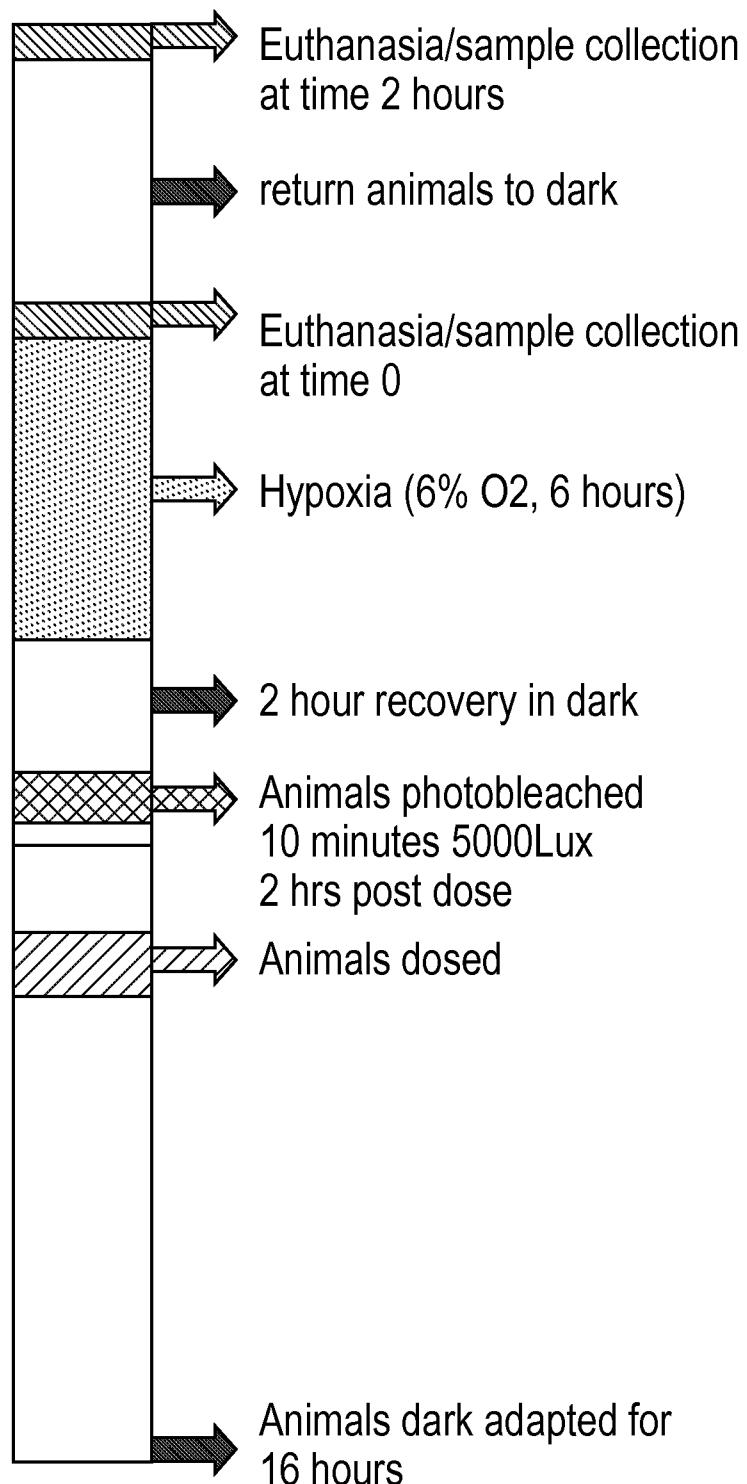
**FIG. 4A**

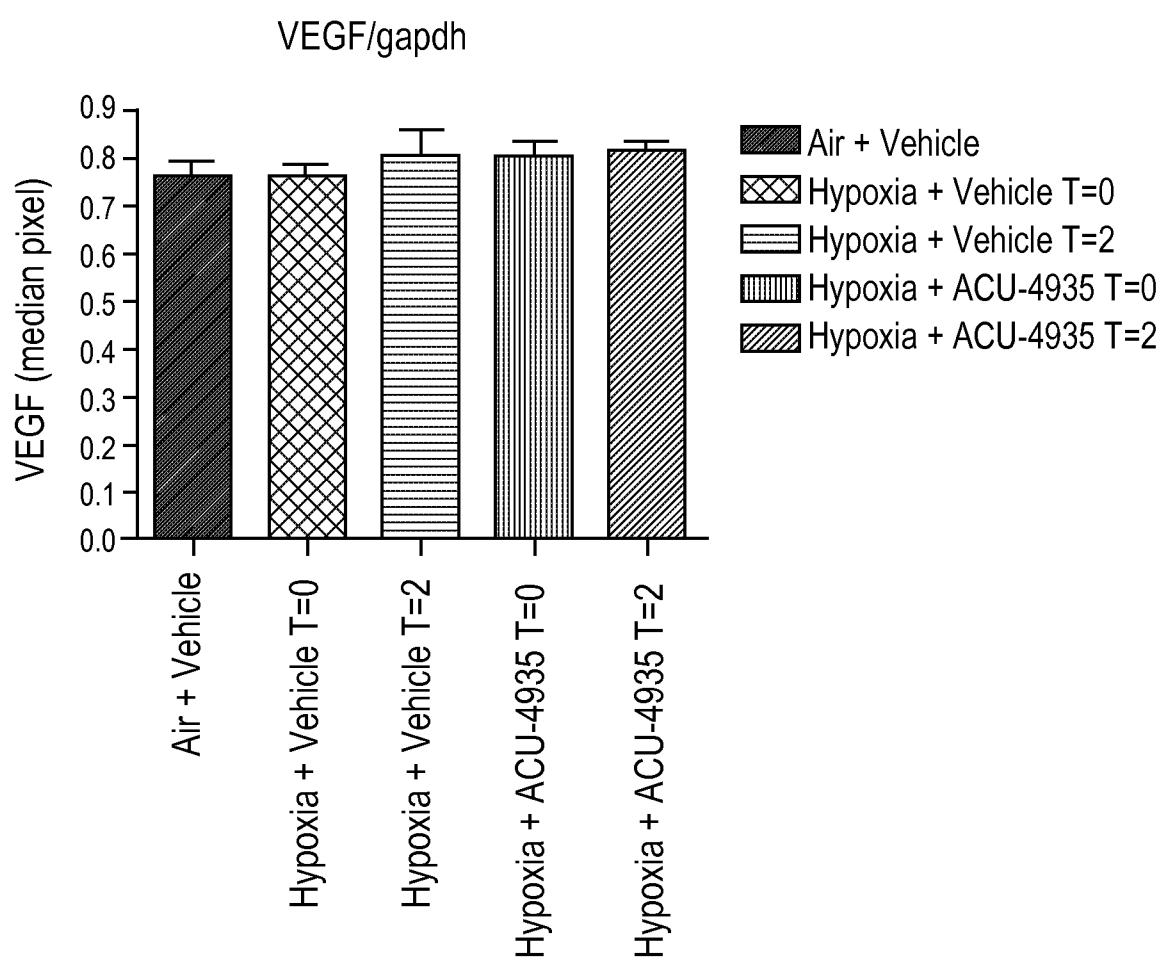
**FIG. 4B**

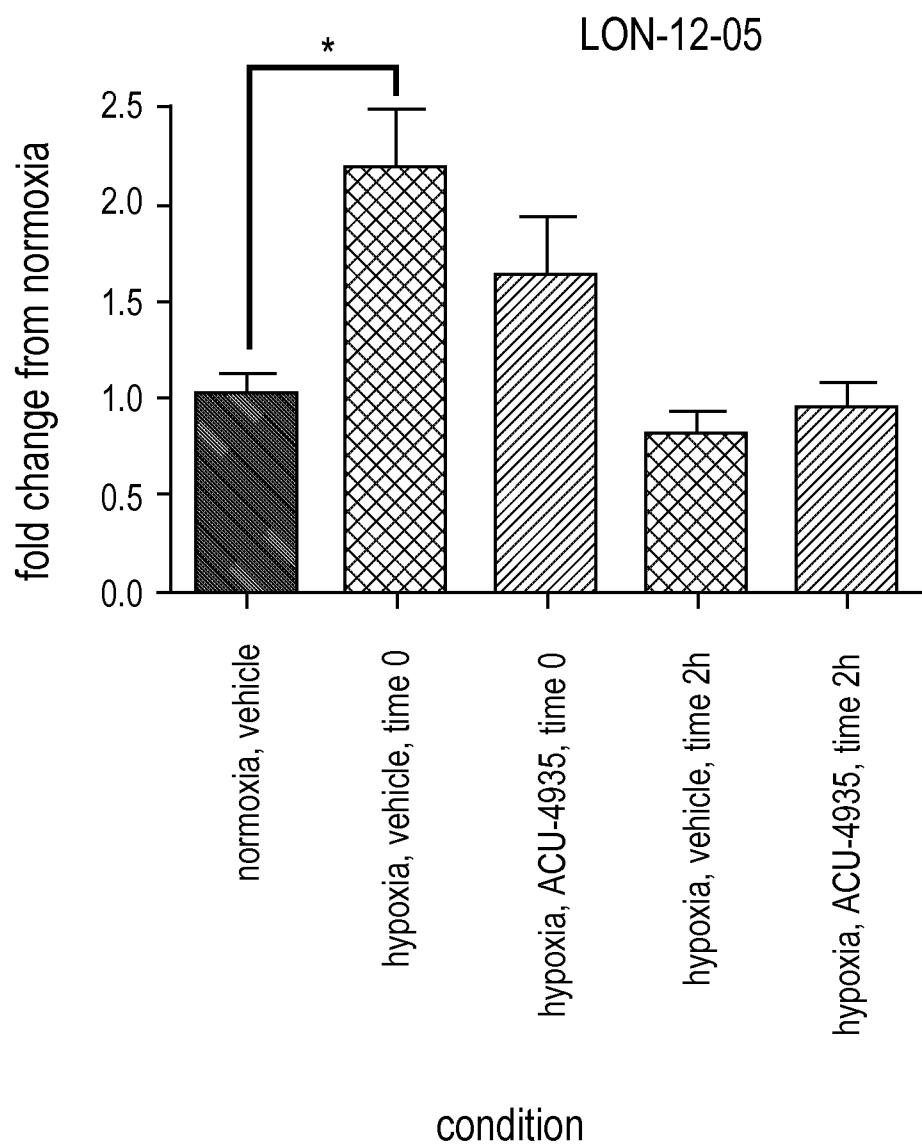
**FIG. 5**

**FIG. 6**

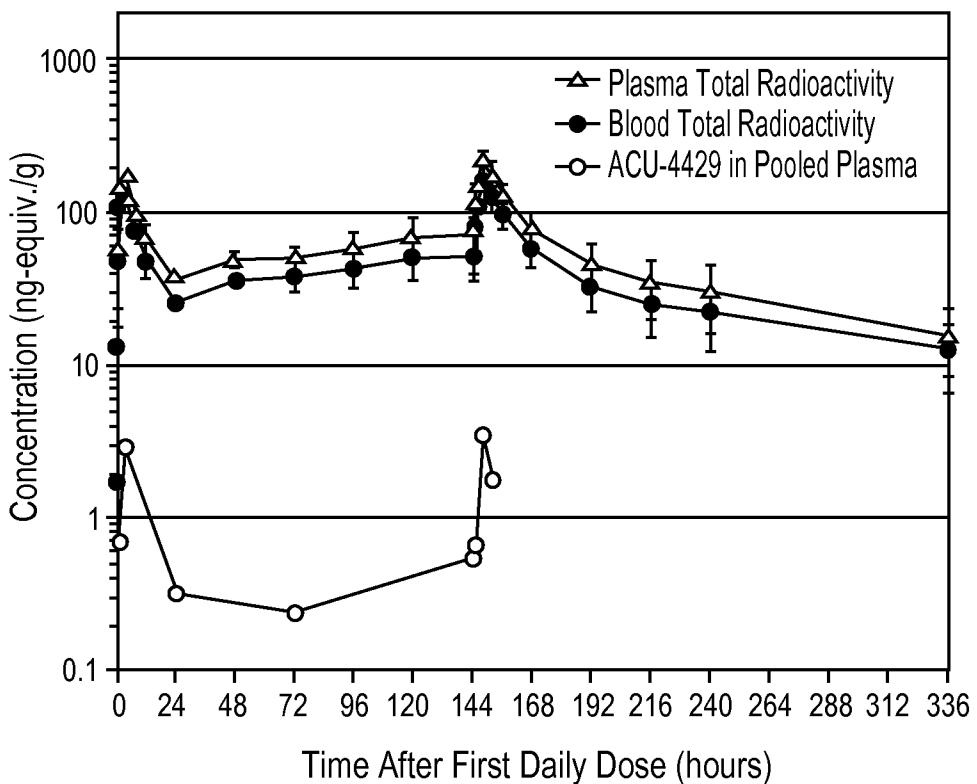
**FIG. 7**

**FIG. 8**

**FIG. 9**

**FIG. 10**

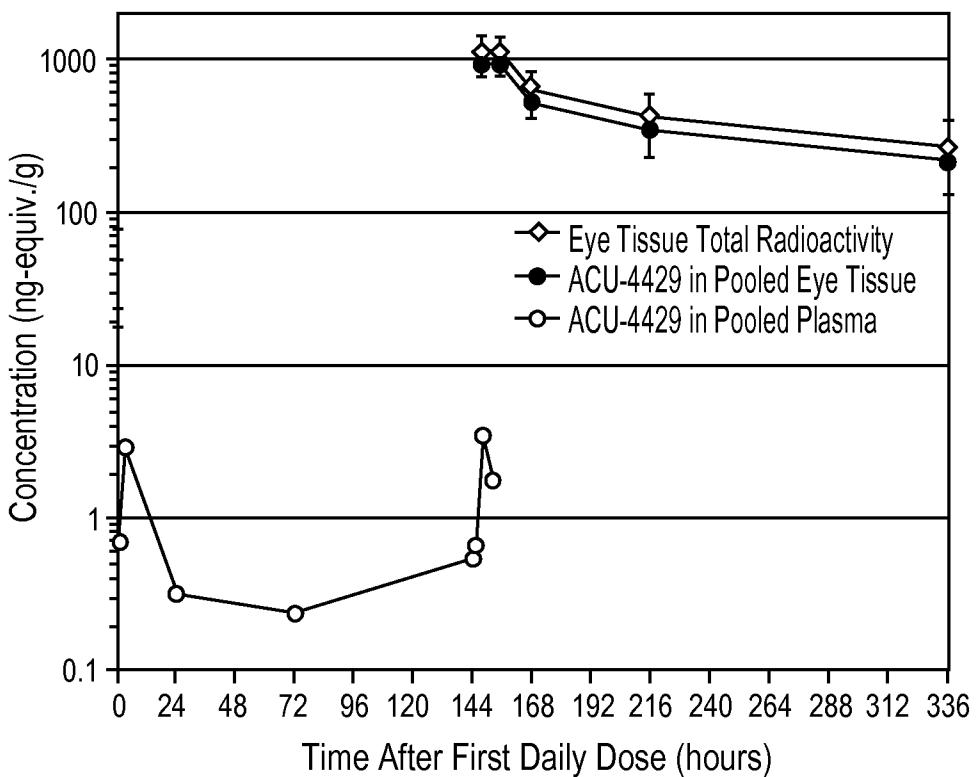
**Blood or Plasma**  
Mean Concentration-Time Profiles



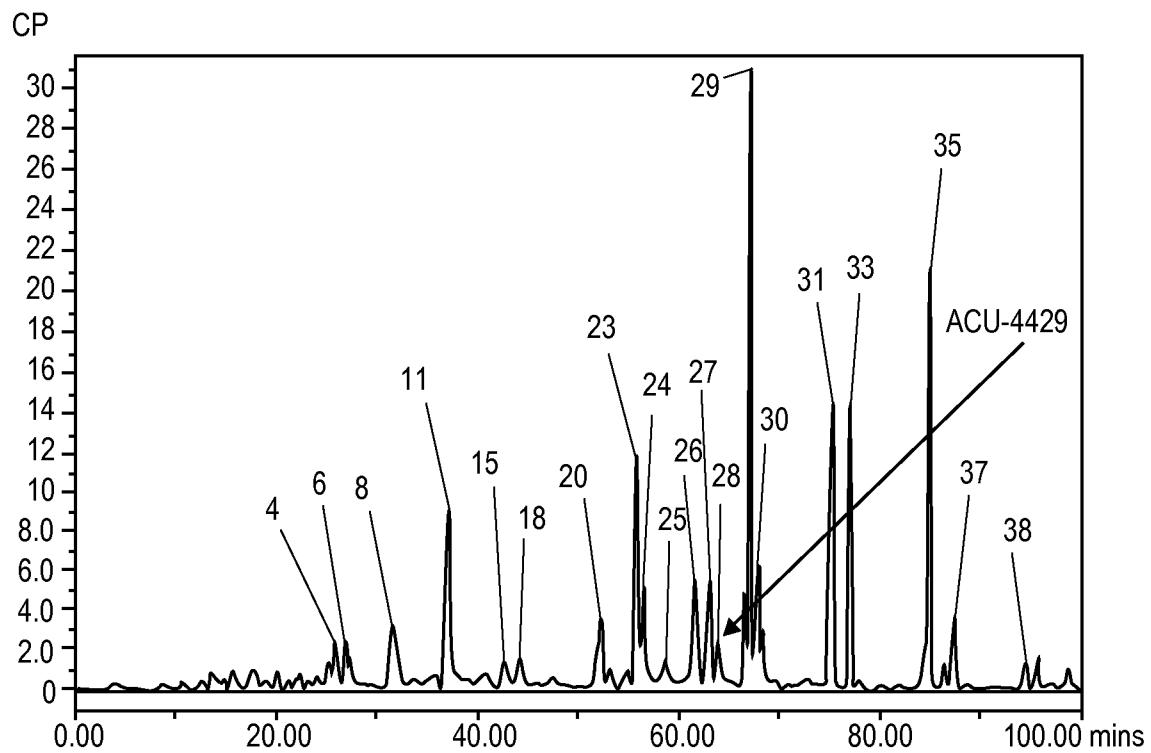
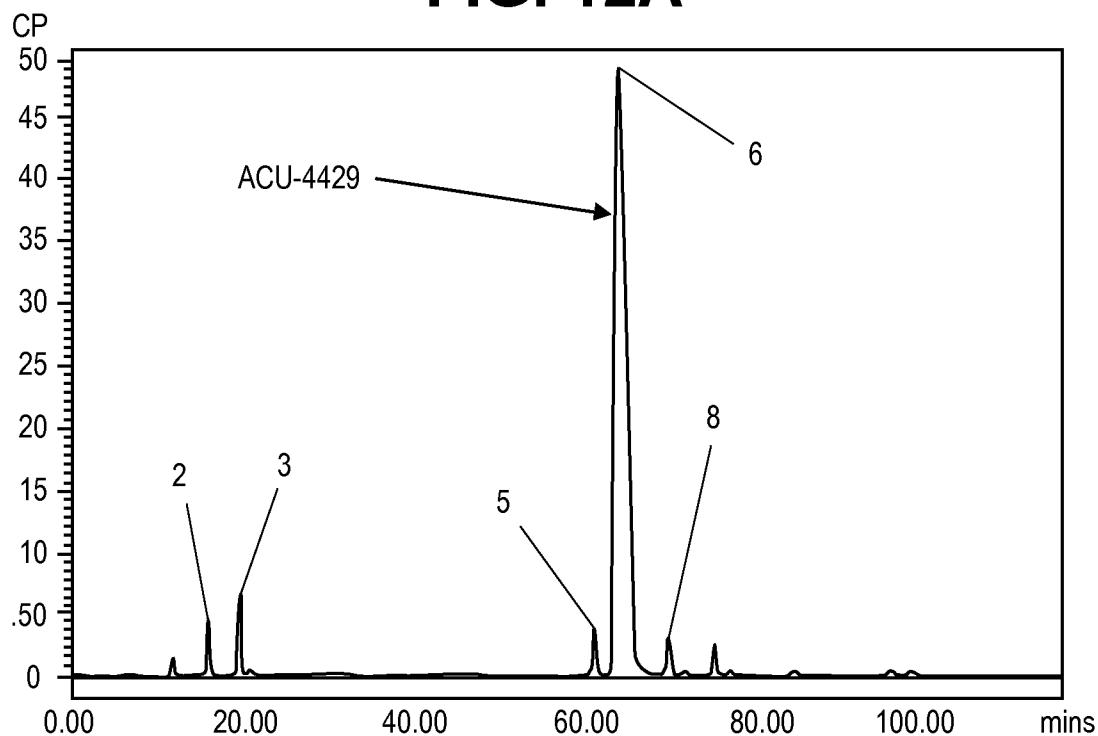
**FIG. 11A**

**Eye Tissues**

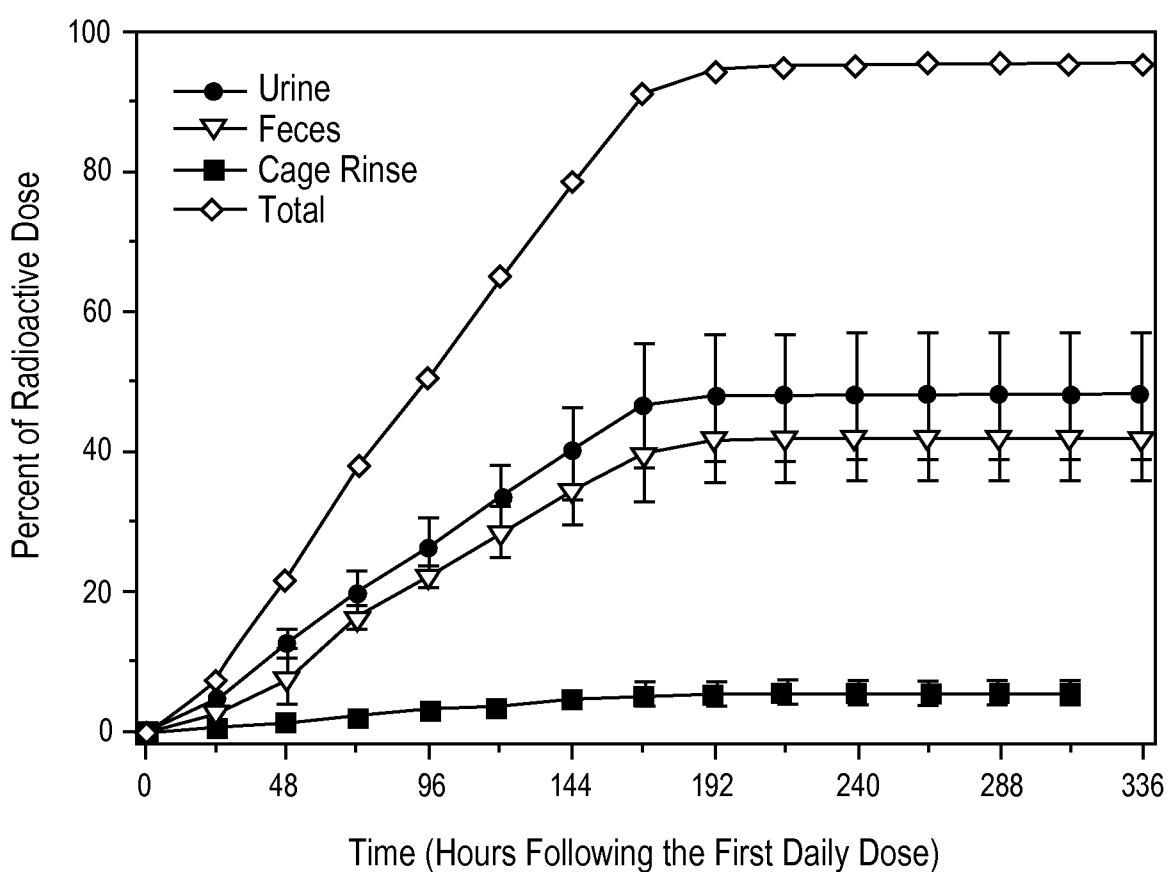
Mean Concentration-Time Profiles



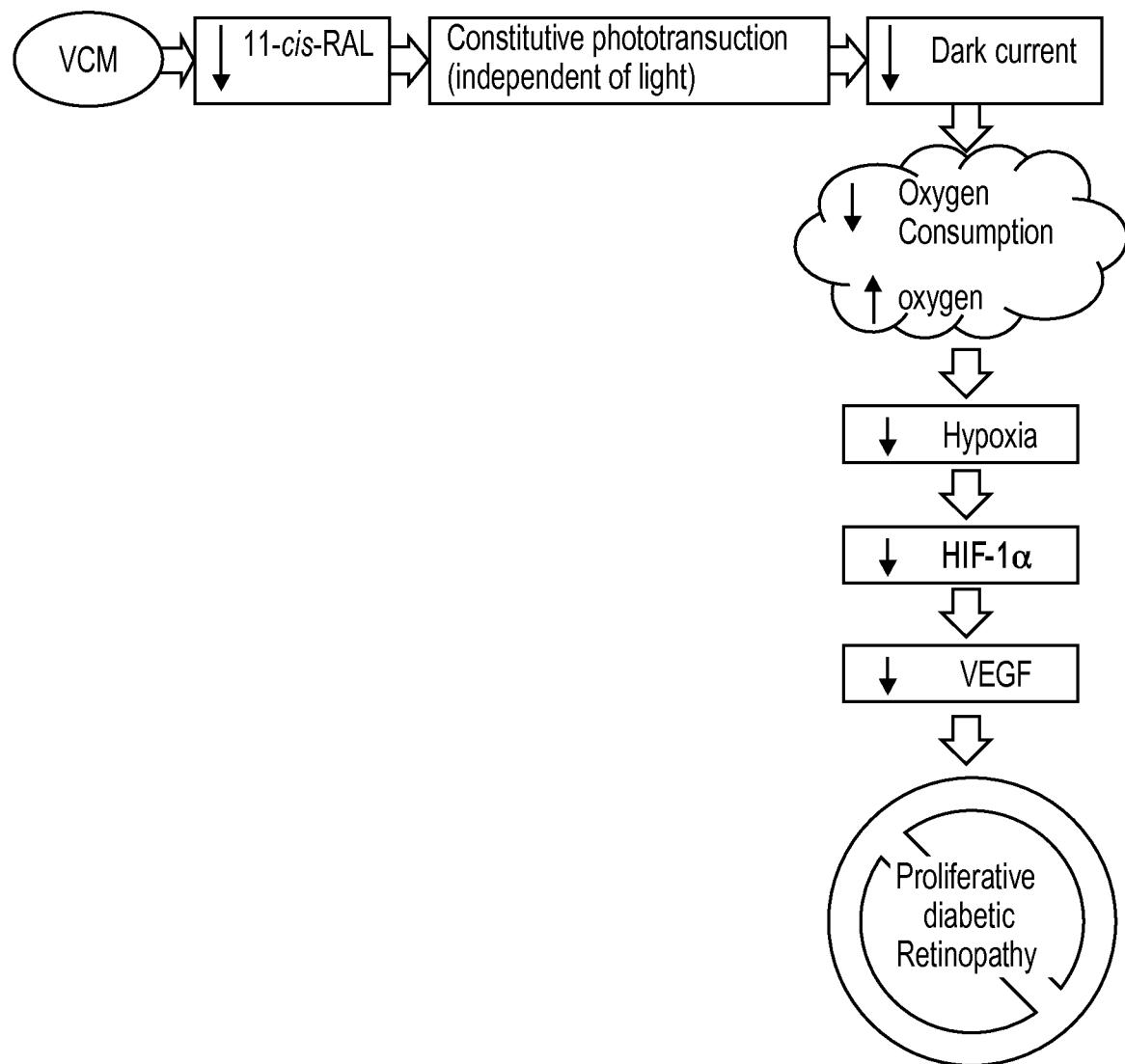
**FIG. 11B**

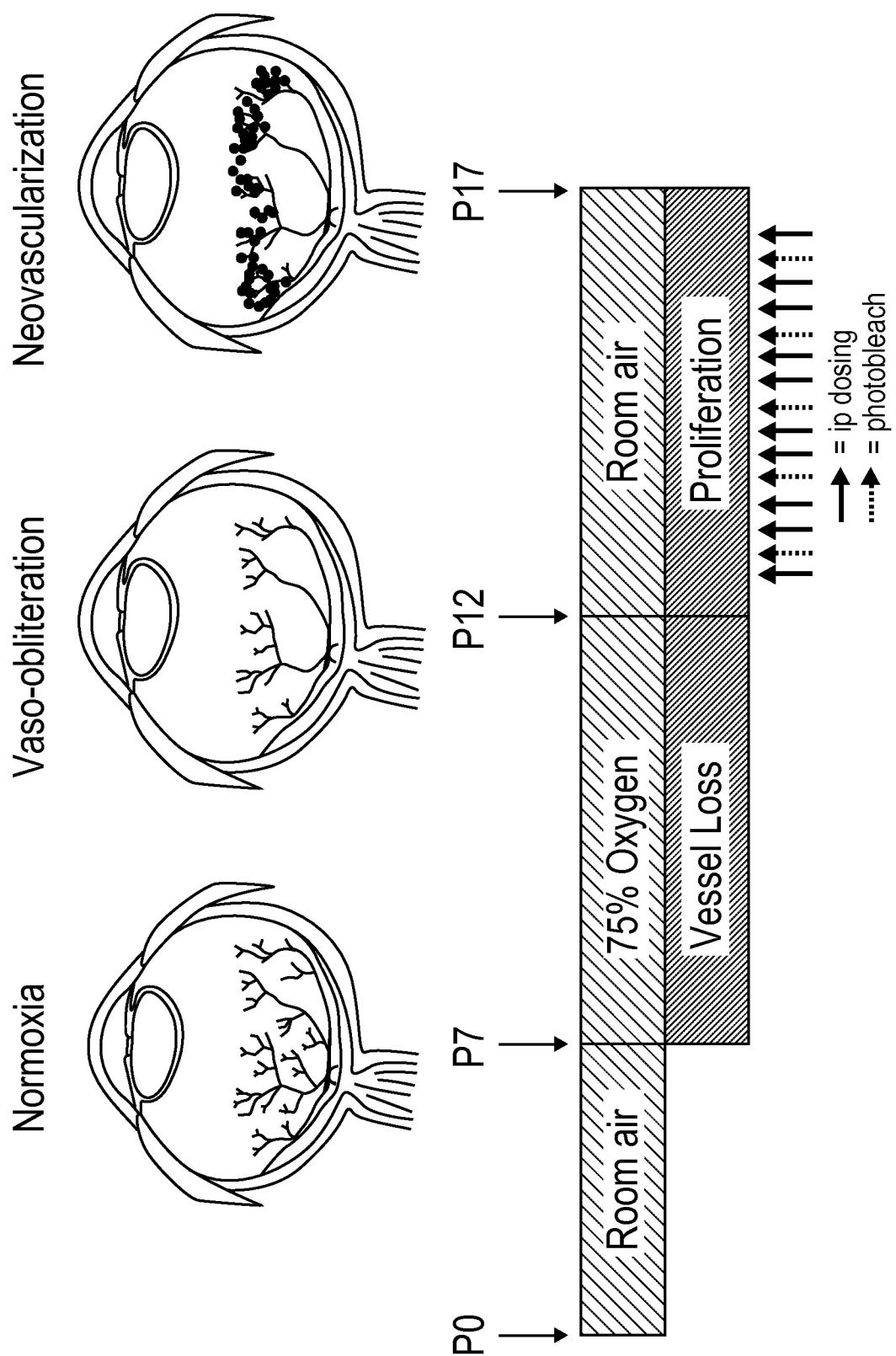
**Metabolite Radioprofiles at 4 Hours Post-dose on Day 7****FIG. 12A****FIG. 12B**

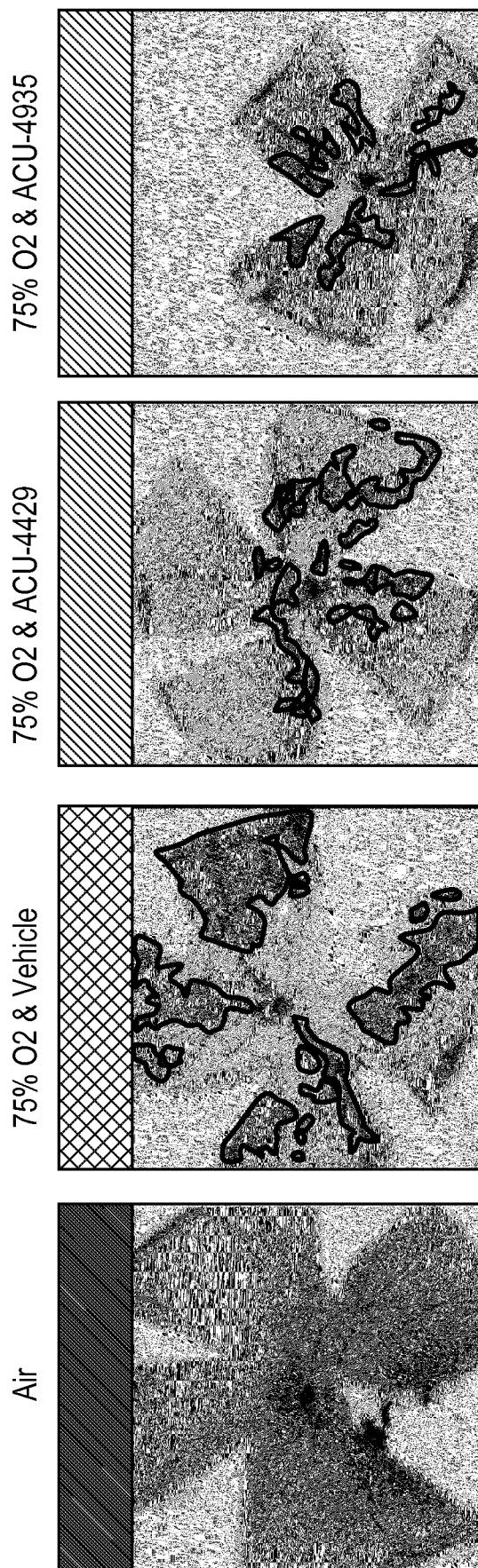
### Mean Cumulative Percentage of Radioactive Dose Recovered



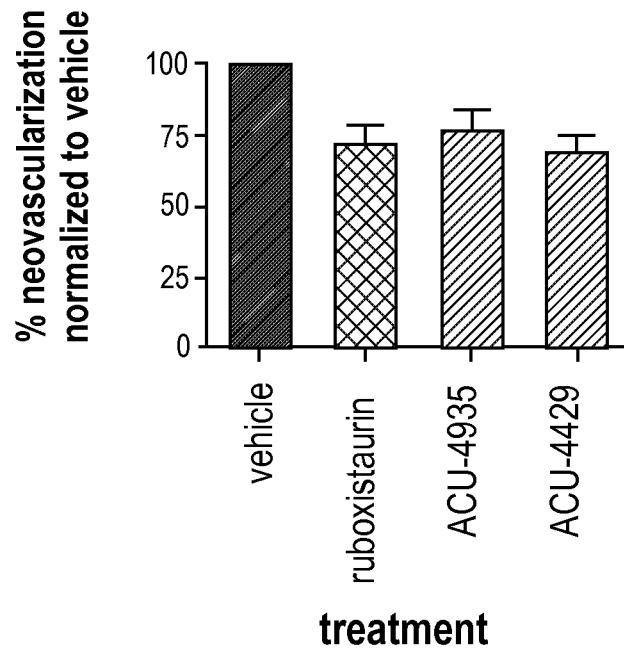
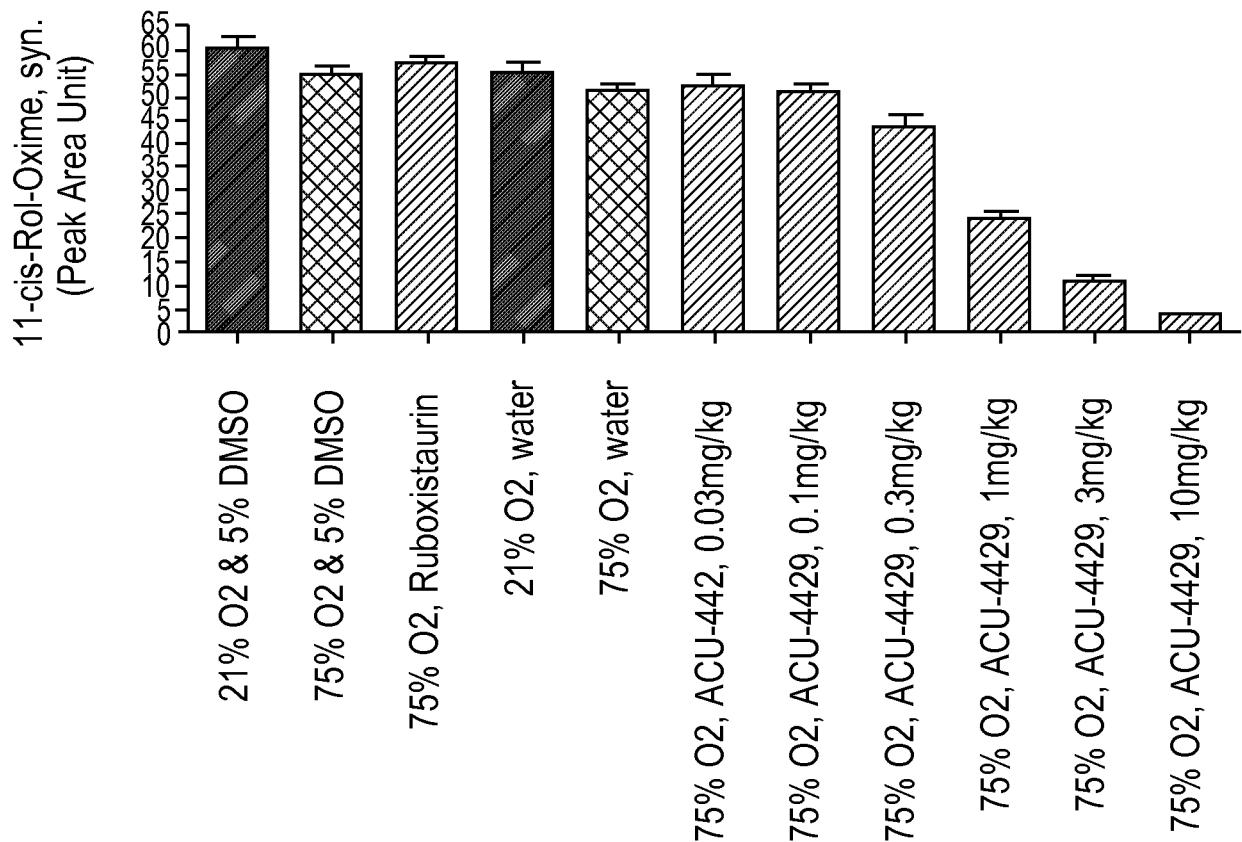
**FIG. 13**

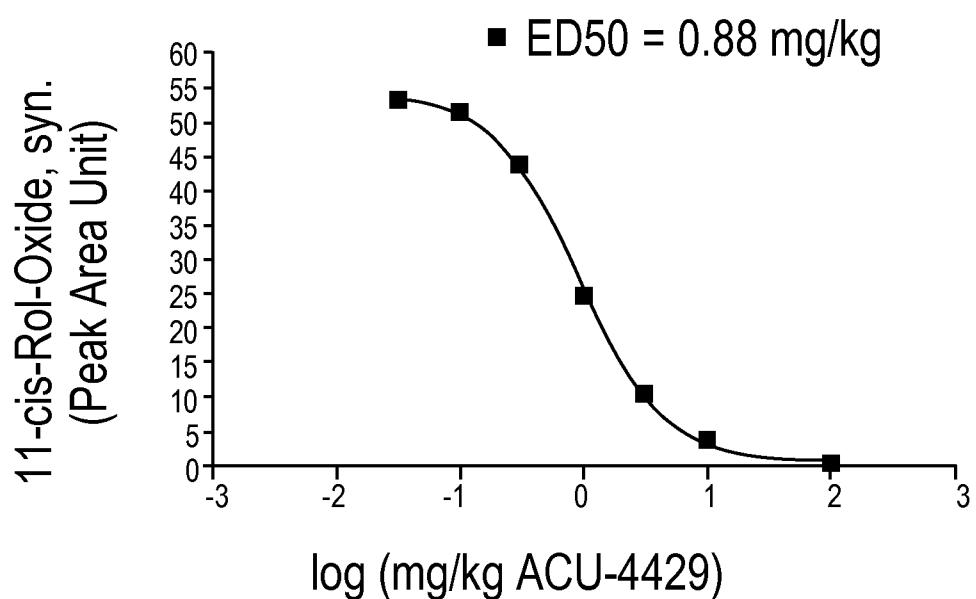
**FIG. 14**

**FIG. 15**

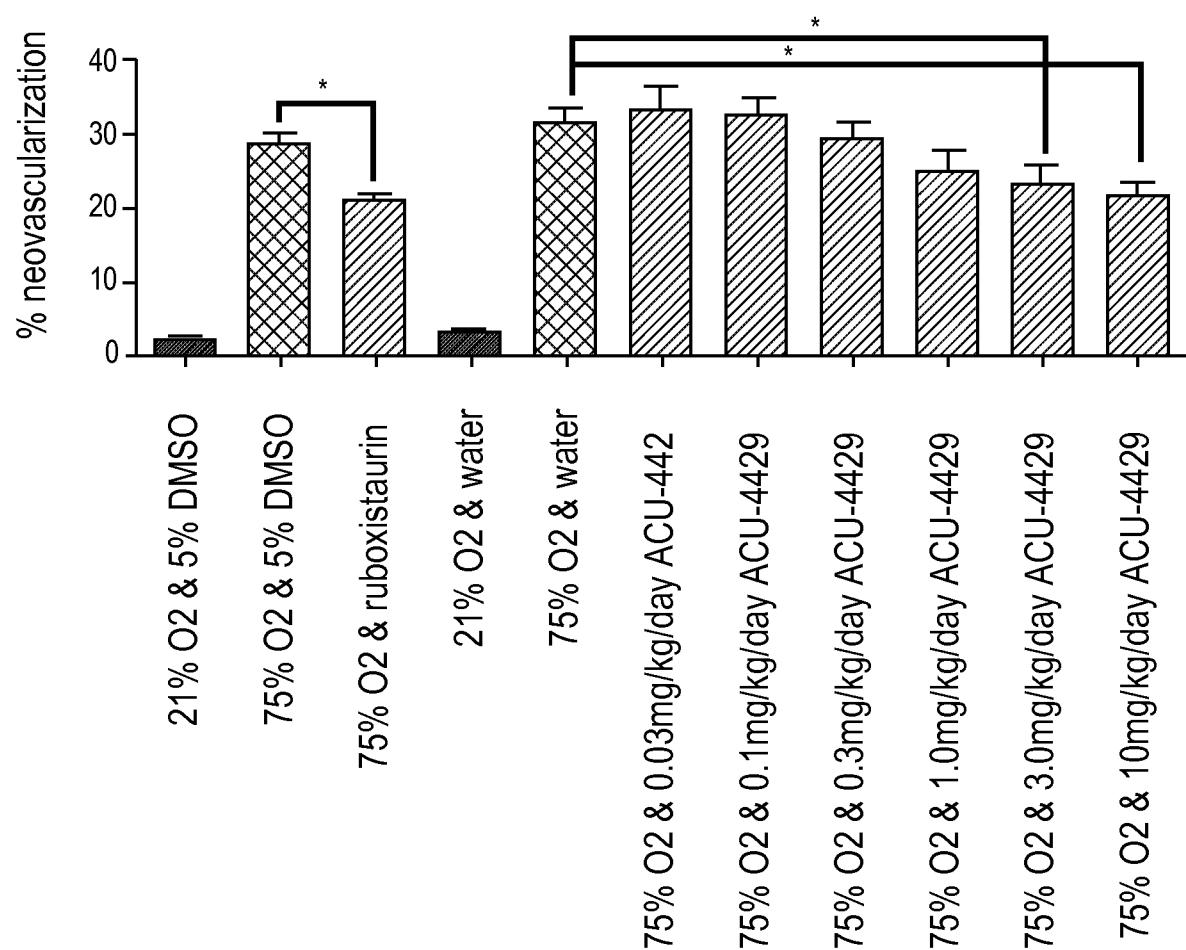


**FIG. 16A**

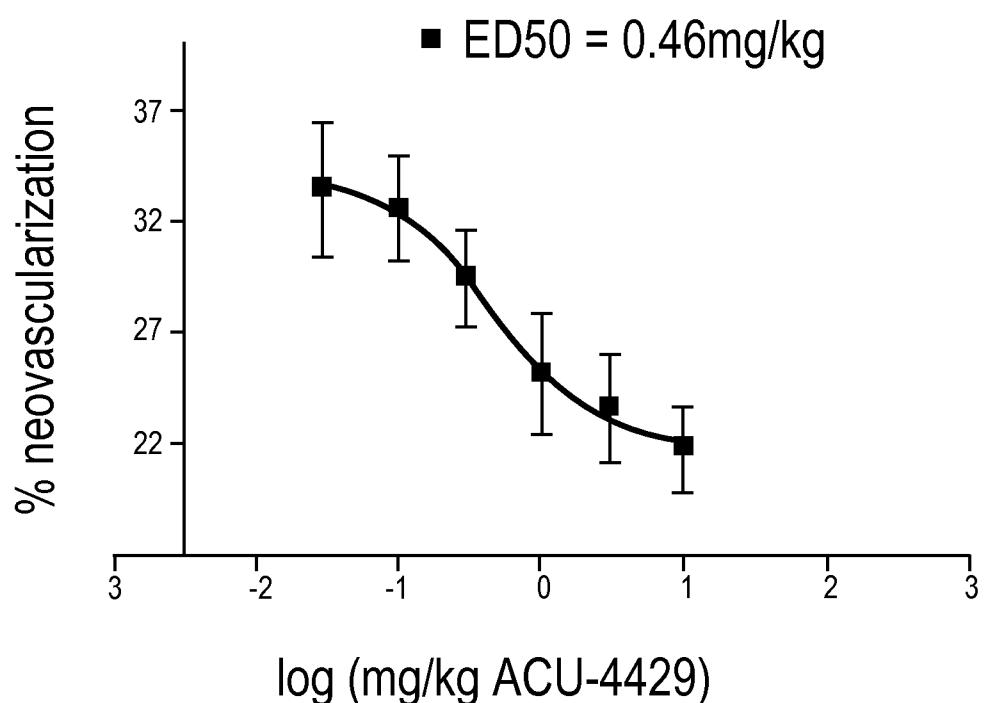
**FIG. 16B****FIG. 16C**

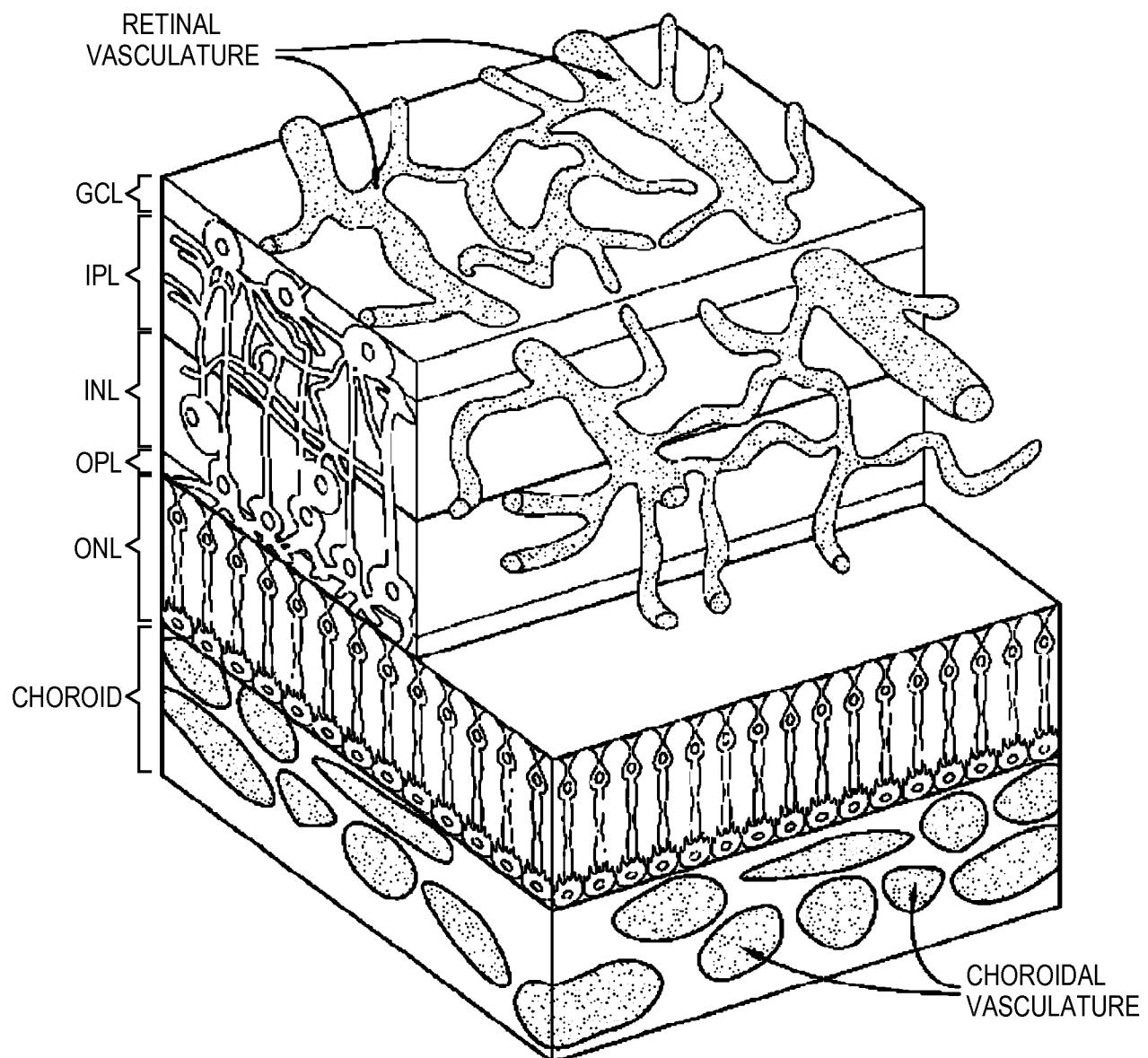
**FIG. 16D**

19/27

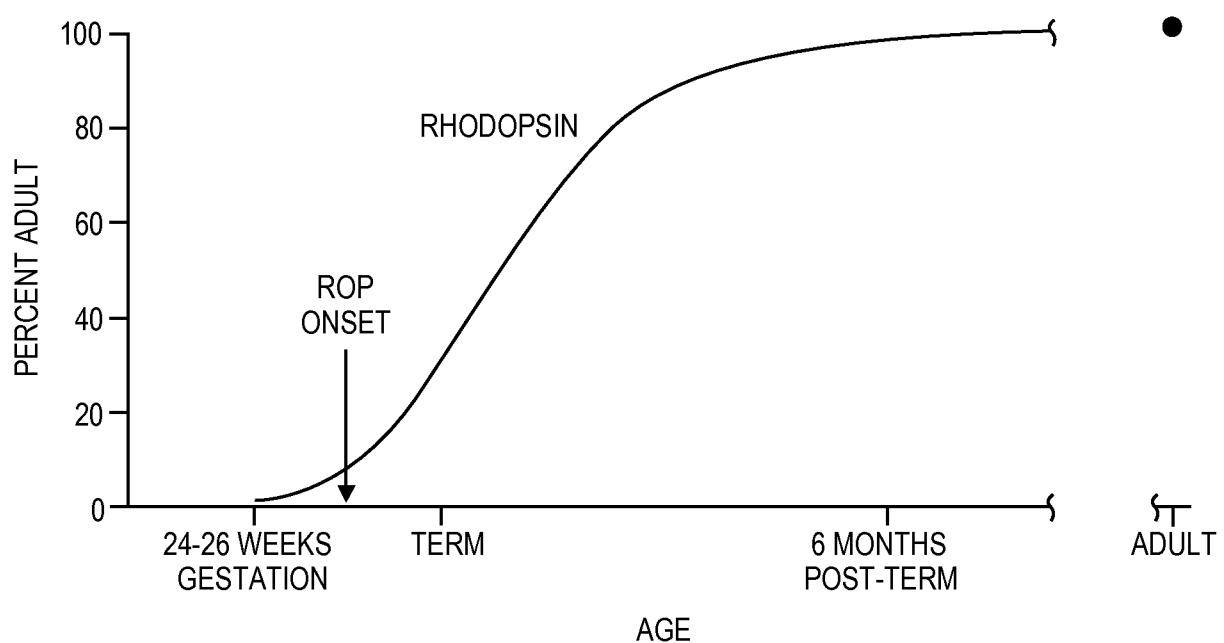
**FIG. 16E**

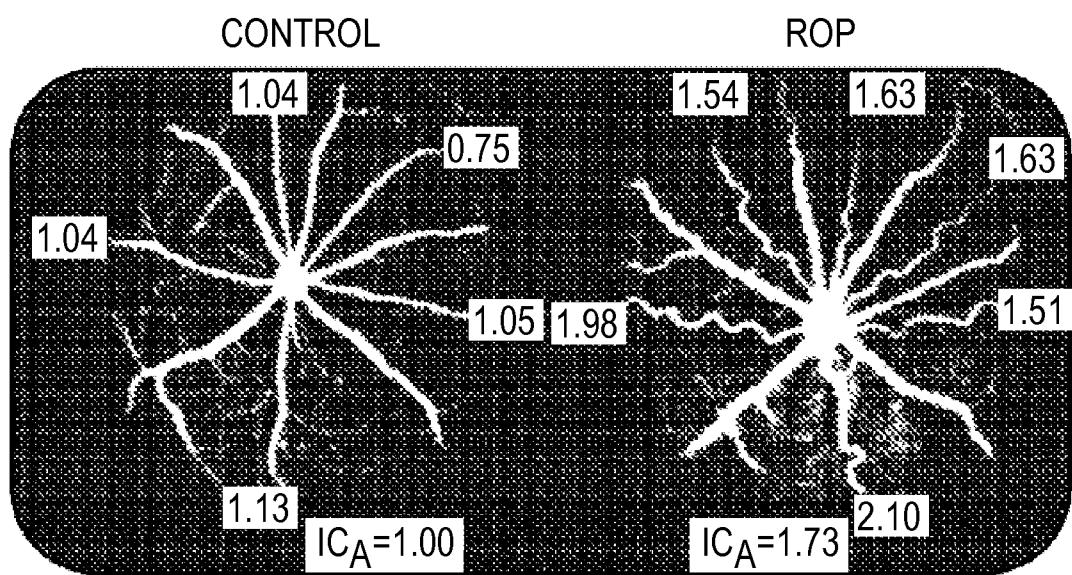
20/27

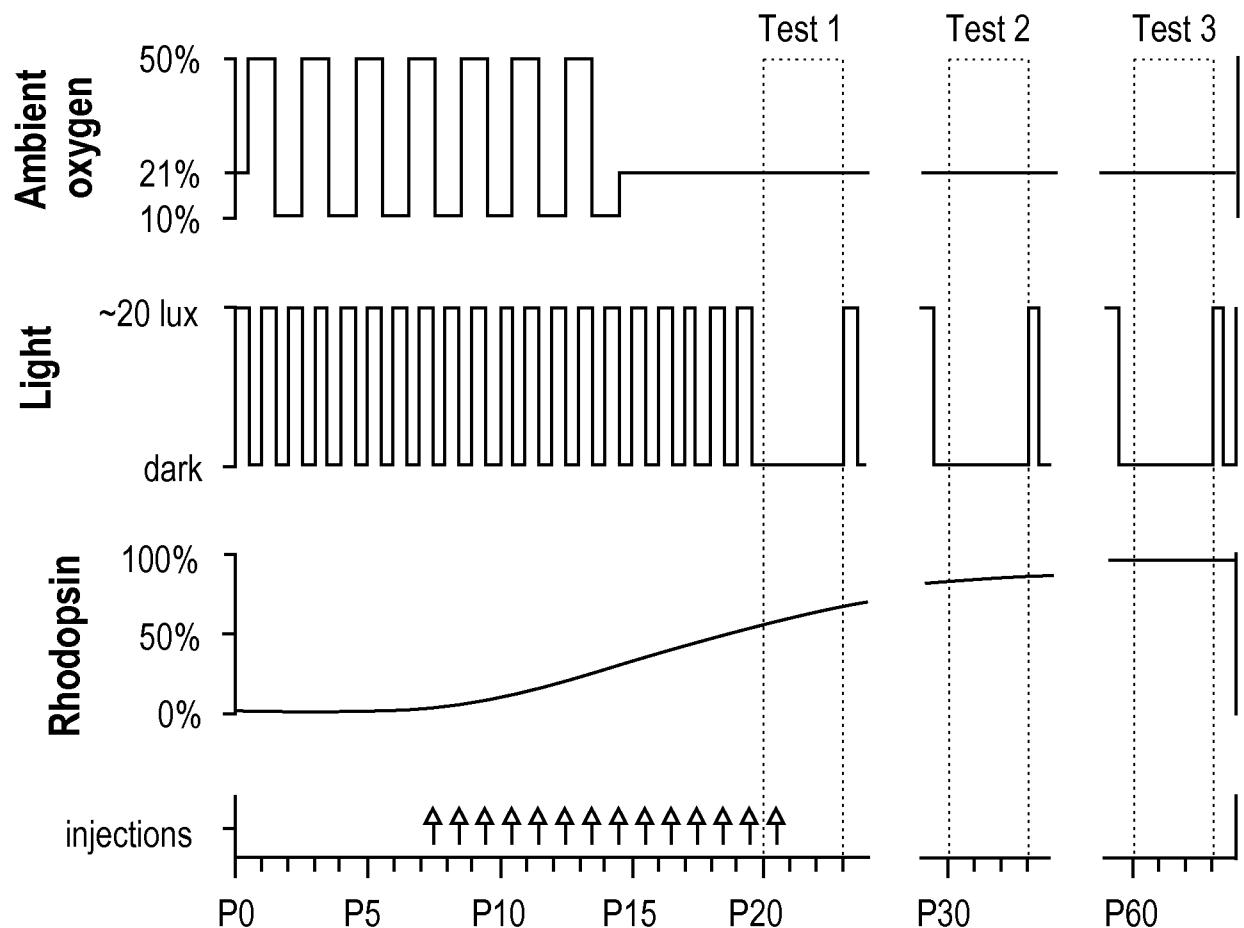
**FIG. 16F**

**FIG. 17**

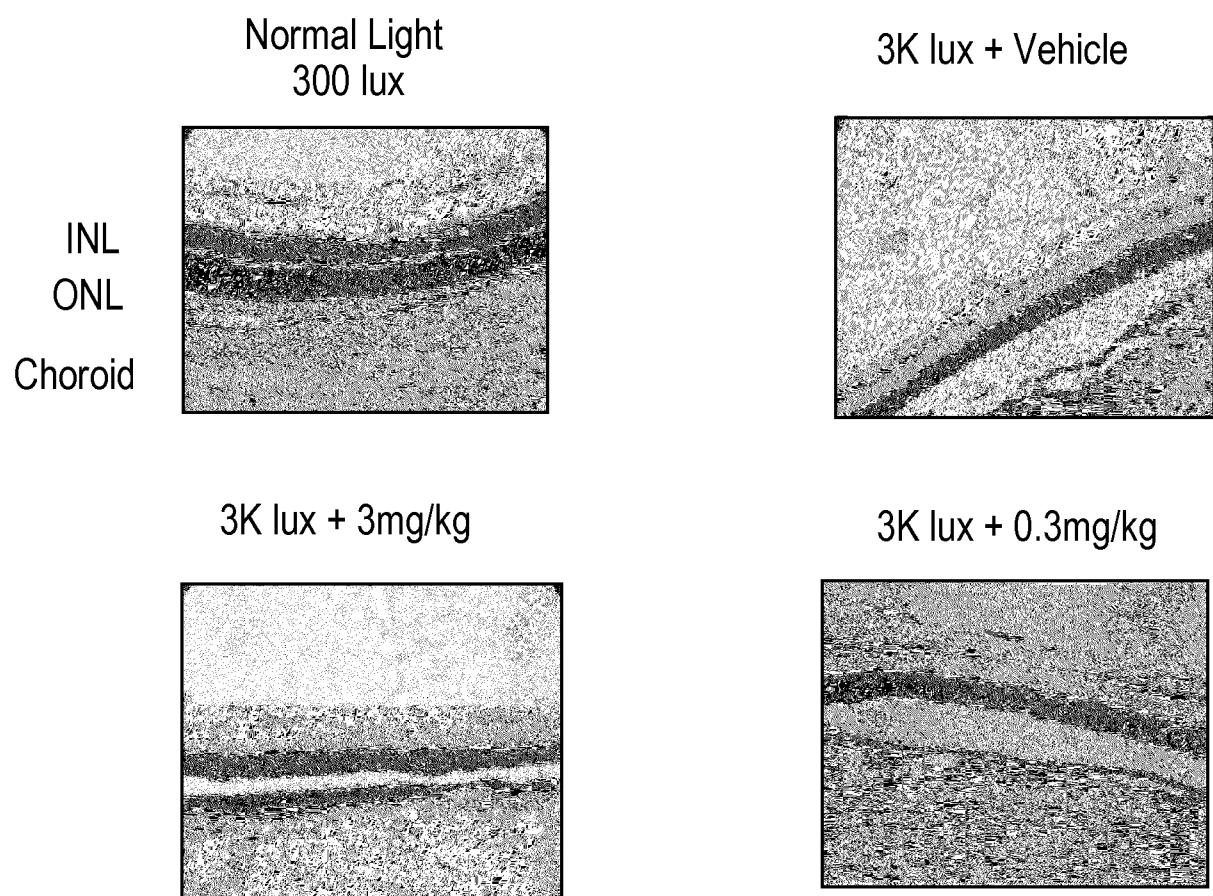
22/27

**FIG. 18**

**FIG. 19**

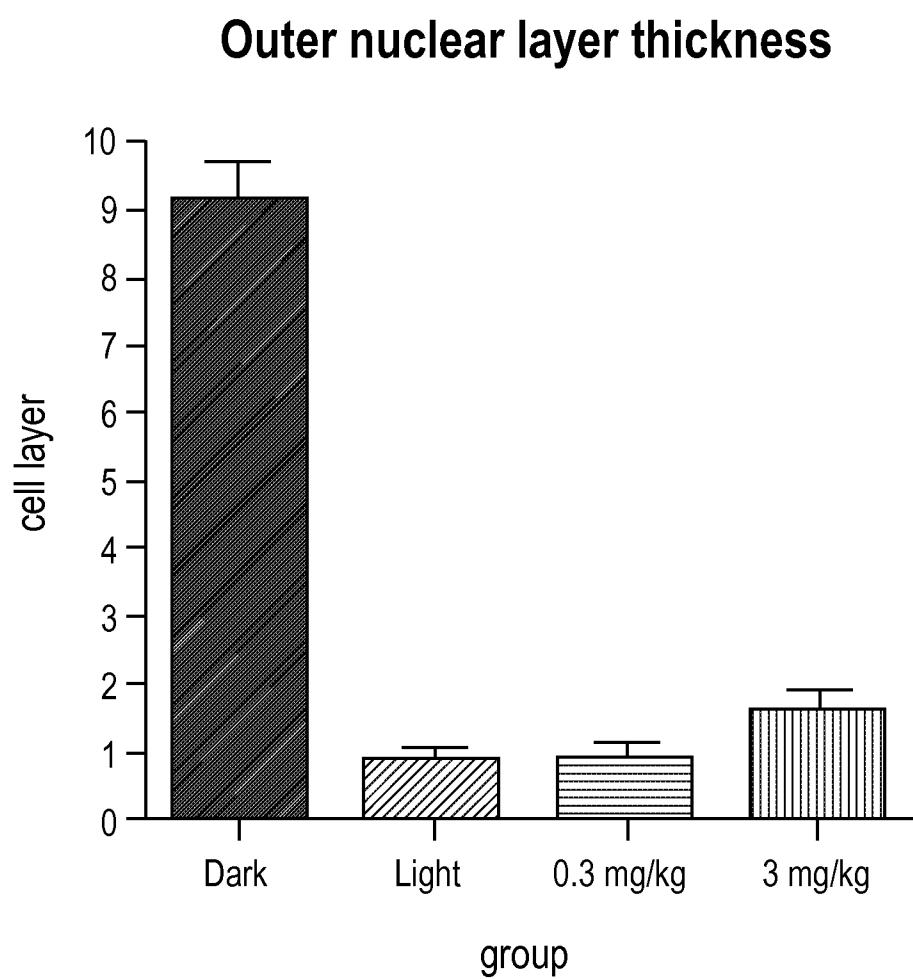
**FIG. 20**

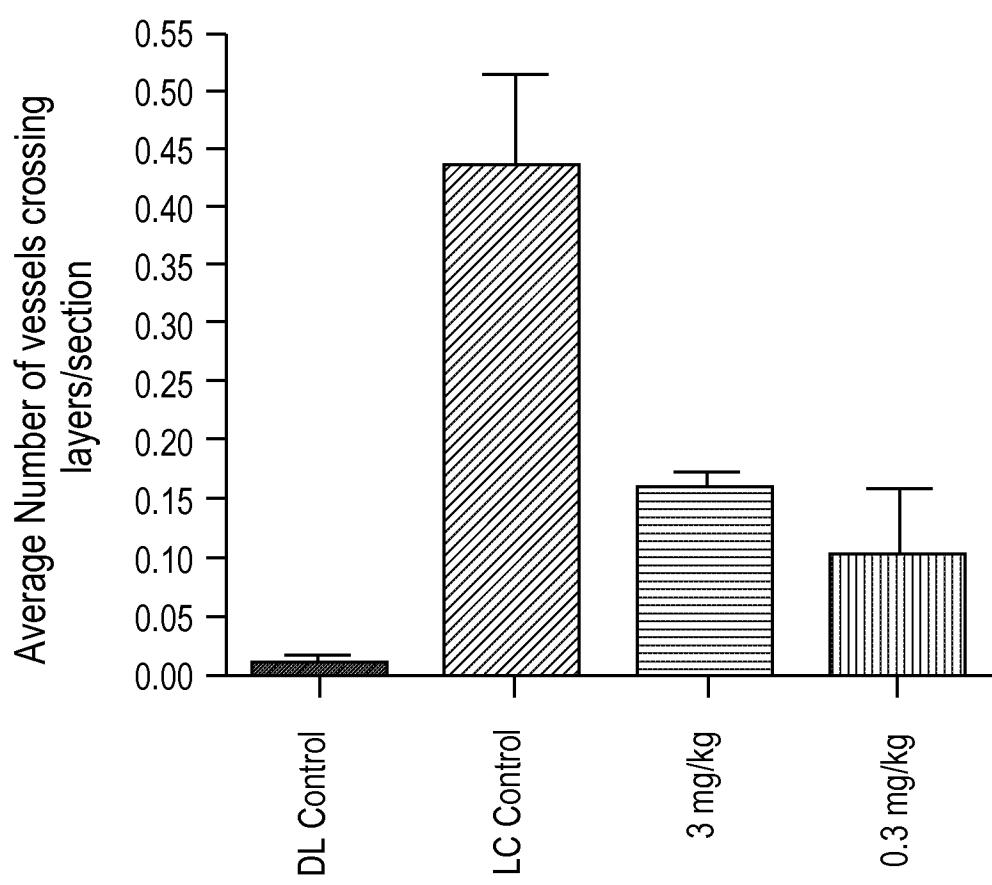
## Comparison of ONL



**FIG. 21**

26/27

**FIG. 22**

**WIS-11-04****FIG. 23**

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization

International Bureau



(10) International Publication Number

WO 2013/166449 A3

(43) International Publication Date  
7 November 2013 (07.11.2013)

(51) International Patent Classification: C07C 215/30 (2006.01)

(52) International Application Number: PCT/US2013/039562

(53) International Filing Date: 3 May 2013 (03.05.2013)

(54) Filing Language: English

(55) Publication Language: English

(56) Priority Data:

61/643,178	4 May 2012 (04.05.2012)	US
61/643,058	4 May 2012 (04.05.2012)	US
61/643,051	4 May 2012 (04.05.2012)	US
61/781,907	14 March 2013 (14.03.2013)	US

(71) Applicant: ACUCELA INC. [US/US]; 1301 Second Ave., Ste. 1900, Seattle, WA 98101 (US).

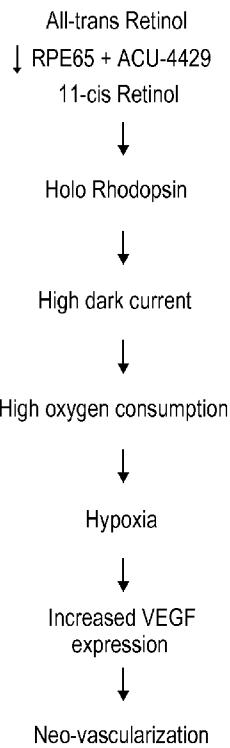
(72) Inventors: BAVIK, Claes, Olof; 20216 41st Place NE, Lake Forest Park, WA 98155 (US). HENRY, Susan, Hayes; 9425 126th Place, Kirkland, WA 98034 (US). KUBOTA, Ryo; 1301 Second Ave., Suite 1900, Seattle, WA 98101 (US). KUKSA, Vladimir, A.; 417 203rd Place SE, Bothell, WA 98101 (US).

(74) Agent: CLARK, David, L., D.; Wilson Sonsini Goodrich & Rosati, 650 Page Mill Road, Palo Alto, CA 94304 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,

[Continued on next page]

(54) Title: METHODS FOR THE TREATMENT OF DIABETIC RETINOPATHY AND OTHER OPHTHALMIC DISEASES



(57) Abstract: Methods are provided herein for the treatment of ophthalmic diseases or conditions such as an ophthalmic disease or disorder associated with diabetes in a patient. Also provided herein are methods of treating retinopathy of prematurity in a patient. Further, provided herein are methods for treating wet age-related macular degeneration in a patient. The methods comprise administration of compounds disclosed herein to a patient in need thereof that inhibit or slow one or more signs or symptoms of such conditions.

WO 2013/166449 A3

**FIG. 4B**

**Declarations under Rule 4.17:**

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

**Published:**

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

**(88) Date of publication of the international search report:**

30 January 2014

HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

**(84) Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2013/039562

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07C 215/30 (2013.01)

USPC - 514/653

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)

IPC(8) - A61K 31/137; A61P 27/02; C07C 211/27, 215/08, 215/30 (2013.01)

USPC - 514/653, 866; 564/355

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  
CPC - A61K 31/137; C07C 211/27, 215/08, 215/30 (2013.01)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase, Google Patents, Google Scholar, PubChem

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/0170841 A1 (SCOTT et al) 02 July 2009 (02.07.2009) entire document	1, 4, 5, 24, 48-54
A	US 2010/0113539 A1 (SCOTT et al) 06 May 2010 (06.05.2010) entire document	1, 4, 5, 24, 48-54
A	US 2011/0003895 A1 (KUBOTA et al) 06 January 2011 (06.01.2011) entire document	1, 4, 5, 24, 48-54

 Further documents are listed in the continuation of Box C. 

\* 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
- “L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- “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

- “T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- “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  20 November 2013	Date of mailing of the international search report  05 DEC 2013
Name and mailing address of the ISA/US  Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer:  Blaine R. Copenheaver  PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US2013/039562

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.: 12-21, 26, 43-47, 55, 62-64 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

See Extra Sheet

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1, 4, 5, 24, 48-54

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US2013/039562

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

**Group I+:** Claims 1-11, 22-25, 27-42, 48-54, and 56-61 are drawn to methods for treating ophthalmic diseases or disorders.

The first invention of Group I+ is restricted to a method for treating an ophthalmic disease or disorder comprising administering to a patient a therapeutically effective amount of a composition comprising a compound, or tautomer, stereoisomer, geometric isomer, N-oxide or a pharmaceutically acceptable salt thereof of Formula A wherein X is selected to be  $-C(R9)=C(R9)-$ , Y is selected to be substituted or unsubstituted carbocyclyl, R1 is hydrogen, R2 is hydroxyl, R7 is hydrogen, R8 is hydrogen, each R9 is independently hydrogen, and R33 is absent where  $n = 0$ . It is believed that claims 1, 4, 5, 24, and 48-54 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

Should an additional fee(s) be paid, Applicant is invited to elect additional formulas to be searched in a specified combination. The exact claims searched will depend on Applicant's election. An exemplary election would be a method for treating an ophthalmic disease or disorder comprising administering to a patient a therapeutically effective amount of a composition comprising a compound, or tautomer, stereoisomer, geometric isomer, N-oxide or a pharmaceutically acceptable salt thereof, of Formula A wherein X is selected to be  $-C(R9)=C(R9)-$ , Y is selected to be substituted or unsubstituted carbocyclyl, R1 is hydrogen, R2 is hydroxyl, R7 is hydrogen, R8 is  $CH_3$ , each R9 is independently hydrogen, and R33 is absent where  $n = 0$ . Additional Formulas will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The Group I+ methods do not share a significant structural element that is responsible for the represented effect of treating ophthalmic diseases or disorders requiring the selection of alternate compounds.

Group I+ share the technical features of a method for treating an ophthalmic disease or disorder associated with diabetes in a patient or associated with neovascularization in the eye of a patient or treating or preventing retinopathy of prematurity comprising administering to the patient a therapeutically effective amount of a composition comprising a compound of Formula (A), or tautomer, stereoisomer, geometric isomer, N-oxide or a pharmaceutically acceptable salt thereof. However, these technical features do not represent a contribution over the prior art.

Specifically, US 2011/0003895 A1 to Kubota et al. teach a method for treating an ophthalmic disease or disorder associated with diabetes in a patient (see Para. [0053]), the method wherein the ophthalmic disease or disorder is...diabetic retinopathy...retinal blood vessel occlusion, retinopathy of prematurity...ophthalmic disease or disorder is dry age-related macular degeneration) or associated with neovascularization in the eye of a patient (Para. [0053]) or treating or preventing retinopathy of prematurity (Para. [0053]) comprising administering to the patient a therapeutically effective amount of a composition comprising a compound of Formula (A), or tautomer, stereoisomer, geometric isomer, N-oxide or a pharmaceutically acceptable salt thereof: wherein, X is  $-C(R9)2-O-$ ; Y is a) substituted or unsubstituted carbocyclyl, optionally substituted with -OH; R1 is hydrogen and R2 is hydroxyl; R7 is hydrogen; R8 is hydrogen; each R9 is independently hydrogen; each R33 is independently selected from halogen or substituted or unsubstituted C 1-C4 alkyl, and n is 0 (see Para. [0047] method for treating an ophthalmic disease or disorder comprising administration of a non-retinoid compound wherein the non-retinoid compound, or tautomer, stereoisomer, geometric isomer, pharmaceutically acceptable solvate, hydrate, salt, polymorph, N-oxide or prodrug thereof, is selected from the group consisting of the first shown compound).

The inventions listed in Group I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.



(12) 发明专利申请

(10) 申请公布号 CN 104411678 A

(43) 申请公布日 2015. 03. 11

(21) 申请号 201380035735. 1

(74) 专利代理机构 北京金信知识产权代理有限公司 11225

(22) 申请日 2013. 05. 03

代理人 朱梅 徐琳

(30) 优先权数据

61/643, 051 2012. 05. 04 US

(51) Int. Cl.

61/643, 058 2012. 05. 04 US

C07C 215/30(2006. 01)

61/643, 178 2012. 05. 04 US

61/781, 907 2013. 03. 14 US

(85) PCT国际申请进入国家阶段日

2015. 01. 04

(86) PCT国际申请的申请数据

PCT/US2013/039562 2013. 05. 03

(87) PCT国际申请的公布数据

W02013/166449 EN 2013. 11. 07

(71) 申请人 奥克塞拉有限公司

地址 美国华盛顿州

(72) 发明人 克拉斯·奥洛夫·贝文

苏珊·海斯·亨利 辽·洼田

弗拉基米拉·A·库克萨

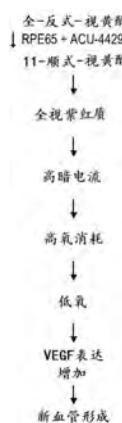
权利要求书14页 说明书70页 附图25页

(54) 发明名称

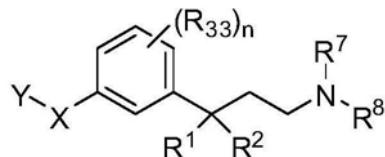
用于治疗糖尿病性视网膜病及其他眼科疾病的方法

(57) 摘要

本申请中提供了用于治疗患者的眼科疾病或病况,如与糖尿病相关的眼科疾病或病症的方法。本文也提供了治疗患者的早产儿视网膜病的方法。此外,本申请中提供了用于治疗患者的湿型年龄相关性黄斑变性的方法。所述方法包括向有需要的患者施用本文所公开的抑制或延缓这类病况的一种或多种迹象或症状的化合物。



1. 一种用于治疗患者中与糖尿病相关的眼科疾病或病症的方法,其包括向该患者施用治疗有效量的组合物,该组合物包含式 (A) 的化合物或其互变异构体、立体异构体、几何异构体、N- 氧化物或药学上可接受的盐:



式 (A)

其中,

X 选自  $-C(R^9) = C(R^9) -$ 、 $-C \equiv C -$ 、 $-C(R^9)_2O -$ 、 $-C(R^9)_2C(R^9) -$ 、 $-C(R^9)_2S -$ 、 $-C(R^9)_2S(O) -$  或  $-C(R^9)_2NR^9 -$ ;

Y 选自:

- a) 取代或未取代的碳环基,其任选地被  $C_1-C_4$  烷基、卤素、 $-OH$  或  $C_1-C_4$  烷氧基取代;
- b) 取代或未取代的碳环基烷基,其任选地被  $C_1-C_4$  烷基、卤素、 $-OH$  或  $C_1-C_4$  烷氧基取代;
- c) 取代或未取代的芳烷基,其任选地被  $C_1-C_4$  烷基、卤素、 $-OH$  或  $C_1-C_4$  烷氧基取代;或
- d) 取代或未取代的  $C_3-C_{10}$  烷基,其任选地被卤素、 $-OH$  或  $C_1-C_4$  烷氧基取代;

$R^1$  为氢且  $R^2$  为羟基;或者  $R^1$  与  $R^2$  形成氧代基团;

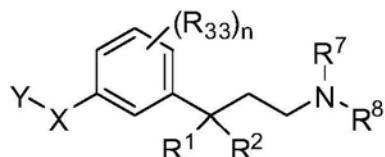
$R^7$  为氢;

$R^8$  为氢或  $CH_3$ ;

各个  $R^9$  独立地为氢或取代或未取代的  $C_1-C_4$  烷基;

各个  $R^{33}$  独立地选自卤素或取代或未取代的  $C_1-C_4$  烷基,且  $n$  为 0、1、2、3 或 4。

2. 一种用于治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法,其包括施用治疗有效量的组合物,该组合物包含式 (A) 的化合物或其互变异构体、立体异构体、几何异构体、N- 氧化物或药学上可接受的盐:



式 (A)

其中,

X 选自  $-C(R^9) = C(R^9) -$ 、 $-C \equiv C -$ 、 $-C(R^9)_2O -$ 、 $-C(R^9)_2C(R^9) -$ 、 $-C(R^9)_2S -$ 、 $-C(R^9)_2S(O) -$  或  $-C(R^9)_2NR^9 -$ ;

Y 选自:

- a) 取代或未取代的碳环基,其任选地被  $C_1-C_4$  烷基、卤素、 $-OH$  或  $C_1-C_4$  烷氧基取代;
- b) 取代或未取代的碳环基烷基,其任选地被  $C_1-C_4$  烷基、卤素、 $-OH$  或  $C_1-C_4$  烷氧基取代;
- c) 取代或未取代的芳烷基,其任选地被  $C_1-C_4$  烷基、卤素、 $-OH$  或  $C_1-C_4$  烷氧基取代;或
- d) 取代或未取代的  $C_3-C_{10}$  烷基,其任选地被卤素、 $-OH$  或  $C_1-C_4$  烷氧基取代;

$R^1$  为氢且  $R^2$  为羟基;或者  $R^1$  与  $R^2$  形成氧代基团;

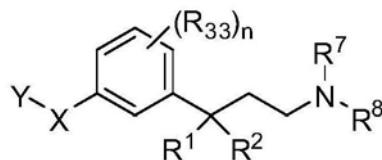
$R^7$  为氢;

$R^8$  为氢或  $CH_3$ ；

各个  $R^9$  独立地为氢或取代或未取代的  $C_1-C_4$  烷基；

各个  $R^{33}$  独立地选自卤素或取代或未取代的  $C_1-C_4$  烷基, 且  $n$  为 0、1、2、3 或 4。

3. 一种用于治疗或预防早产儿视网膜病的方法, 其包括向患者施用治疗有效量的组合物, 该组合物包含式 (A) 的化合物或其互变异构体、立体异构体、几何异构体、 $N$ - 氧化物或药学上可接受的盐：



式 (A)

其中,

$X$  选自  $-C(R^9)=C(R^9)-$ 、 $-C\equiv C-$ 、 $-C(R^9)_2O-$ 、 $-C(R^9)_2-C(R^9)_2-$ 、 $-C(R^9)_2S-$ 、 $-C(R^9)_2S(0)$  $_2-$  或  $-C(R^9)_2NR^9-$ ；

$Y$  选自：

- 取代或未取代的碳环基, 其任选地被  $C_1-C_4$  烷基、卤素、 $-OH$  或  $C_1-C_4$  烷氧基取代；
- 取代或未取代的碳环基烷基, 其任选地被  $C_1-C_4$  烷基、卤素、 $-OH$  或  $C_1-C_4$  烷氧基取代；
- 取代或未取代的芳烷基, 其任选地被  $C_1-C_4$  烷基、卤素、 $-OH$  或  $C_1-C_4$  烷氧基取代；或
- 取代或未取代的  $C_3-C_{10}$  烷基, 其任选地被卤素、 $-OH$  或  $C_1-C_4$  烷氧基取代；

$R^1$  为氢且  $R^2$  为羟基; 或者  $R^1$  与  $R^2$  形成氧代基团；

$R^7$  为氢；

$R^8$  为氢或  $CH_3$ ；

各个  $R^9$  独立地为氢或取代或未取代的  $C_1-C_4$  烷基；

各个  $R^{33}$  独立地选自卤素或取代或未取代的  $C_1-C_4$  烷基, 且  $n$  为 0、1、2、3 或 4。

4. 如权利要求 1-3 中任一项所述的方法, 其中

$n$  为 0、1 或 2。

5. 如权利要求 4 所述的方法, 其中

$X$  为  $-C(R^9)=C(R^9)-$ 。

6. 如权利要求 4 所述的方法,

其中  $X$  为  $-C\equiv C-$ 。

7. 如权利要求 4 所述的方法,

其中  $X$  为  $-C(R^9)_2O-$ 。

8. 如权利要求 4 所述的方法,

其中  $X$  为  $-C(R^9)_2-C(R^9)_2-$ 。

9. 如权利要求 4 所述的方法,

其中  $X$  为  $-C(R^9)_2S-$ 。

10. 如权利要求 4 所述的方法,

其中  $X$  为  $-C(R^9)_2S(0)_2-$ 。

11. 如权利要求 4 所述的方法,

其中 X 为  $-C(R^9)_2-NR^9-$ 。

12. 如权利要求 5、7、8、9、10 或 11 中任一项所述的方法,其中 Y 为取代或未取代的碳环基,或取代或未取代的  $C_3-C_{10}$  烷基。

13. 如权利要求 12 所述的方法,其中 Y 为取代或未取代的碳环基。

14. 如权利要求 12 所述的方法,其中 Y 为取代或未取代的  $C_3-C_{10}$  烷基。

15. 如权利要求 13 所述的方法,其中所述取代或未取代的碳环基为取代或未取代的 4 元、5 元、6 元或 7 元环。

16. 如权利要求 14 所述的方法,其中所述取代或未取代的  $C_3-C_{10}$  烷基为取代或未取代的  $C_3-C_6$  烷基。

17. 如权利要求 16 所述的方法,其中所述取代的  $C_3-C_6$  烷基被  $C_1-C_2$  烷氧基取代。

18. 如权利要求 18 所述的方法,其中所述取代的  $C_3-C_6$  烷基为  $-CH_2CH_2CH_2OCH_3$ 。

19. 如权利要求 15 所述的方法,其中所述取代或未取代的碳环基为 6 元环。

20. 如权利要求 19 所述的方法,其中所述取代或未取代的 6 元环为取代或未取代的环己基。

21. 如权利要求 20 所述的方法,其中 X 为  $-C(R^9)_2-O-$ 。

22. 如权利要求 1-3 中任一项所述的方法,其中  $R^1$  为氢且  $R^2$  为羟基。

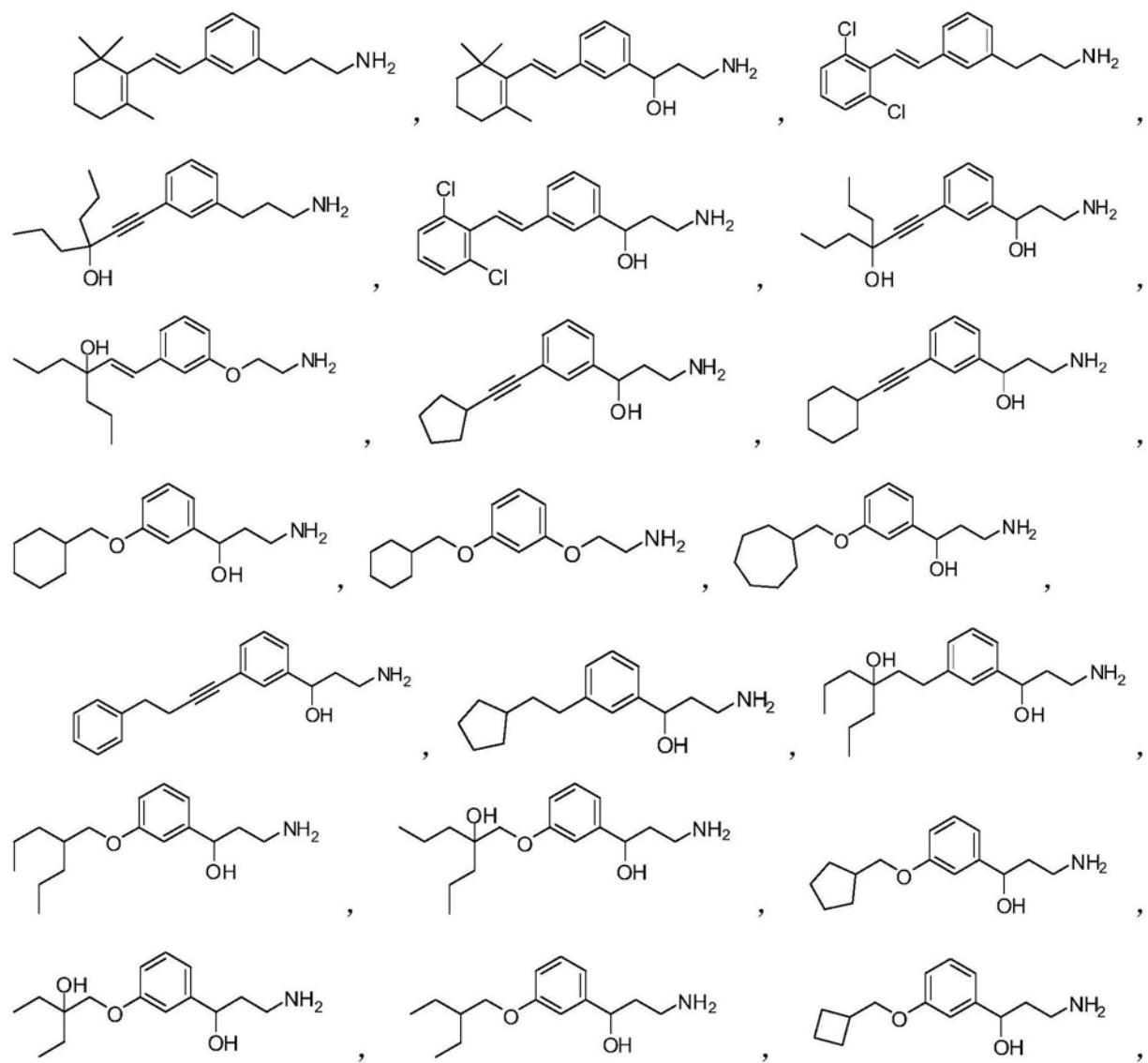
23. 如权利要求 1-3 中任一项所述的方法,其中  $R^1$  与  $R^2$  形成氧代基团。

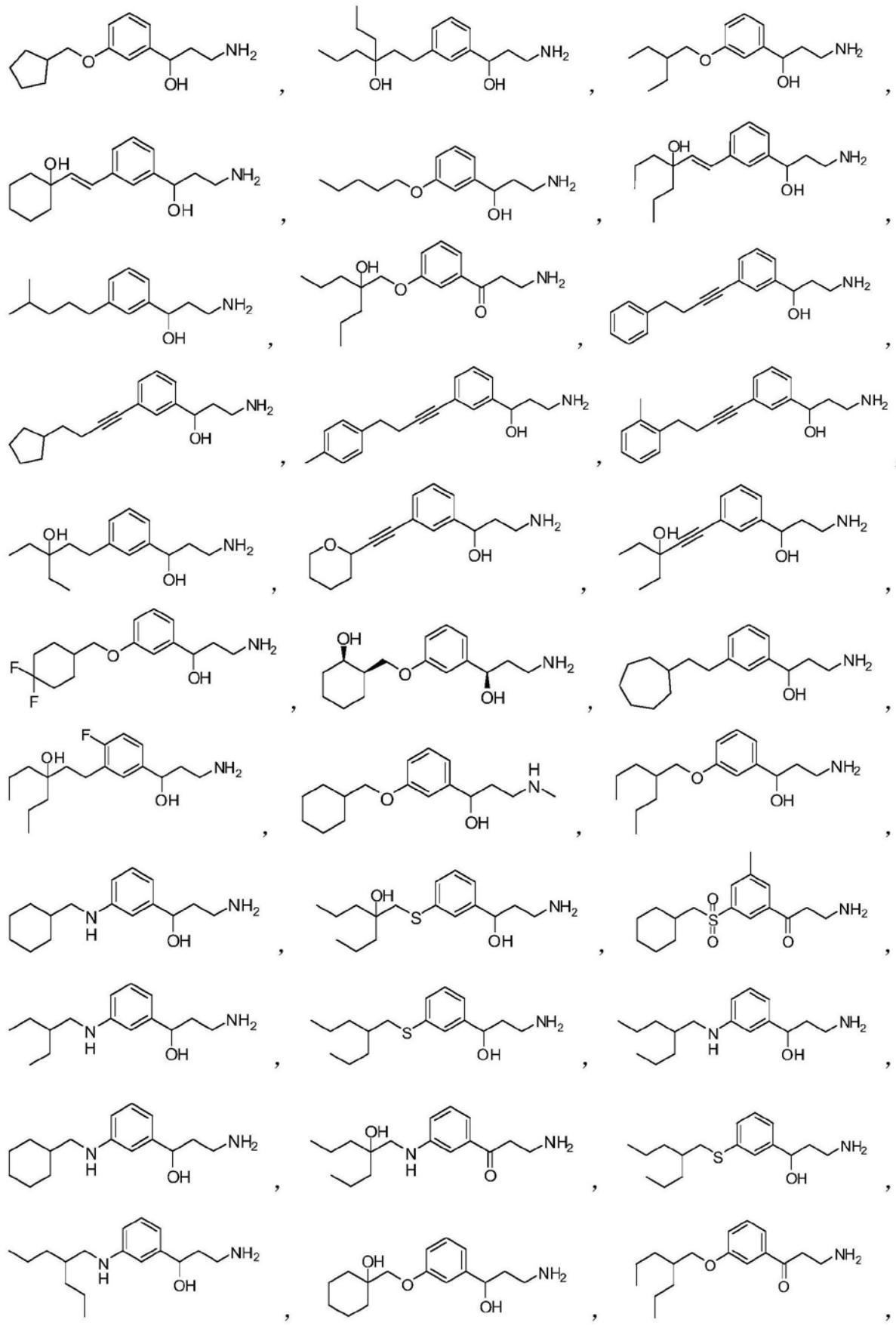
24. 如权利要求 1-3 中任一项所述的方法,其中  $R^8$  为氢。

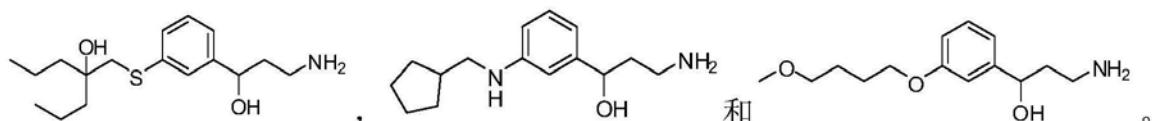
25. 如权利要求 1-3 中任一项所述的方法,其中  $R^8$  为甲基。

26. 如权利要求 22-25 中任一项所述的方法,其中 X 为  $-C(R^9)_2-O-$ 。

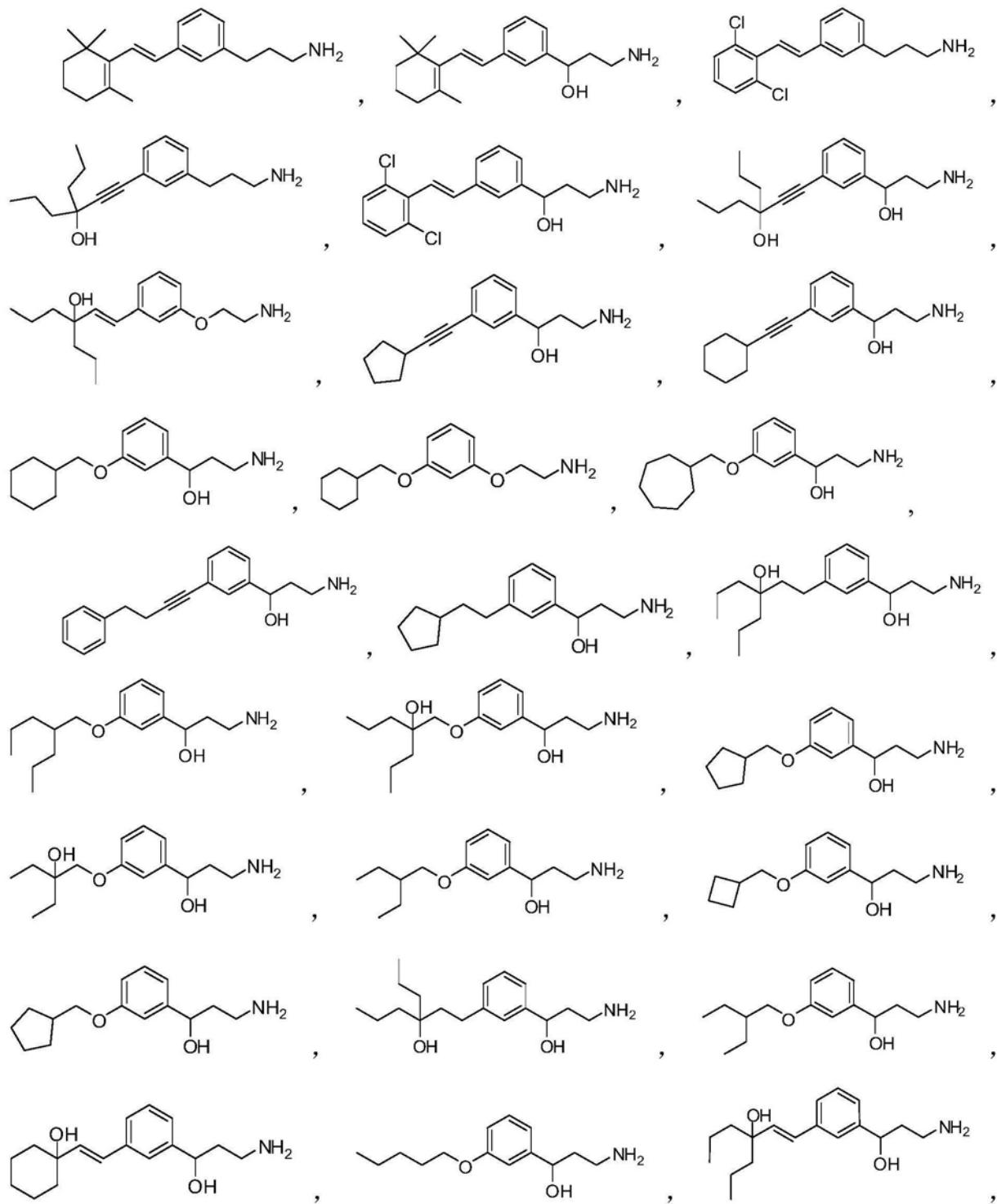
27. 一种用于治疗患者中与糖尿病相关的眼科疾病或病症的方法,其包括向患者施用治疗有效量的组合物,该组合物包含选自以下的化合物或其互变异构体、立体异构体、几何异构体、N- 氧化物或药学上可接受的盐:

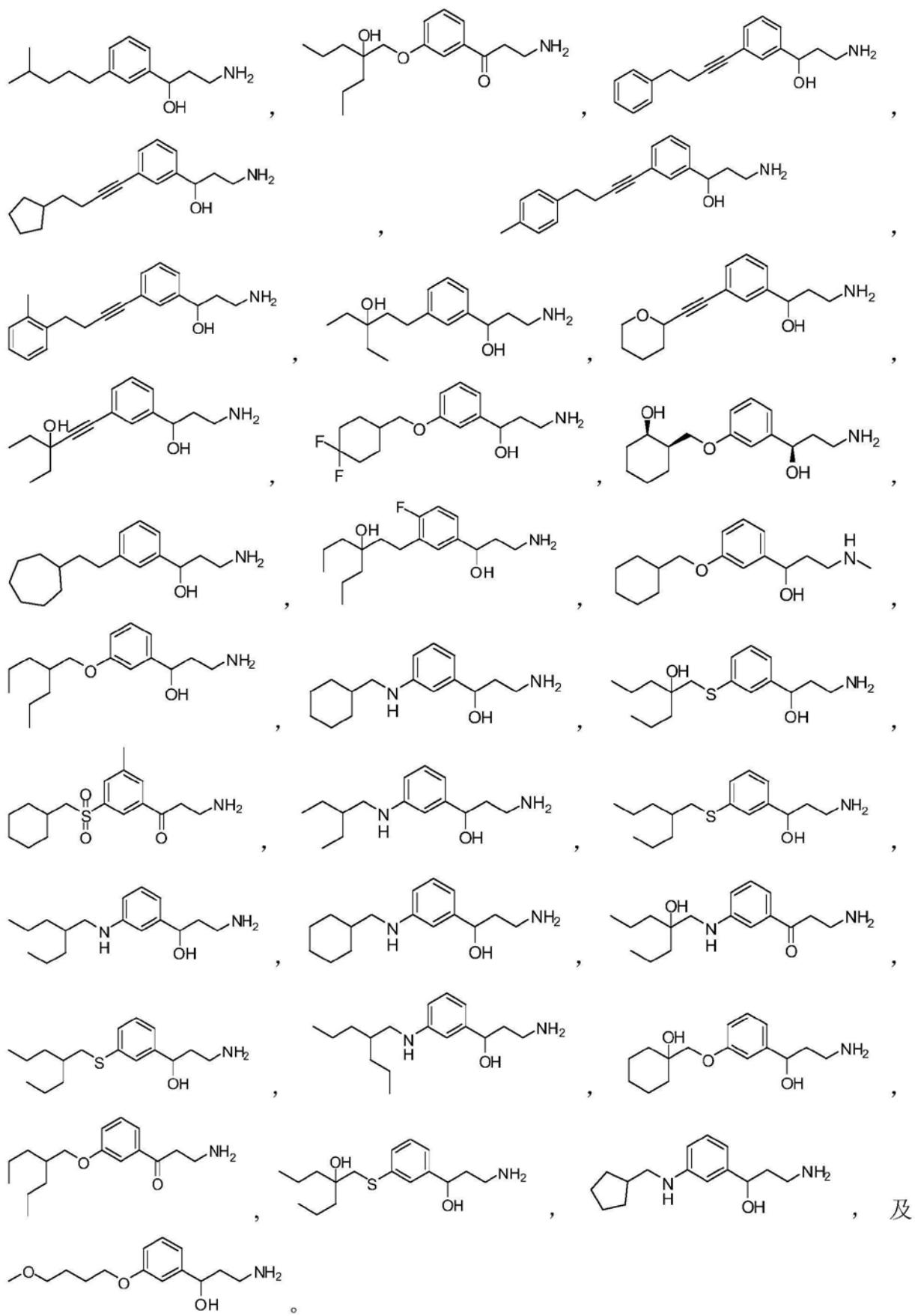






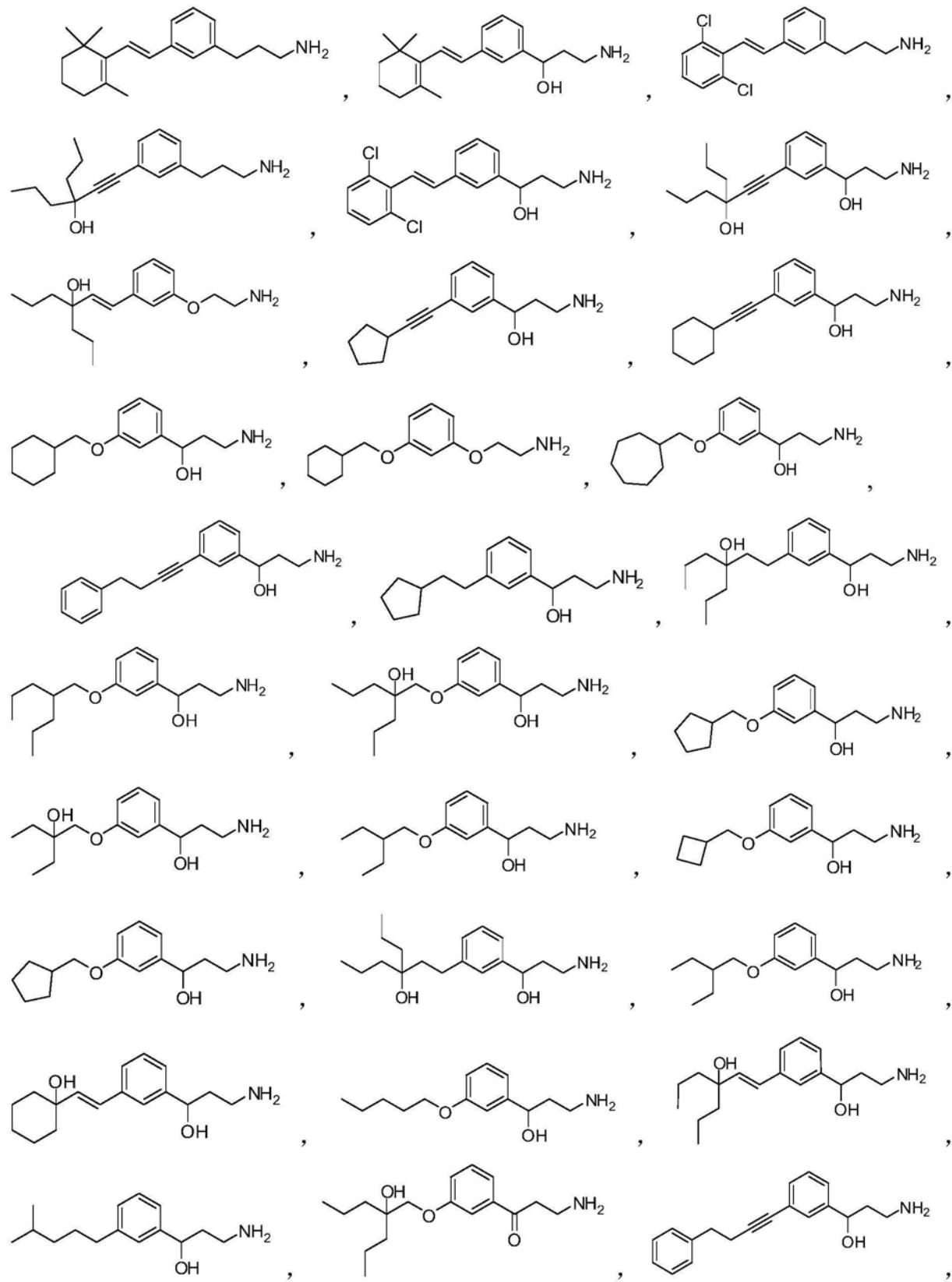
28. 一种用于治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法,其包括向患者施用治疗有效量的组合物,该组合物包含选自以下的化合物或其互变异构体、立体异构体、几何异构体、N-氧化物或药学上可接受的盐:

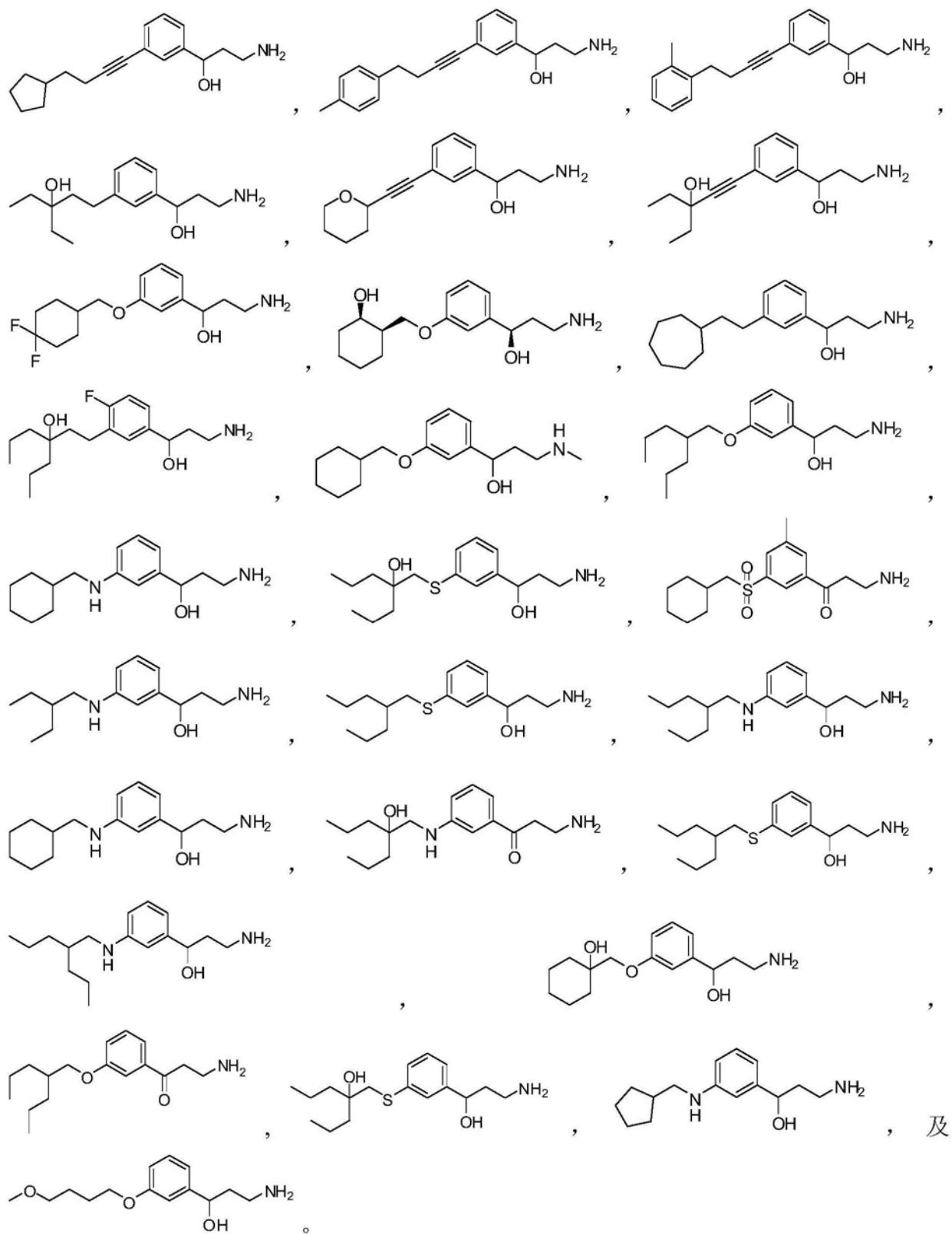




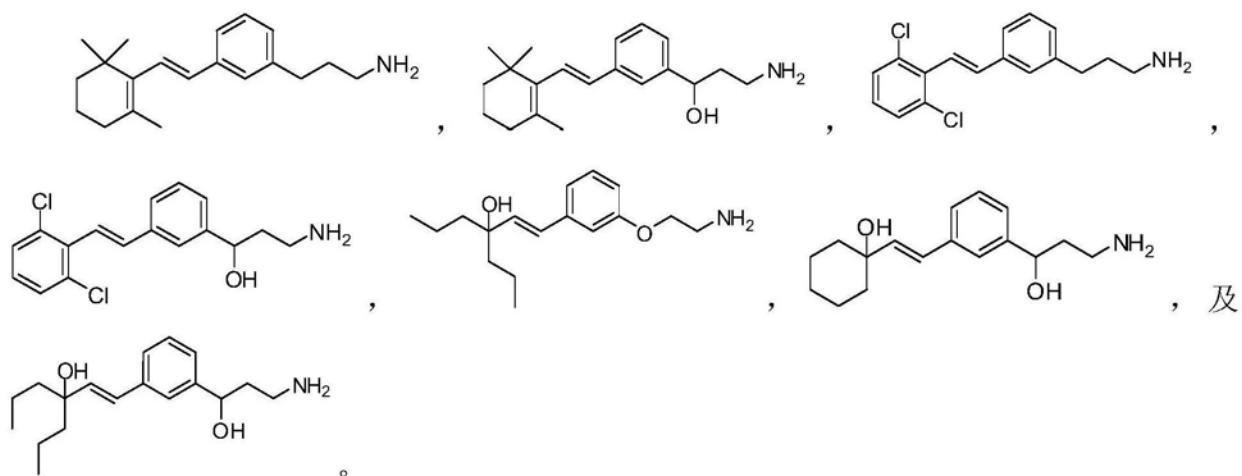
29. 一种用于治疗或预防早产儿视网膜病的方法,其包括向患者施用治疗有效量的组合物,该组合物包含选自以下的化合物或其互变异构体、立体异构体、几何异构体、N- 氧

化物或药学上可接受的盐：

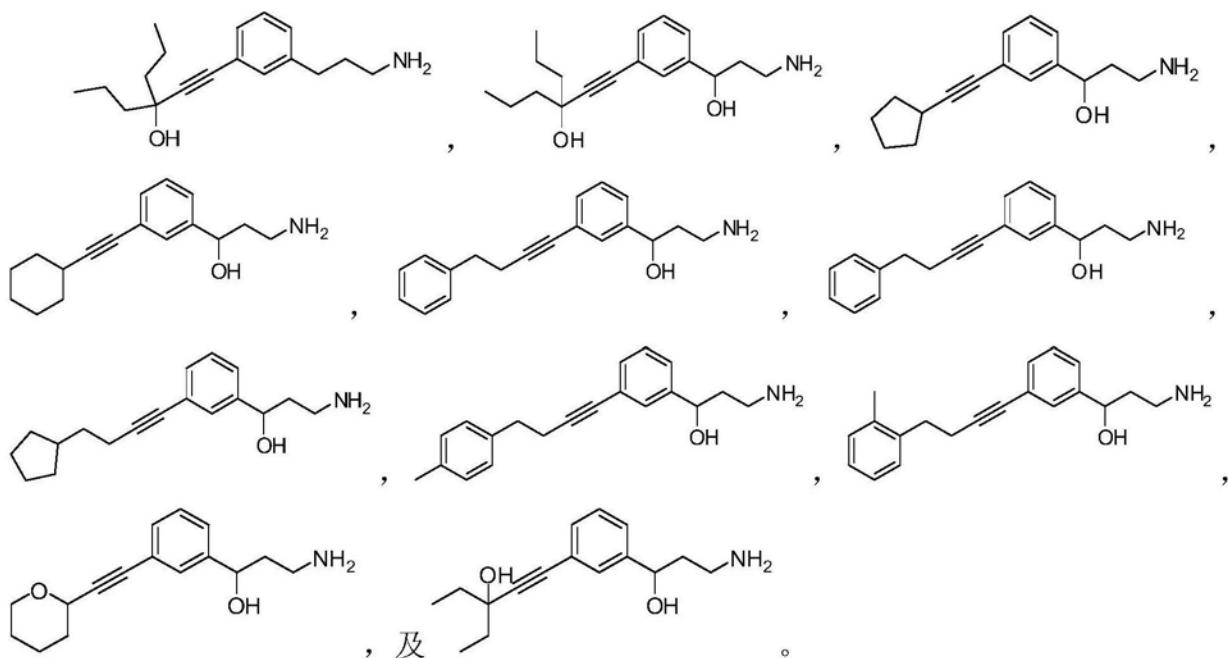




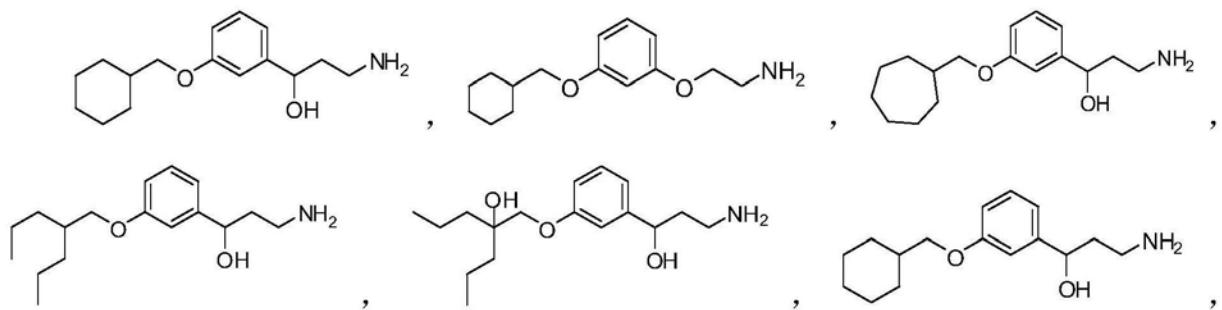
30. 如权利要求 27-29 中任一项所述的方法,其中所述组合物包含选自以下的化合物或其立体异构体、几何异构体、N- 氧化物或药学上可接受的盐:

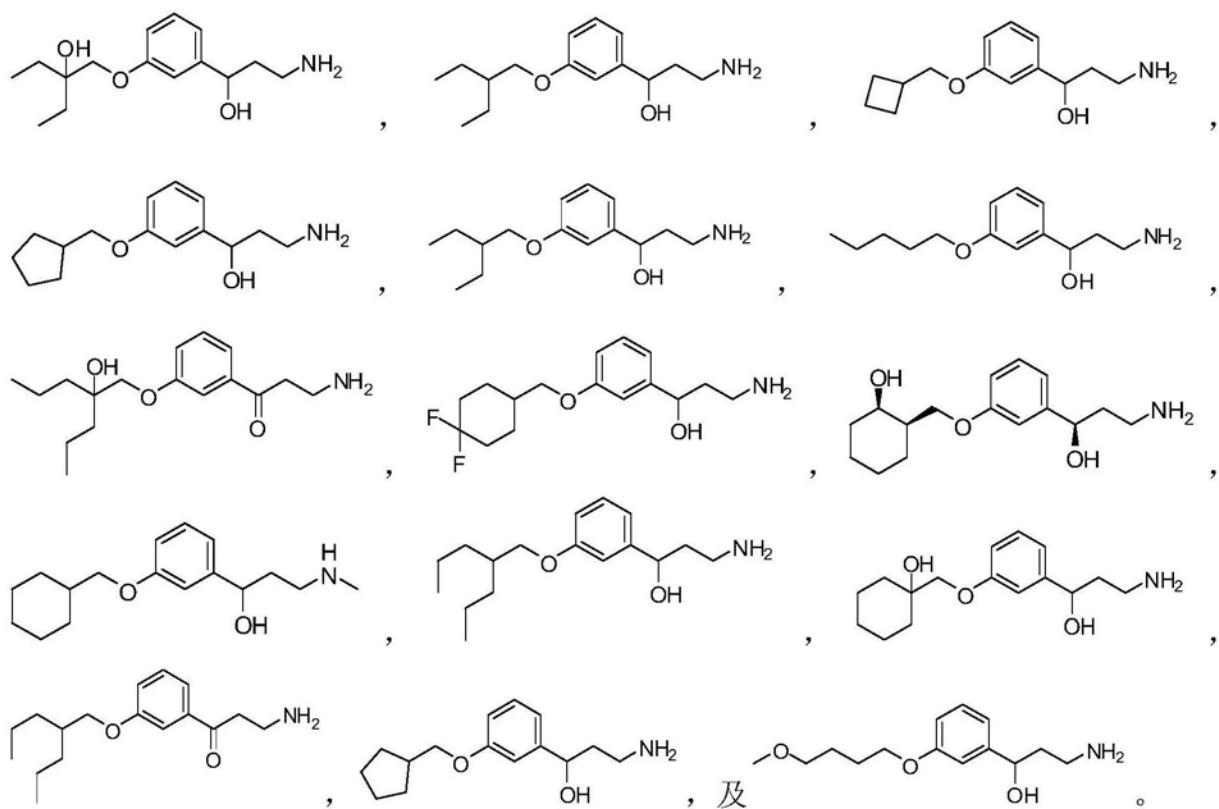


31. 如权利要求 27-29 中任一项所述的方法,其中所述组合物包含选自以下的化合物或其立体异构体、N- 氧化物或药学上可接受的盐:

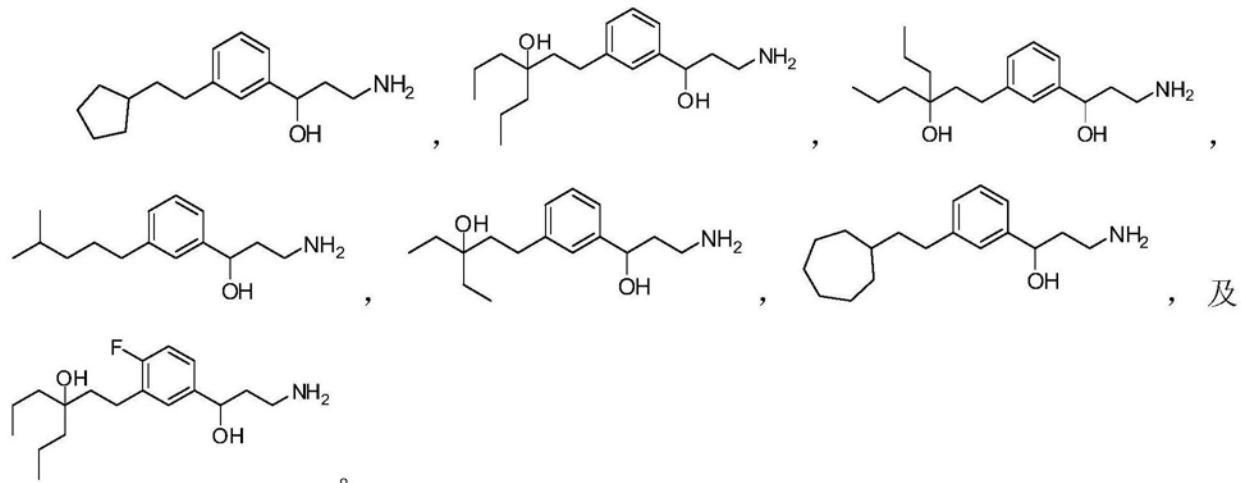


32. 如权利要求 27-29 中任一项所述的方法,其中所述组合物包含选自以下的化合物或其互变异构体、立体异构体、N- 氧化物或药学上可接受的盐:

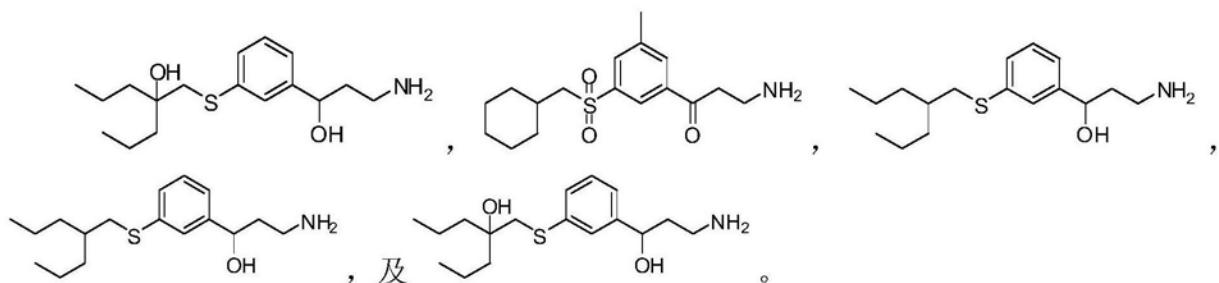




33. 如权利要求 27-29 中任一项所述的方法, 其中所述组合物包含选自以下的化合物或其立体异构体、N- 氧化物或药学上可接受的盐 :

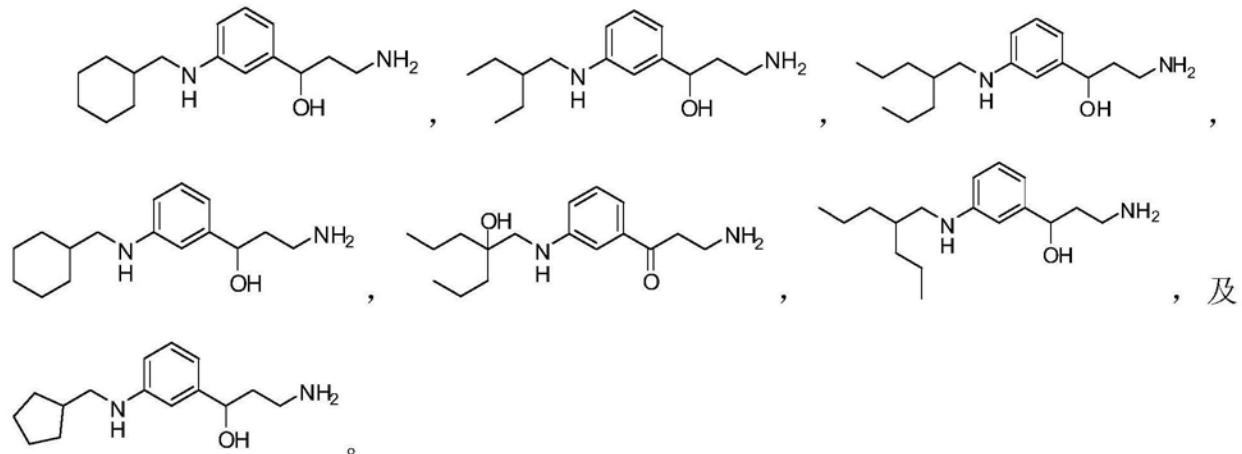


34. 如权利要求 27-29 中任一项所述的方法, 其中所述组合物包含选自以下的化合物或其互变异构体、立体异构体、N- 氧化物或药学上可接受的盐 :

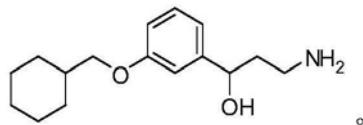


35. 如权利要求 27-29 中任一项所述的方法, 其中所述组合物包含选自以下的化合物

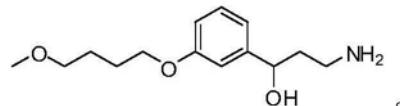
或其互变异构体、立体异构体、N- 氧化物或药学上可接受的盐：



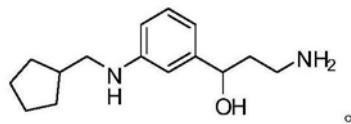
36. 如权利要求 27-29 中任一项所述的方法, 其中所述组合物包含具有下列结构的化合物或其立体异构体、N- 氧化物或药学上可接受的盐：



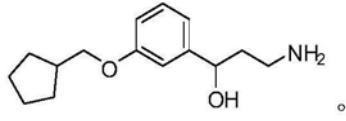
37. 如权利要求 27-29 中任一项所述的方法, 其中所述组合物包含具有下列结构的化合物或其立体异构体、N- 氧化物或药学上可接受的盐：



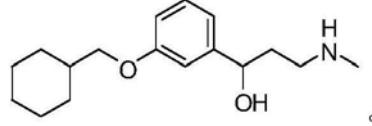
38. 如权利要求 27-29 中任一项所述的方法, 其中所述组合物包含具有下列结构的化合物或其立体异构体、N- 氧化物或药学上可接受的盐：



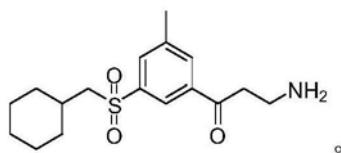
39. 如权利要求 27-29 中任一项所述的方法, 其中所述组合物包含具有下列结构的化合物或其立体异构体、N- 氧化物或药学上可接受的盐：



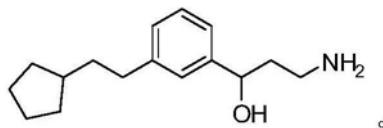
40. 如权利要求 27-29 中任一项所述的方法, 其中所述组合物包含具有下列结构的化合物或其立体异构体、N- 氧化物或药学上可接受的盐：



41. 如权利要求 27-29 中任一项所述的方法, 其中所述组合物包含具有下列结构的化合物或其立体异构体、N- 氧化物或药学上可接受的盐：



42. 如权利要求 27-29 中任一项所述的方法,其中所述组合物包含具有下列结构的化合物或其立体异构体、N- 氧化物或药学上可接受的盐 :



43. 如权利要求 1-42 中任一项所述的方法,其中所述组合物通过口服施用于患者。

44. 如权利要求 1-43 中任一项所述的方法,其中所述组合物每天施用一次。

45. 如权利要求 1-44 中任一项所述的方法,其中治疗导致患者的中心视力的改善。

46. 如权利要求 1-45 中任一项所述的方法,其进一步包括施用一种或多种额外的治疗方案。

47. 如权利要求 46 所述的方法,其中所述一种或多种治疗方案为激光疗法、冷冻疗法、荧光素血管造影术、玻璃体切割术、皮质类固醇、抗血管内皮生长因子 (VEGF) 治疗、用于持久性弥漫性糖尿病性黄斑水肿的玻璃体切割术、糖尿病性视网膜病处置中的药理性玻璃体溶解术、贝特类药物、肾素 - 血管紧张素系统 (ras) 阻滞剂、过氧化物酶体增殖物激活受体  $\gamma$  激动剂、抗蛋白激酶 C (PKC)、胰岛细胞移植、治疗性寡核苷酸、生长激素及胰岛素生长因子 (IGF)、系统性因子的控制或它们的组合。

48. 如权利要求 1 所述的方法,其中所述与糖尿病相关的眼科疾病或病症为糖尿病性视网膜病。

49. 如权利要求 1 所述的方法,其中所述与糖尿病相关的眼科疾病或病症为非增生性糖尿病性视网膜病。

50. 如权利要求 1 所述的方法,其中所述与糖尿病相关的眼科疾病或病症为增生性糖尿病性视网膜病。

51. 如权利要求 1 所述的方法,其中所述与糖尿病相关的眼科疾病或病症为糖尿病性黄斑病。

52. 如权利要求 1 所述的方法,其中所述与糖尿病相关的眼科疾病或病症为糖尿病性黄斑水肿。

53. 如权利要求 1 所述的方法,其中所述与糖尿病相关的眼科疾病或病症为新生血管性青光眼。

54. 如权利要求 1 所述的方法,其中所述与糖尿病相关的眼科疾病或病症为黄斑缺血。

55. 如权利要求 47 所述的方法,其中所述一种或多种治疗方案包括施用雷珠单抗、贝伐珠单抗或培加尼布。

56. 如权利要求 2 所述的方法,其中所述与新血管形成相关的眼科疾病或病症为湿型年龄相关性黄斑变性。

57. 如权利要求 2 所述的方法,其中所述与新血管形成相关的眼科疾病或病症为脉络膜新血管形成。

58. 如权利要求 2 所述的方法,其中所述与新血管形成相关的眼科疾病或病症选自:布鲁赫膜中的缺陷、眼血管内皮生长因子 (VEGF) 量的增加、近视、近视性变性、中心视力恶化、视物变形症、颜色干扰、血管出血或它们的组合。

59. 如权利要求 2 所述的方法,其中所述与新血管形成相关的眼科疾病或病症为视网膜新血管形成。

60. 如权利要求 3 所述的方法,其中所述患者为早产儿。

61. 如权利要求 3 所述的方法,其中所述患者进一步以补充氧治疗。

62. 一种用于在需要让眼睛暴露于亮光、激光的医疗程序、导致瞳孔长期和 / 或过度放大的程序或以其他方式使眼睛对光敏感的程序期间保护眼睛的方法,该方法包括将包含本文所述的化合物的组合物施用于有需要的患者。

63. 如权利要求 62 所述的方法,其中所述组合物在所述医疗程序之前口服施用。

64. 如权利要求 62 所述的方法,其中所述组合物在所述医疗程序之前 24 小时内和 / 或所述医疗程序之后 48 小时内口服施用。

## 用于治疗糖尿病性视网膜病及其他眼科疾病的方法

[0001] 相关申请的交叉引用

[0002] 本申请要求 2013 年 3 月 14 日提交的美国临时申请号 61/781,907、2012 年 5 月 4 日提交的美国临时申请号 61/643,178、2012 年 5 月 4 日提交的美国临时申请号 61/643,051 和 2012 年 5 月 4 日提交的美国临时申请号 61/643,058 的权益，所有这些申请均通过引用整体并入本文。

### 背景技术

[0003] 糖尿病性视网膜病是糖尿病的常见并且特异的微血管并发症，并且是工作年龄人群中的可预防性失明的首要原因。此疾病在三分之一糖尿病患者中被鉴定出且与危及生命的全身性血管并发症（包括中风、冠心病和心力衰竭）的风险增加有关。对血糖、血压及可能的血脂的最佳控制仍然是降低视网膜病发展和进展的风险的基础。

[0004] 早产儿视网膜病 (ROP) 在美国每年使约 400–800 名婴儿失明，而在全世界使数以千计的更多人视力减弱。此问题在发展中国家日益严重，因为尽管新生儿重症监护中的稳定改善已导致极低出生体重的婴儿的存活率增加，但他们恰恰是 ROP 风险最高的患者。

[0005] 视网膜包含将光转换成神经信号的光感受器，并且还具有丰富的血管供应。ROP 的临床标志为异常的视网膜脉管系统，其出现在早产年龄 (pre-term ages)。此异常脉管系统不足以在视杆光感受器（体内所有细胞中最需要氧的细胞）的成熟过程中供给氧。在最严重的 ROP 病例中，视力丧失是由渗漏的视网膜血管触动的视网膜脱离而造成的。然而，在较轻度的 ROP 病例中，视网膜血管异常通常不需要治疗就能解决，但患者却终身苦于一系列的视觉障碍，即使有最佳的光学矫正。

[0006] 年龄相关性黄斑变性 (AMD) 为美国 55 岁以上的个体中严重视力丧失的主要原因。AMD 以萎缩性或（较少见）渗出性的形式发生。在渗出性 AMD 中，血管从脉络膜毛细血管层通过布鲁赫膜中的缺陷生长，并且在一些情况下是从下方的视网膜色素上皮生长（脉络膜新血管形成或血管发生）。从这些血管渗出的浆液性或出血性渗出物的组织化造成黄斑区的纤维血管瘢痕化，伴随神经视网膜的变性、视网膜色素上皮的剥离及撕裂、玻璃体出血及中心视力永久丧失。此过程造成 AMD 患者中显著视力丧失的超过 80% 的病例。

[0007] 脉络膜新血管形成 (CNV) 在大多数病例中已被证明对治疗有抗性。激光治疗可以在不涉及视网膜中心的选定的病例中消融 CNV 并有助于保持视力，但这仅限于所述病例的约 10%。遗憾的是，即使有成功的激光光凝术，但约 50%–70% 的眼睛会复发新血管形成（超过 3 年为 50%，而第 5 年时 >60%）。(Macular Photocoagulation Study Group, Arch. Ophthalmol. 204:694–701 (1986))。此外，许多产生 CNV 的患者并非激光治疗的良好候选者，因为 CNV 对于激光治疗而言过大，或者不能确定位置，使得医生无法准确地瞄准激光。

[0008] 视网膜新血管形成 (RNV) 在许多与视网膜缺血有关的视网膜病中发生，诸如镰状细胞性视网膜病、伊尔斯病、眼缺血综合征、颈动脉海绵窦瘤、家族性渗出性玻璃体视网膜病、高粘稠度综合征、特发性闭塞性小动脉炎、放射性视网膜病、视网膜静脉闭塞、视网膜动脉闭塞、视网膜栓塞。视网膜新血管形成也可伴随炎性疾病（伯尔绍视网膜脉络膜病

(birdshot retinochoroidopathy)、视网膜血管炎、结节病、弓形体病及葡萄膜炎)、脉络膜黑色素瘤、慢性视网膜脱离、色素失调症及很少地在色素性视网膜炎中发生。

[0009] 几乎所有 RNV 的共同因素为视网膜缺血, 其释放出可扩散的血管生成因子 (如 VEGF)。新血管形成从视网膜内开始, 然后侵害视网膜内界膜。新血管在内视网膜上生长, 并且在玻璃体已脱离 (玻璃体脱离) 后在其后表面上生长。新血管形成可从视盘表面或视网膜爆发。RNV 通常进展成玻璃体视网膜新血管形成。虹膜新血管形成往往是在视网膜新血管形成之后。

## 发明内容

[0010] 本申请中提供了用于治疗患者的各种眼科疾病或病况, 如与糖尿病相关的眼科疾病或病症的方法。本文还提供了治疗患者的早产儿视网膜病的方法。此外, 本申请中提供了用于治疗患者的湿型年龄相关性黄斑变性的方法。

[0011] 在一方面, 本申请中提供了通过向有需要的患者施用视觉周期调节 (VCM) 化合物来治疗未成熟眼睛中的早产儿视网膜病的方法。本文所描述的方法涉及施用本文所描述的、为视觉周期调节剂 (VCM) 的化合物, 该化合物减少或抑制视杆光感受器中的需要能量的过程。在一个实施方式中, 该 VCM 化合物是通过口服施用的。

[0012] 在另一方面, 本文描述了通过向具有不成熟的视网膜的患者施用 VCM 化合物来改善由视杆介导的视网膜功能的方法。本文所描述的方法减少发育中的视网膜的视杆能量需求, 从而在视网膜成熟后, 相对于未以该药剂治疗的患者改善由视杆介导的视网膜功能。

[0013] 在另一方面, 本文描述了通过向有需要的患者施用包含本文所描述的化合物的组合物来调节视觉周期的方法, 其中视觉周期的调节治疗早产儿视网膜病。

[0014] 本文还描述了通过将细胞与抑制视杆细胞中的能量需求的 VCM 化合物接触来改善发育中的视杆细胞的功能和 / 或抑制其视觉周期的方法。在这类方法的一个实施方式中, 该治疗局部施用于眼睛。在这类方法的另一个实施方式中, 该治疗施用于远离眼睛的部位或全身性施用。

[0015] 在一个实施方式中, 向欲以本文所描述的化合物治疗的患者施用一种或多种额外的化合物或治疗。例如, 在一个实施方式中, 用补充供氧来治疗患者。

[0016] 在进一步的方面中是用于治疗患者的湿型年龄相关性黄斑变性的方法, 其包括向患者施用治疗有效量的视觉周期调节 (VCM) 化合物。

[0017] 待治疗的患者包括人类和非人类 (例如家养或野生动物)。

[0018] 在一个实施方式中, VCM 化合物的组合物通过口服施用。组合物可以施用一次或多次。施用可以每天多于一次、每天一次、每隔一天一次、每周或每月一次进行。

[0019] 在这样的方法中, 治疗导致患者的一种或多种症状的改善。可通过这类方法改善的症状包括但不限于出血、渗漏、瘢痕形成、光感受器受损、视力丧失或它们的组合。

[0020] 在一个实施方式中是用于减少或抑制患者中的血管形成 (例如, 新血管形成) 的方法, 其包括向患者施用治疗有效量的视觉周期调节 (VCM) 化合物。在一个实施方式中, 血管形成与脉络膜新血管形成有关。在一个实施方式中, 血管形成与视网膜新血管形成有关。血管形成的抑制或减少可以是, 例如, 至少约 1%、2%、5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95% 或 100%。

[0021] 在一个实施方式中是用于治疗患者中的脉络膜新血管形成的方法,其包括向患者施用治疗有效量的视觉周期调节 (VCM) 化合物。

[0022] 本文所描述的一个实施方式为在需要使眼睛暴露于亮光、激光的医疗程序期间、导致瞳孔延长和 / 或过度放大或以其他方式使眼睛对光敏感的程序期间保护眼睛的方法,该方法包括向有需要的患者施用包含本文所述化合物的组合物。足够剂量的本文所述的化合物将视觉周期抑制至少 50%。因此,在一些实施方式中,有效剂量将经历该医疗程序的受试者的眼睛中的视觉周期抑制至少 50%、至少 75% 或至少 90%。此外,抑制的持续时间也取决于剂量。因此,在一个实施方式中,抑制持续至少 1 小时、至少 2 小时、至少 4 小时、至少 8 小时、至少 12 小时、至少 24 小时或至少 48 小时。最后,本文的化合物为视觉周期的可逆抑制剂,因此受试者的视觉周期在 3 个半衰期内回复至正常。在一个实施方式中,与上述这类医疗程序一起使用的化合物为艾美舒司塔 (emixustat)。

[0023] 在另一方面是针对本文所述眼科疾病和病况的治疗的给药方案 (例如,每天施用的次数)。在一个实施方式中,该化合物每天施用一次 (其包括大致同时施用的该化合物的多个分剂量);在另一个实施方式中,该化合物每隔两天施用一次 (其包括大致同时施用的该化合物的多个分剂量);而在另一个实施方式中,该化合物每隔三天或更多天施用一次 (其包括大致同时施用的该化合物的多个分剂量)。

[0024] 在另一方面是针对本文所述眼科疾病和病况的治疗的给药方案 (例如,后续施用的剂量的量之间的变化)。在一个实施方式中,该化合物在第一天施用的剂量水平高于后续天数施用的剂量水平 (例如,负荷剂量)。在另一个实施方式中,该化合物在第一天施用的剂量水平是后续天数施用的剂量水平的两倍。在另一个实施方式中,该化合物在第一天施用的剂量水平是后续天数施用的剂量水平的三倍。

[0025] 在另一方面是针对本文所述眼科疾病和病况的治疗的给药方案 (例如,一天中施用化合物的时间)。在一个实施方式中,该化合物在早上施用;在另一个实施方式中,该化合物在晚上施用;在另一个实施方式中,该化合物在醒后施用;而在另一个实施方式中,该化合物在临睡前施用。在一个实施方式中,该化合物在晚上作为控释制剂施用。在另一个实施方式中,该化合物在餐前施用,或备选地在餐中或备选地在餐后施用。在一些实施方式中,该餐是早餐;在其他实施方式中,该餐是午餐;在另外其他的实施方式中,该餐是晚餐 / 晚饭。

[0026] 在一方面, (R)-3-氨基-1-(3-(环己基甲氧基)苯基)丙-1-醇的每日剂量为约 4mg 至约 100mg。在另一方面, (R)-3-氨基-1-(3-(环己基甲氧基)苯基)丙-1-醇的每日剂量为约 2mg、约 5mg、约 7mg、约 10mg、约 15mg、约 20mg、约 40mg、约 60mg、约 75mg 或约 100mg。

[0027] 在一些实施方式中,视觉周期的抑制通过 ERG 来测定。关于足以将视觉周期抑制至少 50% 的本文所述化合物的剂量以及用于在受试者中测定视觉周期抑制的方法 (包括 ERG) 的信息描述于美国专利申请公开 US 2011/0003895 中,其公开内容通过引用并入本文。

[0028] 在一个实施方式中,该组合物在医疗程序前通过口服施用。在一个实施方式中,该组合物在医疗程序后 24 小时和 / 或 48 小时施用。

[0029] 在一个实施方式中,该 VCM 化合物的组合物通过口服施用。组合物可施用一次或

多次。施用可每天多于一次、每天一次、每隔一天一次、每周一次或每月一次进行。

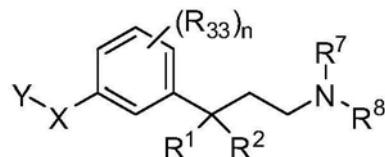
[0030] 在这类方法中,治疗导致患者的一种或多种症状改善。可由这类方法改善的症状包括但不限于布鲁赫膜中的缺陷、眼血管内皮生长因子 (VEGF) 量的增加、近视、近视性变性、中心视力恶化、视物变形症、颜色干扰、血管出血或它们的组合。

[0031] 在一个实施方式中是用于治疗患者的视网膜新血管形成的方法,其包括向患者施用治疗有效量的视觉周期调节 (VCM) 化合物。

[0032] 在一个实施方式中,视网膜新血管形成与一种或多种视网膜病有关,该视网膜病包括但不限于镰状细胞性视网膜病、伊尔斯病、眼缺血综合征、颈动脉海绵窦瘤、家族性渗出性玻璃体视网膜病、高粘稠度综合征、特发性闭塞性小动脉炎、放射性视网膜病、视网膜静脉闭塞、视网膜动脉闭塞、视网膜栓塞、伯尔绍视网膜脉络膜病、视网膜血管炎、结节病、弓形体病、葡萄膜炎、脉络膜黑色素瘤、慢性视网膜脱离、色素失调症及色素性视网膜炎。

[0033] 在另一方面是用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法,其包括向患者施用治疗有效量的组合物,该组合物包含式 (A) 的化合物或其互变异构体、立体异构体、几何异构体、N- 氧化物或药学上可接受的盐:

[0034]



[0035] 式 (A)

[0036] 其中,

[0037] X 选自  $-C(R^9) = C(R^9) -$ 、 $-C \equiv C -$ 、 $-C(R^9)_2 -$ 、 $-O -$ 、 $-C(R^9)_2 - C(R^9)_2 -$ 、 $-C(R^9)_2 - S -$ 、 $-C(R^9)_2 - S(O)_2 -$  或  $-C(R^9)_2 - NR^9 -$ ;

[0038] Y 选自:

[0039] a) 取代或未取代的碳环基,其任选地被  $C_1 - C_4$  烷基、卤素、 $-OH$  或  $C_1 - C_4$  烷氧基取代;

[0040] b) 取代或未取代的碳环基烷基,其任选地被  $C_1 - C_4$  烷基、卤素、 $-OH$  或  $C_1 - C_4$  烷氧基取代;

[0041] c) 取代或未取代的芳烷基,其任选地被  $C_1 - C_4$  烷基、卤素、 $-OH$  或  $C_1 - C_4$  烷氧基取代;或

[0042] d) 取代或未取代的  $C_3 - C_{10}$  烷基,其任选地被卤素、 $-OH$  或  $C_1 - C_4$  烷氧基取代;

[0043]  $R^1$  为氢且  $R^2$  为羟基;或者  $R^1$  与  $R^2$  形成氧代基团;

[0044]  $R^7$  为氢;

[0045]  $R^8$  为氢或  $CH_3$ ;

[0046] 各个  $R^9$  独立地为氢或取代或未取代的  $C_1 - C_4$  烷基;

[0047] 各个  $R^{33}$  独立地选自卤素或取代或未取代的  $C_1 - C_4$  烷基,且  $n$  为 0、1、2、3 或 4。

[0048] 另一个实施方式提供用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法,其中  $n$  为 0、1 或 2。

[0049] 另一个实施方式提供其中 X 为  $-C(R^9) = C(R^9)-$  的方法。另一个实施方式提供其中 X 为  $-C \equiv C-$  的方法。另一个实施方式提供其中 X 为  $-C(R^9)_2-0-$  的方法。另一个实施方式提供其中 X 为  $-C(R^9)_2-C(R^9)_2-$  的方法。另一个实施方式提供其中 X 为  $-C(R^9)_2-S-$  的方法。另一个实施方式提供其中 X 为  $-C(R^9)_2-S(O)_2-$  的方法。另一个实施方式提供其中 X 为  $-C(R^9)_2-NR^9-$  的方法。

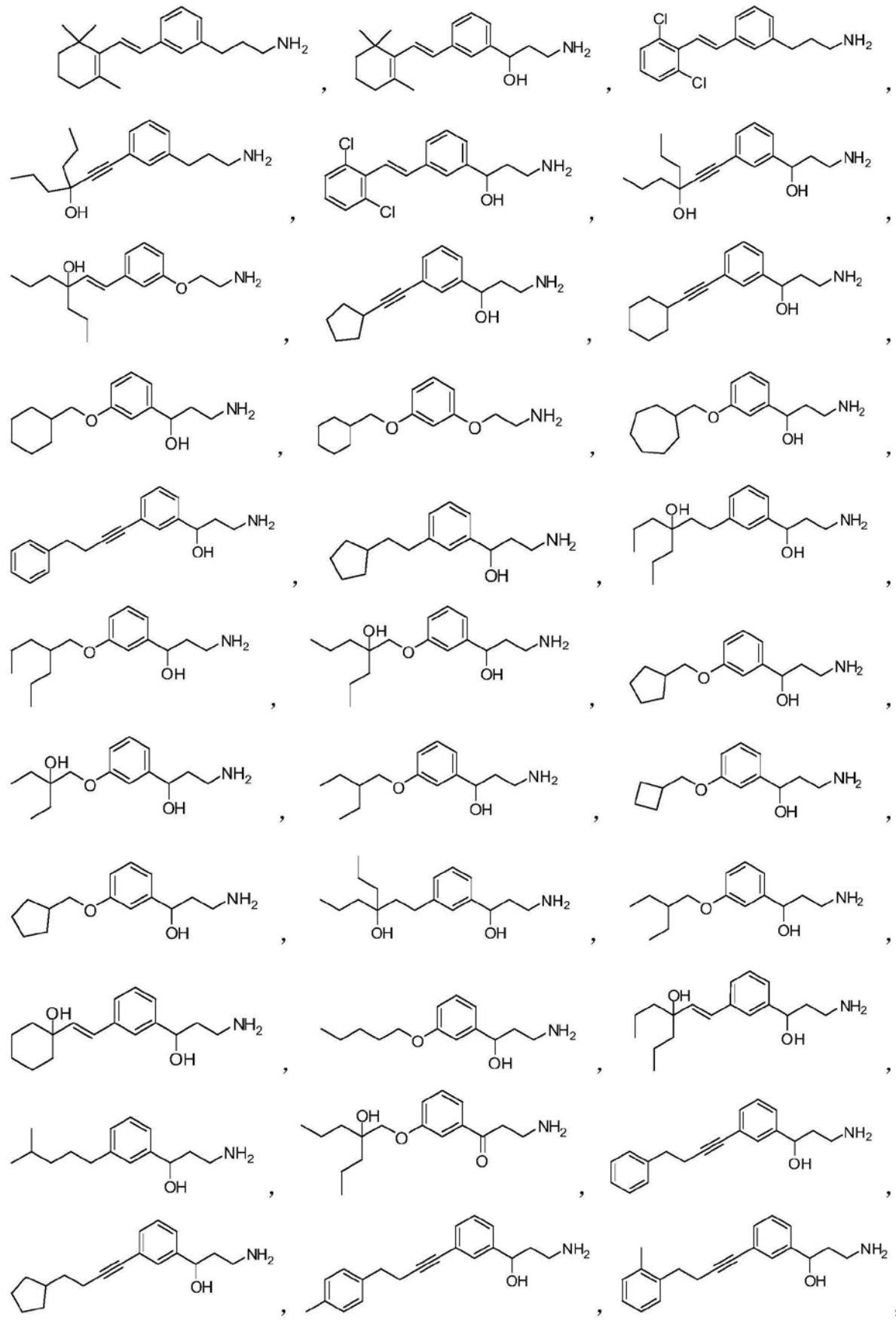
[0050] 另一个实施方式提供其中 Y 为取代或未取代的碳环基或者取代或未取代的  $C_3-C_{10}$  烷基的方法。另一个实施方式提供其中 Y 为取代或未取代的碳环基的方法。另一个实施方式提供其中该取代或未取代的碳环基为取代或未取代的 4 元、5 元、6 元或 7 元环的方法。另一个实施方式提供其中该取代或未取代的 6 元环为取代或未取代的环己基的方法。另一个实施方式提供其中该取代或未取代的 6 元环为取代或未取代的环己基且 X 为  $-C(R^9)_2-0-$  的方法。

[0051] 另一个实施方式提供其中 Y 为取代或未取代的  $C_3-C_{10}$  烷基的方法。另一个实施方式提供其中该取代或未取代的  $C_3-C_{10}$  烷基为取代或未取代的  $C_3-C_6$  烷基的方法。另一个实施方式提供其中该取代的  $C_3-C_6$  烷基是被  $C_1-C_2$  烷氧基取代的方法。另一个实施方式提供其中该取代的  $C_3-C_6$  烷基为  $-CH_2CH_2CH_2OCH_3$  的方法。

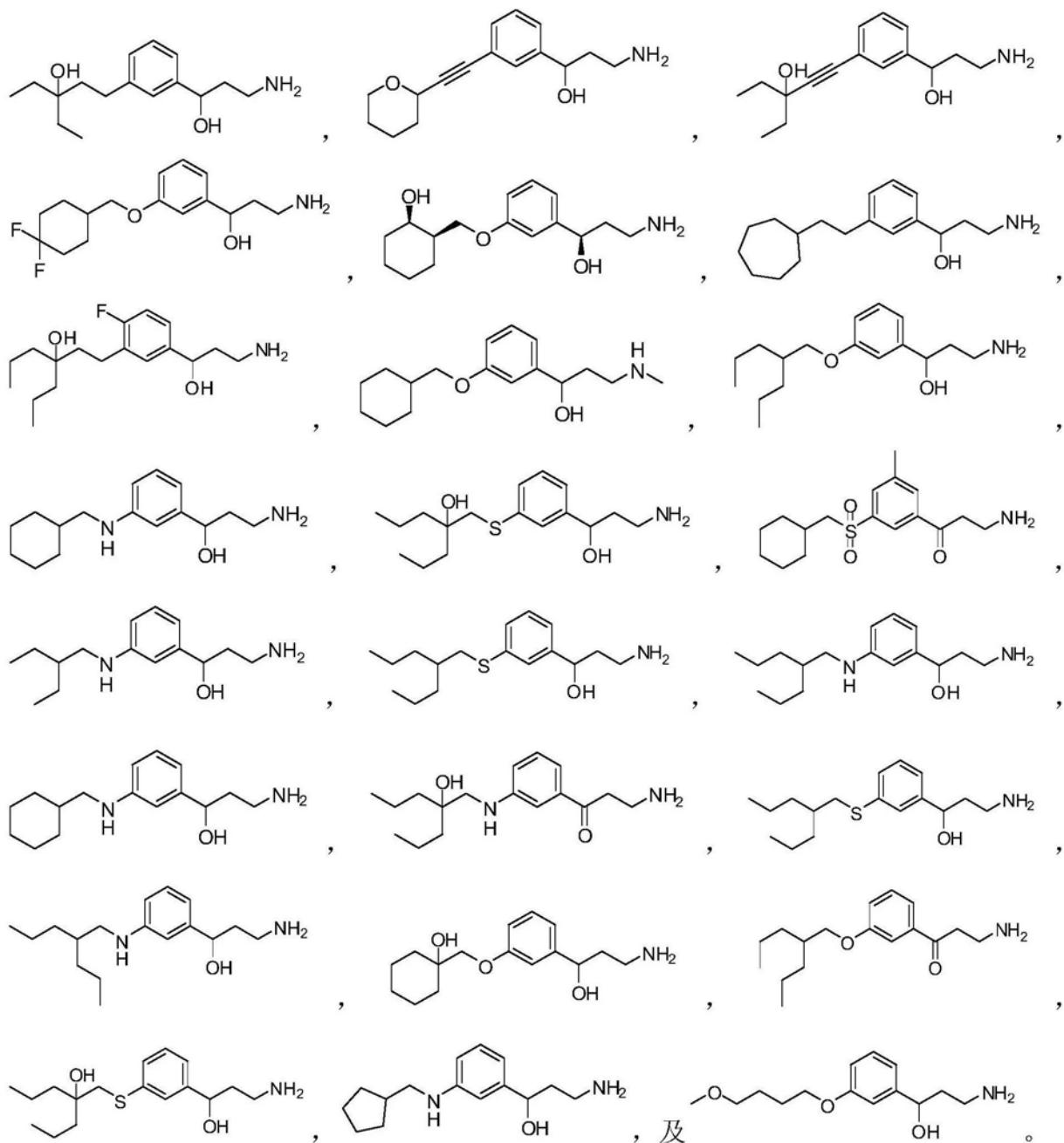
[0052] 另一个实施方式提供其中  $R^1$  为氢且  $R^2$  为羟基的方法。另一个实施方式提供其中  $R^1$  与  $R^2$  形成氧代基团的方法。另一个实施方式提供其中  $R^8$  为氢的方法。另一个实施方式提供其中  $R^8$  为甲基的方法。另一个实施方式提供其中  $R^1$  为氢、 $R^2$  为羟基且 X 为  $-C(R^9)_2-0-$  的方法。

[0053] 一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法，其包括向患者施用治疗有效量的组合物，该组合物包含选自以下的化合物或其互变异构体、立体异构体、几何异构体、N- 氧化物或药学上可接受的盐：

[0054]

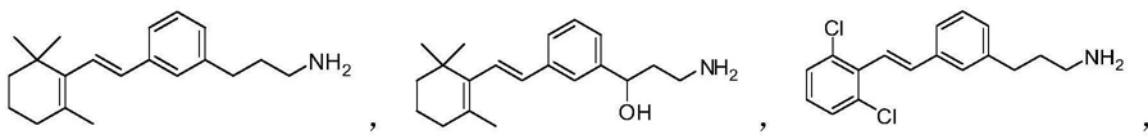


[0055]

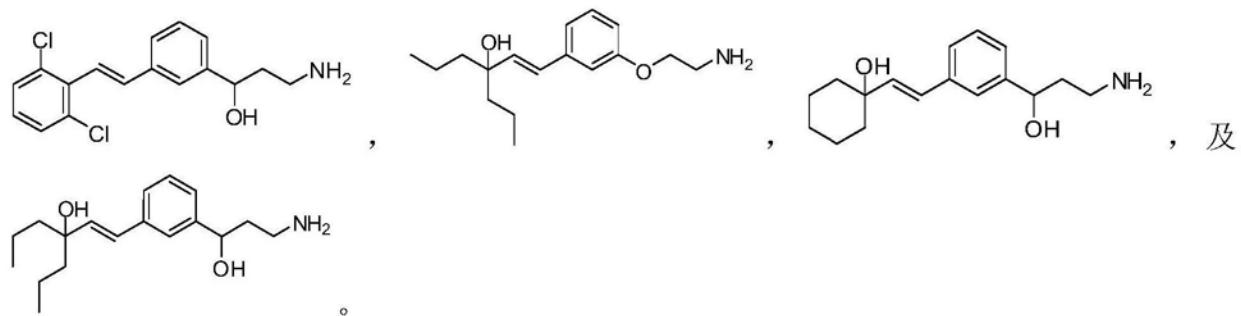


[0056] 另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法，其中所述组合物包含选自以下的化合物或其立体异构体、几何异构体、N- 氧化物或药学上可接受的盐：

[0057]

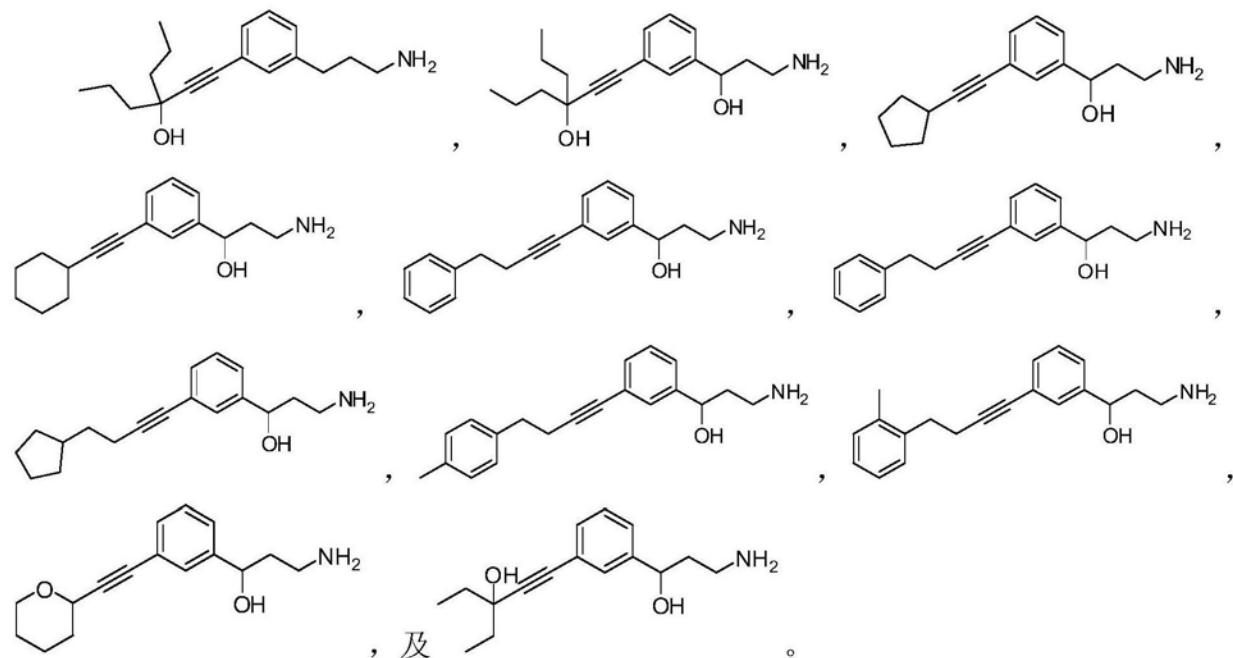


[0058]



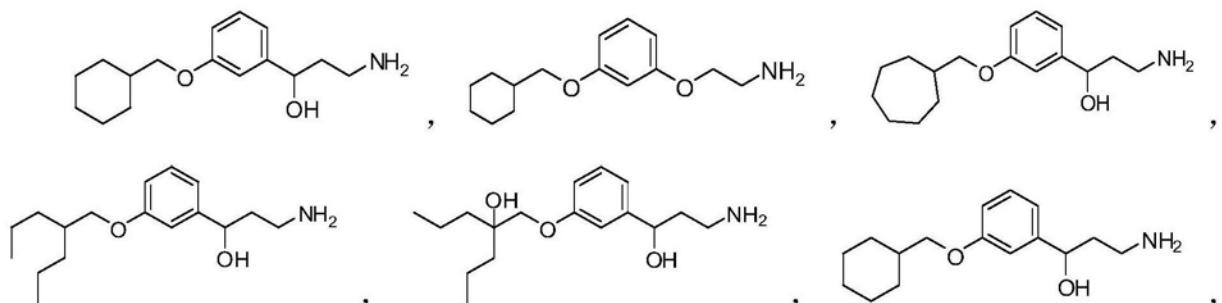
[0059] 另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法,其中所述组合物包含选自以下的化合物或其立体异构体、N- 氧化物或药学上可接受的盐:

[0060]

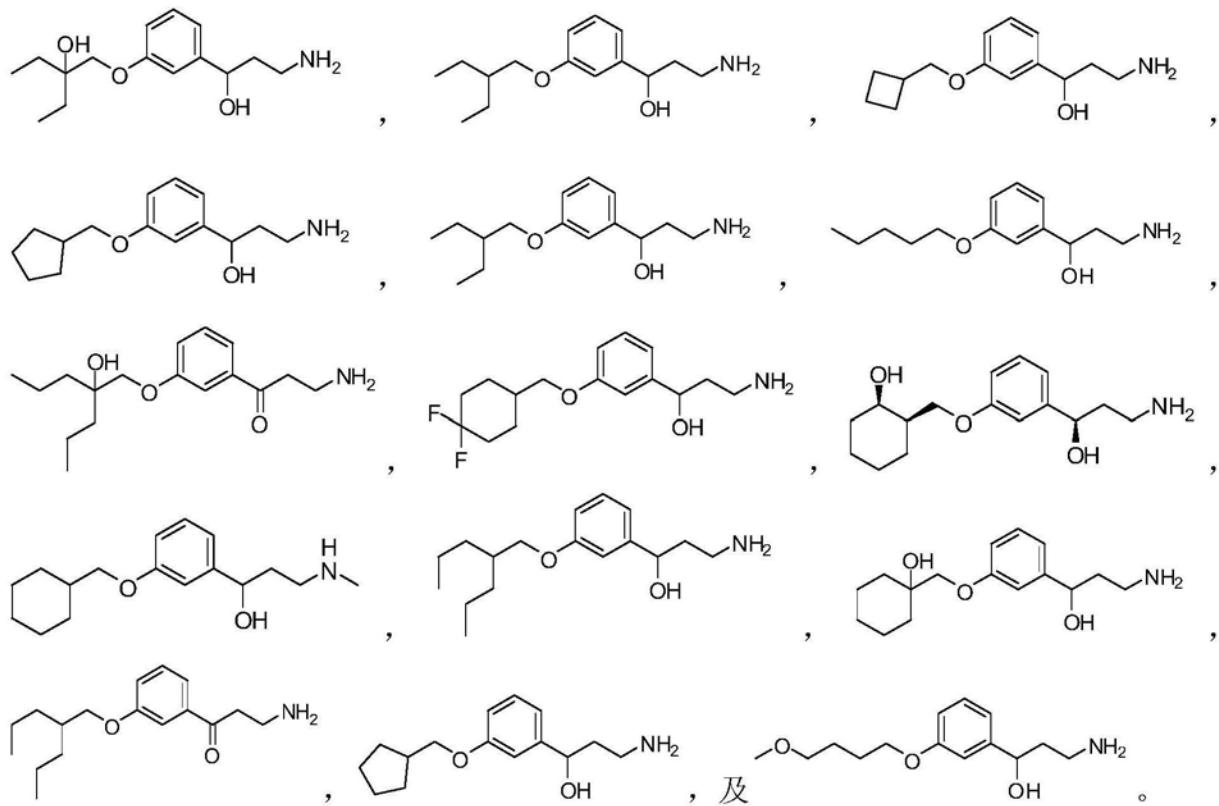


[0061] 另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法,其中所述组合物包含选自以下的化合物或其互变异构体、立体异构体、N- 氧化物或药学上可接受的盐:

[0062]

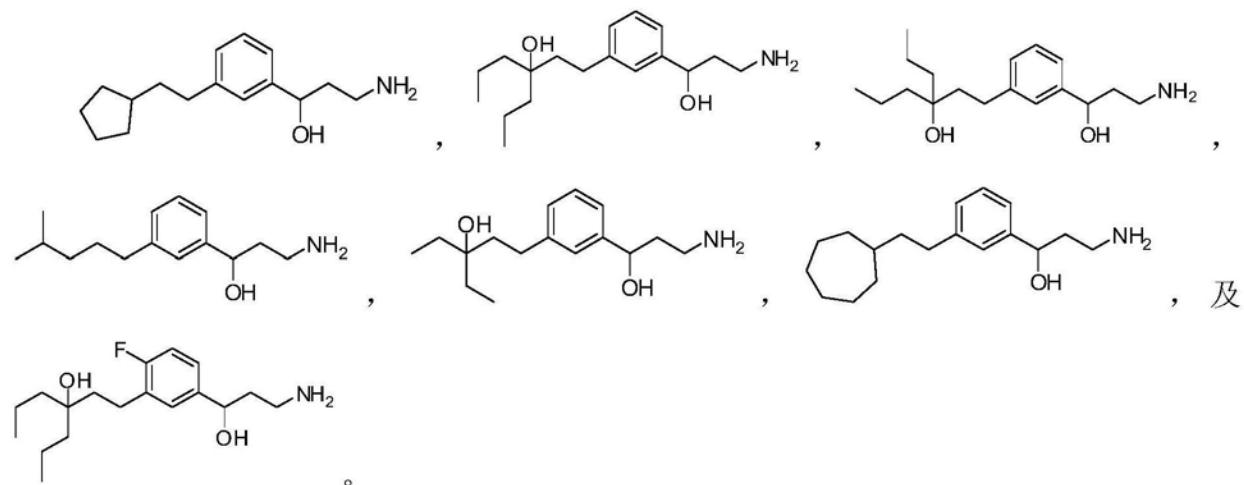


[0063]



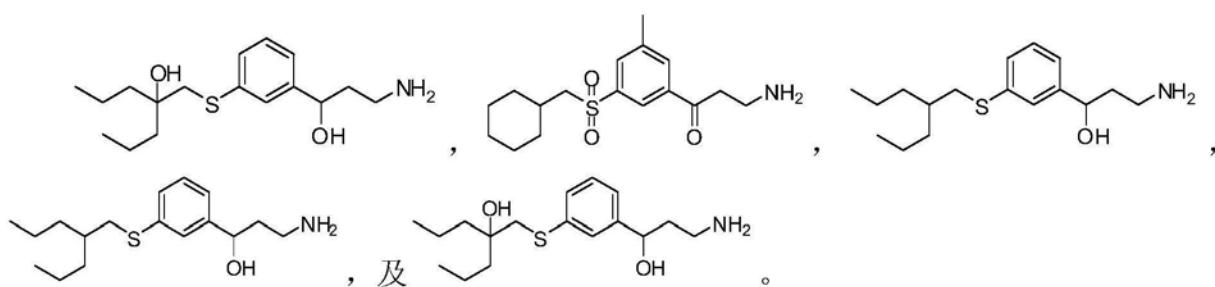
[0064] 另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法,其中所述组合物包含选自以下的化合物或其立体异构体、N-氧化物或药学上可接受的盐:

[0065]



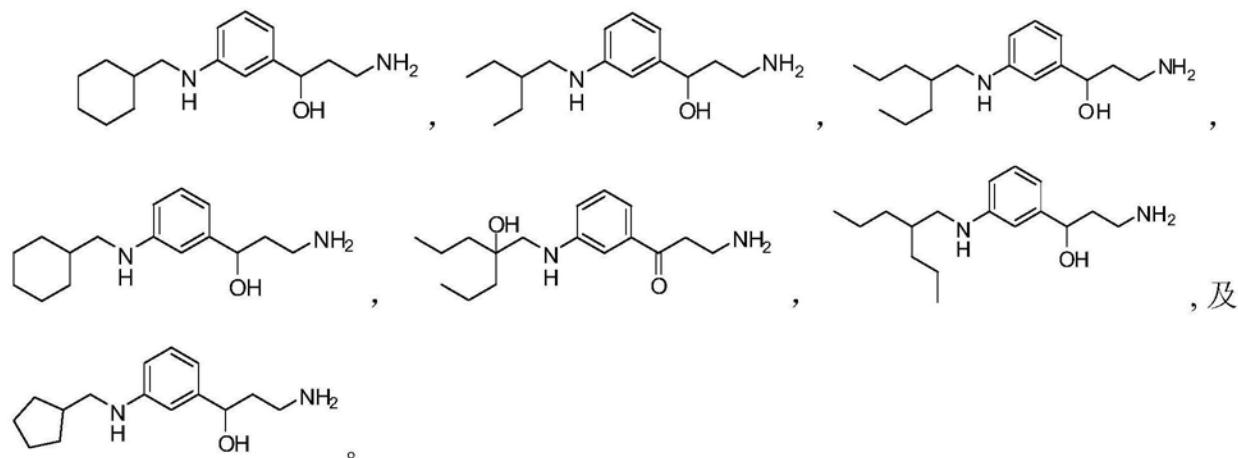
[0066] 另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法,其中所述组合物包含选自以下的化合物或其互变异构体、立体异构体、N-氧化物或药学上可接受的盐:

[0067]



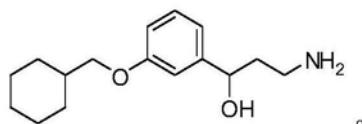
[0068] 另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病；或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法，其中所述组合物包含选自以下的化合物或其互变异构体、立体异构体、N-氧化物或药学上可接受的盐：

[0069]



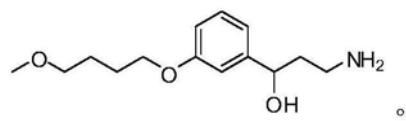
[0070] 另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法，其中所述组合物包含具有下列结构的化合物或其立体异构体、N-氧化物或药学上可接受的盐：

[0071]



[0072] 另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法，其中所述组合物包含具有下列结构的化合物、其立体异构体、N-氧化物或药学上可接受的盐：

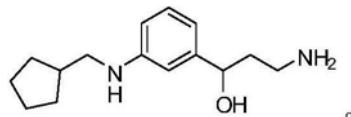
[0073]



[0074] 另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方

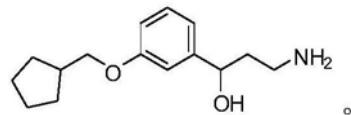
法,其中所述组合物包含其有下列结构的化合物或其立体异构体、N- 氧化物或药学上可接受的盐 :

[0075]



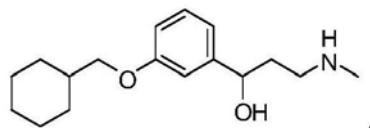
[0076] 另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法,其中所述组合物包含具有下列结构的化合物或其立体异构体、N- 氧化物或药学上可接受的盐 :

[0077]



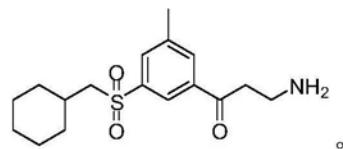
[0078] 另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法,其中所述组合物包含具有下列结构的化合物或其立体异构体、N- 氧化物或药学上可接受的盐 :

[0079]



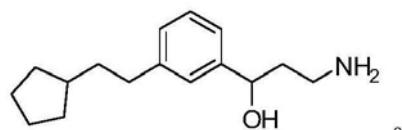
[0080] 另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法,其中所述组合物包含具有下列结构的化合物或其立体异构体、N- 氧化物或药学上可接受的盐 :

[0081]



[0082] 另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法,其中所述组合物包含具有下列结构的化合物或其立体异构体、N- 氧化物或药学上可接受的盐 :

[0083]



[0084] 另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或

预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法,其中所述组合物通过口服施用于患者。另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法,其中所述组合物每天施用一次。另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法,其中治疗导致患者的中心视力改善。

[0085] 另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管形成相关的眼科疾病或病症的方法,其进一步包括施用一种或多种额外的治疗方案。另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症、治疗或预防患者的早产儿视网膜病或治疗与患者眼睛中新血管生成相关的眼科疾病或病症的方法,其中所述一种或多种治疗方案为激光疗法、冷冻疗法、荧光素血管造影术、玻璃体切割术、皮质类固醇、抗血管内皮生长因子(VEGF)治疗、用于持久性弥漫性糖尿病性黄斑水肿的玻璃体切割术、糖尿病性视网膜病处置中的药理性玻璃体溶解术、贝特类药物(fibrates)、肾素-血管紧张素系统(ras)阻滞剂、过氧化物酶体增殖物激活受体 $\gamma$ 激动剂、抗蛋白激酶C(PKC)、胰岛细胞移植、治疗性寡核苷酸、生长激素及胰岛素生长因子(IGF)、系统性因子的控制或它们的组合。

[0086] 另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症的方法,其中该与糖尿病相关的眼科疾病或病症为糖尿病性视网膜病。另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症的方法,其中该与糖尿病相关的眼科疾病或病症为非增生性糖尿病性视网膜病。另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症的方法,其中该与糖尿病相关的眼科疾病或病症为增生性糖尿病性视网膜病。另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症的方法,其中该与糖尿病相关的眼科疾病或病症为糖尿病性黄斑病。另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症的方法,其中该与糖尿病相关的眼科疾病或病症为糖尿病性黄斑水肿。另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症的方法,其中该与糖尿病相关的眼科疾病或病症为新生血管性青光眼。另一个实施方式提供了用于治疗患者中与糖尿病相关的眼科疾病或病症的方法,其中该与糖尿病相关的眼科疾病或病症为黄斑缺血(macular ischemia)。

[0087] 援引并入

[0088] 本说明书中提及的所有出版物、专利及专利申请均通过引用并入本文,其并入的程度与具体地且单独地指出各个单独的出版物、专利或专利申请通过引用并入的程度是相同的。

## 附图说明

[0089] 在所附权利要求中特别说明了本发明的新颖的特征。通过参考对利用了本发明原理的示例性实施方式加以阐述的以下详细说明及附图,将会获得对本发明的特征和优点的更好理解,在附图中:

[0090] 图1是描绘如实施例3中所描述的第1-3组的时间轴(timeline)的图。

[0091] 图 2 是描绘如实施例 3 中所描述的第 4 组的时间轴的图。

[0092] 图 3 是描绘如实施例 3 中所描述的第 5-6 组的时间轴的图。

[0093] 图 4A 描绘视觉周期, 其显示出视网膜中视觉活性类维生素 A (retinoid) 的生化转化。

[0094] 图 4B 说明 ACU-4429 的可能的作用方式。

[0095] 图 5 是描绘 ACU-44291a 期的平均口服药代动力学 (PK) 谱 (profile) 的数据的图。

[0096] 图 6 是描绘 ACU-44291a 期的视杆 ERG 抑制的图。

[0097] 图 7 是描绘 1b 期的药代动力学 (PK) 数据的图。

[0098] 图 8 提供测试 ACU-4935 是否降低由低氧条件引起的 VEGF 上调的实验的时间轴。

[0099] 图 9 是说明用 ACU-4935 治疗后由低氧条件造成的 VEGF 蛋白质表达的图。

[0100] 图 10 是说明用 ACU-4935 治疗后由低氧条件造成的 VEGF mRNA 水平的图。

[0101] 图 11 :血或血浆 (图 11A) 或眼组织 (图 11B) 中的平均浓度 - 时间谱。

[0102] 图 12 :如实施例 10 所述在第 7 天给药后 4 小时的代谢物放射性谱。图 12A 提供 G4M 第 8 天 4 小时血浆的结果。图 12B 提供 G3 M4 小时视网膜色素上皮的结果。

[0103] 图 13 是说明如实施例 10 所述回收的放射性剂量的平均累积百分比的图。

[0104] 图 14 :视觉周期调节剂 (VCM) (例如 ACU-4420 及 ACU-4935) 抑制视觉周期异构酶, 从而模拟构成性光转导的状态并减少暗电流。

[0105] 图 15 :说明用 ACU-4420 和 ACU-4935 治疗 129SvE 小鼠幼崽 (PO) 的方案。

[0106] 图 16A-16B 显示 VCM 抑制新血管形成。图 16A 描绘视网膜的平面封固制品 (flatmount preparations) 的异凝集素染色。新生血管区以红色描出轮廓。图 16B 是比较各治疗组中的新血管形成%的柱状图。图 16C-16F 显示 ACU-4429 以剂量依赖的方式抑制新血管形成及 11- 顺式 -RAL。图 16C 和 16D 显示 ACU-4429 以剂量依赖的方式降低眼睛中的 11- 顺式 -RAL 浓度, 因此降低视觉周期异构酶活性 (ED500. 88mg/kg)。ACU-4429 与载体之间的差异在统计上有意义 (P<0.01)。图 16E 和 16F 显示 ACU-4429 以剂量依赖的方式减少左眼的新血管形成 (在异凝集素染色的平面封固制品中测量); 通过 21% O<sub>2</sub> 下的载体 (水)、75% O<sub>2</sub> 下的载体 (水) 及 ACU-4429 治疗的单因素 ANOVA 比较, 此下降在 3.0 和 10.0mg/kg 时具有显著性。

[0107] 图 17 为神经视网膜及其血管供应的图 (不按比例)。示出了神经视网膜的各层 (神经节细胞层、内丛状层、内核层、外丛状层、外核层)。通过脉络膜血管的血流是迅速的。通过检眼镜检查可见的视网膜脉管系统位于视网膜玻璃体表面上的神经节细胞之间并延伸毛细管网络深入感受器后层。视网膜小动脉的口径调节至扰动血氧水平 (“自动调节”)。

[0108] 图 18 说明显示作为年龄函数的人视紫红质含量的逻辑增长曲线 (Fulton 等人, Invest. Ophthalmol. Vis. Sci., (1999) 40:1878-1883)。箭头指示早产儿的 ROP 发病年龄 (Palmer 等人, Ophthalmology, (1991) 98:1628-1640)。

[0109] 图 19 为早产儿视网膜病的大鼠模型。 (a) 在 22 日龄的对照和 ROP 大鼠中注射荧光素后使用蓝色 (488nm) 激光激发 (Seeliger 等人, Vision Res., (2005) 45:3512-9) 所取得的激光扫描检眼镜 (SLO) 图像。(使用有色素的大鼠来协助 SLO 成像。) 各视网膜小动脉的集成曲率表示为对照中的平均值 (ICA) 的比例。ROP 大鼠的 ICA 值越高反映其小动脉的弯曲度越大。对照和 ROP 眼底 (fundus) 中的脉络膜外观类似。 (b) 对照和 ROP 大鼠中对全

视野刺激的样品视网膜电描记 (ERG) 响应。如图所示以相同的闪光强度测试这两种大鼠。垂直的灰线表示闪光出现的时间。

[0110] 图 20 说明实验范例的特性。严格控制并同步环境氧气和光周期。VCM 的给药被设计为针对视网膜中视紫红质的发展性增加的快速增长期 (箭头)。虚线框中的区域表示三个测试窗口。

[0111] 图 21 提供石蜡切片的 H&E 染色的照片 (来自实施例 7, 慢性光诱导 CNV)。外核层在来自经光和载体处理的动物眼睛的切片中最薄。

[0112] 图 22 是描绘来自经环境光和 3000 勒克司 (lux) 加载体或 ACU-4429 处理的动物的 H&E 切片中外核层中的核行数的图。数据为平均值  $\pm$  SEM。

[0113] 图 23 是描绘横跨层 / 切片的血管数目的图。

## 具体实施方式

[0114] 本公开内容涉及用于治疗糖尿病性视网膜病的方法。本文所使用的“糖尿病性视网膜病”是指在糖尿病中所见到的由于微血管变化而造成的视网膜的变化。向眼睛的视网膜供应氧的血管由于长期的高血糖水平 (高血糖症) 而受损。一般而言, 此疾病在几个月的期间内缓慢地发展, 但随着时间推移, 糖尿病性视网膜病可以变得更糟, 并导致视力丧失。糖尿病性视网膜病通常会影响双眼。糖尿病性视网膜病从轻度的非增生性异常 (其特征为血管通透性增加) 进展至中度和重度的非增生性糖尿病性视网膜病 (NPDR) (其特征为血管封闭), 再进展至增生性糖尿病性视网膜病 (PDR) (其特征为视网膜及玻璃体后表面上新血管的生长)。黄斑水肿 (其特征为视网膜从渗漏的血管增厚) 可在视网膜病的所有阶段发生。此外, 其他的病况 (例如妊娠、青春期、血糖控制、高血压及白内障手术) 可加速这些变化。

[0115] 非增生性糖尿病性视网膜病、增生性糖尿病性视网膜病及糖尿病性黄斑病是糖尿病性视网膜病的三种主要类型。

[0116] 非增生性糖尿病性视网膜病 (NPDR) 被视为是视网膜病的早期阶段且最常见于糖尿病患者中。视网膜中的微小血管仅轻微受影响, 但可能会形成凸起 (微动脉瘤) 和彼此连接 (视网膜内微血管异常) 和 / 或渗漏流体 (水肿)、蛋白质沉积物 (渗出物) 及血液 (出血)。非增生性糖尿病性视网膜病 (NPDR) 的另一典型迹象为视网膜上出现肿大的白色斑块 (棉絮斑)。这些变化可能发生在整个视网膜的任何地方, 包括黄斑部。

[0117] 非增生性糖尿病性视网膜病有三个阶段, 其详述于下:

[0118] (1) 轻度非增生性糖尿病性视网膜病: 在这个最早的阶段中, 可能出现至少一个微动脉瘤。微动脉瘤是视网膜血管中的气球样肿胀的小区域。

[0119] (2) 中度非增生性糖尿病性视网膜病: 随着病情进展, 一些滋养视网膜的血管被阻塞。

[0120] (3) 重度非增生性糖尿病性视网膜病: 更多血管被堵塞, 从而剥夺视网膜数个区域的血液供应。这些视网膜区域发送信号给身体以长出用于滋养的新血管。

[0121] 非增生性糖尿病性视网膜病不应对患者造成任何问题, 因为只要黄斑不受影响则视力仍保持正常。然而, 由于糖尿病性视网膜病的症状在这个阶段通常不可见, 建议应进行定期的视网膜筛检眼睛检查以监测进展为视网膜病的更严重阶段的迹象。

[0122] 增生性糖尿病性视网膜病 (PDR) :此阶段在重度非增生性糖尿病性视网膜病之后到来,其特征为眼睛中异常新血管的生长。当糖尿病造成血管堵塞时,眼睛和视网膜的部分因缺乏氧和营养而发展出缺血。眼睛试图通过长出新的血管供应低氧区来回应此种情况。不幸的是,反而形成容易出血的脆弱新血管。此过程被称为新血管形成。这些异常的新血管在视网膜表面上错误的地方生长并长入玻璃体凝胶。当这些新血管出血到玻璃体腔内时会发生玻璃体出血。血液阻挡进入眼内的光线到达视网膜。视力损失的程度可为轻度到重度,且取决于眼睛内有多少血液。当出血在几个月内逐步清除时视力可能会慢慢改善。

[0123] 异常的新血管也导致在视网膜上牵拉的瘢痕组织的形成,并可能导致牵引性视网膜脱离。视网膜脱离可影响视网膜的任何部分。若其影响黄斑,则患者可能会失去他 / 她的中心视力且其只能通过手术治疗。

[0124] 糖尿病性黄斑病 :糖尿病性黄斑病是糖尿病患者视力丧失的最常见的原因。其在当黄斑受到由糖尿病引起的视网膜病的变化影响时发生。黄斑位于视网膜中心,对中心视力及清楚地看见精细细节是至关重要的。因此,发展为糖尿病性黄斑病的患者的中心视力和看到细节的能力将受到影响。例如,受影响的个体可能会发现难以识别在远处的脸孔或阅读小印刷字。视力丧失的程度可能是轻度到重度。然而,即使在最坏的情况下,允许个体在家里和外面走动的周边 (侧) 视觉将保持不受影响。

[0125] 糖尿病性视网膜病 (DR) 为一种眼部疾病,其特征为糖尿病患者中因毛细管基底膜增厚及毛细管的周细胞和内皮细胞之间缺乏接触而发展出的过度血管发生。周细胞的损失增加了毛细管的渗漏并导致血液 - 视网膜屏障的破裂。糖尿病性视网膜病是视网膜微血管变化的结果。由高血糖症诱发的周细胞死亡和基底膜增厚导致血管壁的机能不全。这些损害改变血液 - 视网膜屏障的形成,也使视网膜血管变得通透性更强。小血管—例如眼睛中的小血管—特别容易受到不良血糖 (血液葡萄糖) 控制的损害。葡萄糖和 / 或果糖的过度积累会损害视网膜中的微小血管。当受损的血管渗漏流体和脂质至黄斑上时,也可发生黄斑水肿。这些流体使黄斑肿胀,从而使视力模糊。此损伤也造成视网膜处缺氧。

[0126] 随着病情进展,视网膜中的缺氧刺激沿着视网膜及在填充眼睛内部的透明、凝胶样玻璃体液中的血管发生。若不及时治疗,这些新血管可能会出血、使视力模糊并破坏视网膜。纤维血管增生也可引起牵引性视网膜脱离。新血管也可以长入眼睛前房的角内并造成新生血管性青光眼。

[0127] 由糖尿病性黄斑病引起的视力丧失以 2 种方式发生。

[0128] 糖尿病性黄斑水肿 (DME) 是黄斑的肿胀和增厚。这是由于流体从黄斑中的视网膜血管的渗漏。因为黄斑的光感受器细胞的结构和功能受到破坏而使视觉变得模糊。可以通过激光和向眼球内注射来控制由黄斑水肿造成的视力丧失。

[0129] 当通向黄斑的小视网膜血管 (毛细血管) 闭合时发生黄斑缺血。因为黄斑未接收到足够使其正常工作的血液供应而使视力变得模糊。不幸的是,黄斑缺血还没有有效的治疗。黄斑水肿是由于流体从视网膜血管渗漏。硬性渗出物是在视网膜上所见到的黄色沉积物。它们是由蛋白质物质的渗漏造成的。

[0130] 下列医学状况为糖尿病性视网膜病的一些可能的原因。

[0131] 糖尿病 :长期的高血糖症 (高血糖水平) 影响视网膜毛细管的解剖结构和功能。当葡萄糖转至旁路代谢途径时,过多的葡萄糖被转化成山梨糖醇。山梨糖醇导致视网膜毛

细管的周细胞的死亡或功能障碍。这弱化了毛细管壁,从而容许微动脉瘤形成,这是糖尿病性视网膜病的最早期迹象。薄弱的毛细管壁也可造成通透性及渗出物增加。由于糖尿病造成增加的血小板聚集和黏附(血块形成)的倾向,毛细管循环变得迟缓或者甚至因阻塞而完全受损。这也可促成糖尿病性视网膜病的发展。

[0132] 1型和2型糖尿病:被诊断为1型糖尿病的个体被认为是胰岛素依赖型,因为他们需要注射或其他用药以提供他们身体本身无法产生的胰岛素。由于缺乏胰岛素,血糖不被调节而水平过高。2型糖尿病个体被认为是胰岛素依赖型或胰岛素抗性的。受此类型的糖尿病影响的个体能产生足够的胰岛素,但身体无法正确地对其加以利用。于是身体通过产生更多的胰岛素来补偿,这可能会导致伴随的血糖水平的异常增加。所有I型糖尿病(幼年发病)和II型糖尿病(成年发病)患者均处于发展为糖尿病性视网膜病的风险中。然而,与2型糖尿病患者相比,1型糖尿病患者更可能引起视网膜病。

[0133] 1型糖尿病和2型糖尿病:1型糖尿病和2型糖尿病患者均处于增加的发展为糖尿病性视网膜病的风险中。

[0134] 过量酒精:若使用过量的酒精会降低维生素B12和硫胺素的水平。然而,单独的酒精摄入并不会与糖尿病性视网膜病相关联,来自酒精的空热量的消费使得坚持限制热量的糖尿病饮食非常困难,还不清楚适量的酒精对视网膜病有何影响。

[0135] 高血压及其他血管危险因素如肥胖及血脂异常可影响视网膜病的发生和发展。

[0136] 高胆固醇:胆固醇可通过使大动脉血管硬化而加重视网膜病并可对眼睛的小血管造成损害。

[0137] 肾脏病,如由蛋白尿及升高的尿素/肌酸酐水平所证明的,是出现视网膜病的很好的预测指标。

[0138] 妊娠:虽然可能不会直接导致视网膜病,但其可加重已有的视网膜病。患有糖尿病的妇女在妊娠期间具有略高的风险。建议所有患有糖尿病的孕妇每三个月进行散瞳检查(dilated eye examination)以保护她们的视力。

[0139] 肾脏损伤:与糖尿病性视网膜病有关,然而似乎是糖尿病性视网膜病导致肾脏损伤,而非肾脏损伤导致糖尿病性视网膜病。

[0140] 染色体15q缺失:涉及15号染色体长臂的遗传物质缺失的一种罕见的染色体疾病。

[0141] 眼内手术被认为可能会增加糖尿病性视网膜病进展的风险。

[0142] 在非增生性糖尿病性视网膜病的最早期通常没有症状。糖尿病性视网膜病的迹象和症状常在疾病向晚期或增生性糖尿病性视网膜病进展时出现。糖尿病性视网膜病的诊断迹象包括下列的一项或多项:血管的变化;视网膜肿胀(黄斑水肿);视网膜上的灰白色沉积物;受损的神经组织;渗漏血管的视觉外观;中心或周边视力丧失;暂时或永久性视力丧失;视野中发展出暗点或阴影;斑点、模糊、朦胧或复视;眼痛;与老花眼无关的近距视力问题;浮在视觉上的斑点或暗线(飞蚊症);色觉受损;视力丧失;中心视力中的暗点或盲点;夜视力不佳或衰退;静脉扩张和视网膜内微血管异常;在视网膜病晚期微小血管沿着视网膜、在填充眼睛内部的透明、凝胶样玻璃体液中生长;影响到控制眼睛运动的眼肌的神经损伤(神经病);不随意眼动(眼球震颤);视力波动及进行性恶化;黄斑水肿;黄斑缺血;牵引性视网膜脱离;突然、严重的无痛性视力丧失;血管通透性增加,导致水肿;内皮细胞增

殖；视野中有闪光（闪光幻觉）或缺陷；虹膜上存在异常血管（虹膜发红（rubeosis）或虹膜新血管生成（nvi））、白内障（与糖尿病相关）及玻璃体细胞（若视网膜脱离伴孔形成，玻璃体或色素细胞中有血液）；微动脉瘤—使毛细管壁易渗漏的毛细管壁的物理弱化；硬性渗出物—从视网膜血管渗漏出的脂蛋白/其他蛋白质的沉淀物；出血—弱化的毛细管的破裂，表现为沿着浅表视网膜层中的神经-纤维束经过的小点/较大斑点或“火焰”出血（出血来自较大且较浅表的小动脉）；棉絮斑—因缺血性梗死边缘的不良轴突代谢而引起的轴突碎片的积聚；及新血管形成—（由残余的健康视网膜）重建缺氧视网膜组织的血管的尝试。

[0143] 本公开内容也涉及使用视觉周期调节（VCM）化合物来治疗早产儿视网膜病（ROP）的方法。本文所描述的工作首次证明了用非类维生素A VCM 进行全身性治疗对不成熟的眼睛的视网膜病的效果。当受试者为新生儿时，此方法的一个关键要素为高  $O_2$  含量。早产儿被置于高氧气氛下以支持不成熟的肺功能，其中高氧浓度抑制视网膜脉管系统的正常发育。当婴儿返回正常的空气中时，视网膜由于脉管系统发育不足而变得缺血。缺血触发 VEGF 表达及新血管形成。参见，例如，图 4B。VCM 通过增加能减少暗电流从而降低氧消耗的载脂蛋白-视紫红质而起作用。

[0144] 本文描述了用于治疗或预防视网膜的疾病或病症的 VCM 化合物，特别是用于治疗或预防相关于或涉及血管异常（例如 ROP）的视网膜疾病或病症的 VCM 化合物。本文所描述的方法涉及调节视觉周期的 VCM 化合物的施用。

[0145] 作为一个系统，哺乳动物的视网膜会遭遇影响神经视网膜与滋养它的脉管系统的平衡互连的疾病；当这种平衡被扰乱时就会发生视力丧失。主要影响神经视网膜的疾病，如光感受器变性，也影响视网膜脉管系统。临床特征为脉络膜或视网膜脉管系统异常的疾病，如 ROP，也影响视网膜神经元。这些状况均涉及神经组织的缺氧缺血性病症。光感受器是身体所有细胞中需氧最多的特化细胞 (Steinberg, R., Invest. Ophthalmol. Vis. Sci., (1987) 28:1888-1903)，其在视网膜的所有缺氧缺血性疾病中起作用。

[0146] 在正常发育中，当视杆光感受器分化并开始产生视紫红质（负责光捕获的分子）时；其异常高的需氧量使视网膜缺氧，驱使视网膜血管生长。然而，在 ROP 中，所施用的用于早产儿急性心肺护理的补充氧使视网膜成为高氧的，中断正常的血管生长，并使得周边视网膜无血管。当停止补充氧时，周边视网膜变成缺氧的。缺氧触动导致形成异常的视网膜血管（其在临幊上用来诊断 ROP）的分子级联。虽然早产儿经受高环境氧，但其不成熟的肺及其他医疗并发症往往会导致血氧波动，因而在视网膜发生低氧和高氧事件，这影响敏感的光感受器。发育中的神经视网膜及其脉管系统处于协同分子控制下，而 ROP 的血管异常与神经视网膜的功能有关。最近的研究发现，ROP 中视杆功能障碍的程度有助于预测在视网膜脉管系统中所观察到的异常程度，但在视网膜脉管系统中所观察到的异常程度可能无法帮助预测 ROP 中视杆功能障碍的程度。因此，视杆造成 ROP。

[0147] 本文所使用的“不成熟的视网膜”是指早产儿的视网膜或具有类似于早产儿视网膜的形态/功能的视网膜。不成熟的视网膜的特征可在于存在发育不良或无组织的血管、存在或不存在瘢痕组织。一般而言，人类早产儿为妊娠 37 周或更早出生的婴儿。相反地，术语“视网膜成熟”是指足月婴儿的视网膜或具有类似于足月婴儿的视网膜形态/功能的视网膜。

[0148] 本文所使用的短语“降低视杆的能量需求”或“抑制视杆的能量需求”是指视杆细胞的需氧量降低至少 10%；优选地，视杆细胞的需氧量降低至少 20%、至少 30%、至少 40%、至少 50%、至少 60%、至少 70%、至少 80%、至少 90% 或更多。一般而言，视杆细胞的需氧量优选保持在低于诱发病理性血管发生（例如血管生长）或血管异常所需的水平。

[0149] 本文所使用的术语“血管异常”用来指不允许视网膜正常发育至如同本文所使用的术语“视网膜成熟”的异常或病理性水平的血管生长（例如血管发生）或形态（例如弯曲）。本领域技术人员可滴定测定试剂的施用量或施用时机以使血管的生长和形态维持低于病理性血管生长（例如通过 Laser Doppler Blood Flow 分析所评估的）。在备选的实施方式中，使用视网膜血管的弯曲度水平来评估病理性血管形态和 / 或生长的程度。测量弯曲度的方法在本文中进一步描述。

[0150] 本文所使用的术语“补充氧”是指将受试者的血氧水平维持在所需水平所必要的、高于环境空气的氧浓度（即，约 20–21%）的氧浓度。一般而言，补充氧在临床设置中提供，以维持 100% 的血氧水平，如使用例如经皮氧监测而评估的。监测血氧水平并改变“补充氧”的水平以维持例如 100% 的血氧水平是临床设置（例如新生儿重症监护室）中的标准程序且为医学领域的技术人员所熟知。

[0151] 视网膜的血管和神经疾病

[0152] 尽管视网膜的新生血管性疾病（如早产儿视网膜病（ROP））的医疗处置有所进步，但视网膜神经血管疾病仍然是全世界失明的首要原因。

[0153] 对于 ROP，目前的治疗是本身具有负面后果的外周脉管系统的光凝术及疗效不明的实验性方法，例如以抗血管发生的药物进行的治疗。因为视杆光感受器是眼睛特有的且是身体所有细胞中需氧量最高的，其可能在缺氧缺血性新生血管性视网膜疾病中起作用 (Arden 等人, Br J Ophthalmol (2005) 89:764；及 Fulton 等人, Doc Ophthalmol, (2009) 118(1):55–61)。大鼠 ROP 模型提供了一种体内系统，在其中可以研究和操纵光感受器与视网膜脉管系统的关系。

[0154] 视网膜功能异常是新生血管性视网膜疾病的特征 (Fulton 等人, Doc Ophthalmol, (2009) 118(1):55–61)。新生血管性视网膜疾病中的视力丧失起因于血管异常，而在血管异常解决后仍然存在的终身视网膜功能障碍的严重度与先前血管疾病的严重度有关 (Fulton 等人, Arch Ophthalmol (2001) 119:499)。然而，来自大鼠 ROP 模型的数据显示，视杆光感受器的功能障碍在血管异常之前，传统上通过血管异常来定义 ROP 并预测其严重度 (Reynaud 和 Dorey, Invest Ophthalmol Vis Sci (1994) 35:3169；Akula, Invest Ophthalmol Vis Sci (2007) 48:4351)。血管形态异常是 ROP 的主要诊断标准；然而，ROP 主要为具有继发性血管异常的神经视网膜的病症。表征急性 ROP 的血管异常的外观与视杆光感受器外节段的发育延长以及伴随的视网膜中视紫红质含量的增加相一致 (Lutty 等人, Mol Vis (2006) 12:532；和 Dembinska 等人, Invest Ophthalmol Vis Sci (2002) 43:2481)。

[0155] 视杆细胞生理学及代谢

[0156] 视杆细胞执行三种相关连的、代谢上要求高的过程：暗电流的产生、视色素（视觉周期）的维持及外节段更新 (turnover)，所有这些伴随视杆外节段 (ROS) 的发育延长及眼睛的视紫红质含量的增加而发生。视杆的信号转导机制是生理上独特的。在黑暗中，透过

ROS 中的环鸟苷单磷酸 (cGMP) 门控通道进入的钠和其他阳离子被视杆内节段 (RIS) 中的泵快速地排除, 以致每半分钟使等于整个细胞溶质 (cytosol) 的体积得到循环 (Hagins 等人, Proc Natl Acad Sci USA (1989) 86:1224)。在闪光之后由视紫红质捕获光子所引发并造成 cGMP 减少的分子级联导致暗电流在形成延迟高斯 (delayed Gaussian) 后衰减, 该延迟高斯可通过内在放大常数 A 来描述 (Lamb 和 EPugh, J Physiol (1992) 449:719; 及 Pugh 和 Lamb, Biochem Biophys Acta (1993) 1141:111)。

[0157] 光子捕获后, 视紫红质的发色团 (视黄醇) 经历异构变化, 这种变化使其从视蛋白释出并引发光转导。耗用的发色团从 ROS 经过到达视网膜色素上皮 (RPE), 在此其经历一系列的转化, 之后透过 RPE 的顶端过程 (apical processes) 以视黄醇的形式返回至 ROS。在该处其变成共价连接至视蛋白中的活性位点赖氨酸, 再次变成视紫红质并完成视觉周期 (R. R. Rando, Chem Rev (2001) 101:1881)。视觉周期中的限速步骤由异构水解酶复合物 RPE65 介导 (Moiseyev 等人, Proc Natl Acad Sci USA (2005) 102:12413)。ROS 中的光转导的其他副产物通过 ROS 尖端的昼夜节律脱落过程而排除; 每个 RPE 细胞每天吞噬数千个从 30–50 个包埋的视杆上脱落的视盘 (disk) (R. W. Young, J Cell Biol (1967) 33:61)。通过 RPE65 活性的靶向抑制对视觉周期的受控下调降低了类维生素 A 通过 ROS/RPE 复合物的通量; 这将通过减少视杆的代谢需求而使其较不易遭受来自高氧和低氧的损伤 (Wellard 等人, Vis Neurosci (2005) 22:501)。其也可能减缓吞噬作用, 从而延长视杆外节段。

#### [0158] 从动物模型向患者的转化

[0159] 光感受器紧密贴靠脉络膜脉管系统。高度组织化的感受器后视网膜神经元形成由视网膜血管供应的层。虽然脉络膜为光感受器的主要供应者, 然而, 光感受器变性与视网膜小动脉弱化有关 (Hansen 等人, Vision Research, 48(3):325–31 (2008))。由于光感受器层为特别的氧槽 (oxygen sink), 虽然不希望受理论的约束, 但据推测当光感受器变性时, 其代谢需求减少且视网膜脉管系统因神经视网膜对氧的慢性较低需求而变弱 (Hansen 等人, Vision Research, 48(3):325–31 (2008))。

[0160] 光感受器与视网膜血管网络之间的紧密联系在发育中的视网膜中是明显的。感受器后细胞在光感受器之前分化, 光感受器为最后成熟的视网膜细胞。如同视杆外节段的形成是从后部进展至外围梯度, 血管覆盖也是如此。因此, 神经和血管组分同时且协同的发育是正常视网膜成熟的特征。在早产儿中, ROP 的发病年龄约为视杆外节段长度快速发育增加及随后视紫红质含量增加的年龄。除了不成熟的光感受器及视网膜脉管系统外, 早产儿还具有不成熟的肺, 其产生不稳定的呼吸状态, 伴随对未成熟细胞的低氧损伤风险。临幊上, 这通过施用补充氧来对抗, 但已知高氧和低氧水平均会损害不成熟的光感受器 (Fulton 等人, Invest. Ophthalmol. Vis. Sci., (1999) 40:168–174; 及 Wellard 等人, Vis. Neurosci., (2005) 22:501–507)。

[0161] 通过将幼崽在栖息地饲养, 并在视杆外节段延长的关键期交替相对高和相对低的氧的周期, 来诱导 ROP 的大鼠模型 (Akula 等人, Invest. Ophthalmol. Vis. Sci., (2007) 48:4351–9; Akula 等人, Invest. Ophthalmol. Vis. Sci., (2007) 48:5788–97; Dembinska 等人, Invest. Ophthalmol. Vis. Sci., (2001) 42:1111–1118; Liu 等人, Invest. Ophthalmol. Vis. Sci., (2006) 47:5447–52; Liu 等人, Invest. Ophthalmol. Vis. Sci., (2006) 47:2639–47; Penn 等人, Invest. Ophthalmol.

Vis. Sci., 1995. 36:2063-2070)。诱导后, 视网膜脉管系统的异常随之发生, 也发生神经视网膜的结构和功能的异常 (Fulton 等人, Invest. Ophthalmol. Vis. Sci., (1999) 40:168-174; Akula 等人, Invest. Ophthalmol. Vis. Sci., (2007) 48:4351-9; Akula 等人, Invest. Ophthalmol. Vis. Sci., (2007) 48:5788-97; Dembinska 等人, Invest. Ophthalmol. Vis. Sci., (2001) 42:1111-1118; Liu 等人, Invest. Ophthalmol. Vis. Sci., (2006) 47:5447-52; Liu 等人, Invest. Ophthalmol. Vis. Sci., (2006) 47:2639-47; Reynaud 等人, Invest. Ophthalmol. Vis. Sci., (1995) 36:2071-2079)。ROP 大鼠中视网膜脉管系统的形态及神经视网膜的功能的异常类似于在儿科 ROP 患者中所见 (Dembinska 等人, Invest. Ophthalmol. Vis. Sci., (2001) 42:1111-1118; Liu 等人, Invest. Ophthalmol. Vis. Sci., (2006) 47:5447-52; Liu 等人, Invest. Ophthalmol. Vis. Sci., (2006) 47:2639-47; Reynaud 等人, Invest. Ophthalmol. Vis. Sci., (1995) 36:2071-2079; Barnaby, A. M., Invest. Ophthalmol. Vis. Sci., (2007) 48:4854-60; Fulton 等人, Arch. Ophthalmol., (2001) 119:499-505; Gelman, R., Invest. Ophthalmol. Vis. Sci., (2005) 46(12):4734-4738; Moskowitz 等人, Optometry&Vision Science, (2005) 82:307-317; Fulton, A. B., Invest. Ophthalmol. Vis. Sci., 49(2):814-9(20089))。因此, 大鼠模型可以外推到人类治疗。

[0162] 使用大白鼠 ROP 模型来研究发育期间视网膜的神经和血管特性 (Akula 等人, Invest. Ophthalmol. Vis. Sci., (2007) 48:4351-9; Akula 等人, Invest. Ophthalmol. Vis. Sci., (2007) 48:5788-97; Liu, K, Invest. Ophthalmol. Vis. Sci., (2006) 47:5447-52; Liu 等人, Invest. Ophthalmol. Vis. Sci., (2006) 47:2639-47)。不同的氧暴露安排对视网膜脉管系统及神经视网膜诱导出一系列影响, 其模拟在人类 ROP 病例中所观察到的全部 (轻度到重度) 视网膜病。在视杆外节段延长及视网膜的视紫红质含量增加时的年龄期间定时进行氧暴露以影响视网膜。视网膜电流扫描计 (ERG) 响应及视网膜血管特征的纵向测量在幼年 (约 20 日龄)、青年 (约 30 日龄) 和成年 (约 60 日龄) 大鼠中获得。

[0163] 神经功能的评估

[0164] 使用 ERG 来表征神经功能。如先前所详述的 (Akula 等人, Invest. Ophthalmol. Vis. Sci., (2007) 48:4351-9), 记录暗适应的动物对一系列强度的全视野刺激的 ERG 响应。为了总结视杆光感受器活性, 将光转导活化模型与 a- 波拟合, 并计算所产生的敏感性 (SROD) 及饱和振幅 (RROD) 参数。感受器后活性以 b 波代表。刺激 / 响应函数通过饱和振幅 (Vmax) 及产生半数最大响应的刺激 (log s) 进行总结; 这些参数是从对 b 波振幅拟合的 Michaelis-Menten 函数推导而来的 (Hood Birch, Invest. Ophthalmol. Vis. Sci., (1994) 35:2948-2961; Lamb 和 Pugh, J. Physiol. (Lond). (1992) 449:719-758; Pugh 和 Lamb, Biochim. Biophys. Acta, 1993. 1141:111-149; Pugh 和 Lamb, 于 Handbook of biological physics. Volume 3(2000), Elsevier Science. p. 183-255; Akula 等人, Invest. Ophthalmol. Vis. Sci., (2007) 48:4351-9)。

[0165] 血管特征的评估

[0166] 视网膜血管参数使用图像分析软件推导, 并可应用到数字眼底照片 (Akula 等人, Invest. Ophthalmol. Vis. Sci., (2007) 48:4351-9; Martinez-Perez, M. E., (2001), Imperial College:London; Martinez-Perez 等人, Trans. Biomed.

Eng. , (2002) 49:912-917)。集成曲率 (IC) (其与经验丰富的临床医生所报告的对血管弯曲的主观评估良好相符) 可用于明确说明各眼底的血管状态 (Gelman, R. M. Invest. Ophthalmol. Vis. Sci. , (2005) 46 (12) :4734-4738)。小动脉和小静脉均明显受 ROP 影响。然而, 已经发现小动脉明显受影响, 而小静脉受的影响较少; 因此, 本文所描述的分析中使用小动脉参数 ICA(Akula 等人, Ophthalmol. Vis. Sci. , (2007) 48:4351-9; Liu 等人, Invest. Ophthalmol. Vis. Sci. , (2006) 47:5447-52; Liu 等人, Invest. Ophthalmol. Vis. Sci. , (2006) 47:2639-47; Gelman, R. , M. Invest. Ophthalmol. Vis. Sci. , (2005) 46 (12) :4734-4738)。

[0167] 视网膜敏感性和脉管系统的关系

[0168] 使用年轻 (20 日龄) 时的视杆光感受器敏感性 (SROD) 来预测由 ICA 说明的视网膜血管结果。早期的较佳敏感性与较佳 (较不弯曲) 的血管结果有关 (Akula 等人, Invest. Ophthalmol. Vis. Sci. , (2007) 48:4351-9)。停止诱导氧暴露后, 感受器后神经视网膜敏感度 (b 波 log s) 恢复且血管弯曲度降低。发育中的视网膜神经元和血管的调节在几种生长因子 (例如血管内皮生长因子 (VEGF)、脑信号蛋白) 及其神经毡蛋白受体的协同控制下发生 (Gariano 等人, Gene Expression Patterns, (2006) 6:187-192)。在 ROP 的大鼠模型中, 已发现这些生长因子的表达改变 (Mocko 等人, ARVO Absract, (2008))。

[0169] 本文也描述了用于治疗患者的湿型年龄相关性黄斑变性的方法, 其包括向患者施用治疗有效量的视觉周期调节 (VCM) 化合物。

[0170] 视觉周期调节

[0171] 本文所使用的“视觉周期调节 (VCM)”是指在视网膜中光子向电信号的生物转化。(参见, 例如图 1A 和 1B)。视网膜含有被称为“视杆”(负责夜间视觉) 和“视锥”(负责白昼视觉) 的光感受器细胞。视杆细胞比视锥细胞数目多得多且更活跃。视杆的过度活跃造成毒素在眼内的积聚, 而视锥提供我们的绝大多数视觉信息—包括颜色。VCM 基本上“减缓”视杆的活动, 并减少视网膜中的代谢负荷和氧消耗。图 4B 说明 VCM 影响视觉周期的一种方式。

[0172] 本文公开了可用于改善 ROP 的结果的 VCM 化合物。VCM 化合物单独施用或与一种或多种另外的化合物 / 治疗一起施用, 该另外的化合物 / 治疗包括但不限于: 降低视杆光感受器的能量需求的药物治疗可以减少不适当的血管增生, 及增加患者所暴露的光的环境治疗。由于视杆光感受器的生理学, 在低光度情况下代谢需求最高; 因此, 暴露于增加的光可以降低代谢需求, 从而缓解 ROP 的显现。

[0173] 黄斑变性

[0174] 黄斑变性是指负责高敏锐视力的中心视网膜部分 (称为黄斑) 中光感受器的丧失。黄斑变性与细胞外基质成分及其他碎片在视网膜色素上皮与血管脉络膜之间的膜中的异常沉积有关。此碎片样物质称为玻璃疣。玻璃疣通过眼底镜眼部检查来观察。正常的眼睛可具有不含玻璃疣的黄斑, 然而, 玻璃疣可能富于视网膜周边。在黄斑视觉无任何损失的情况下在黄斑中出现软玻璃疣被认为是 AMD 的早期阶段。

[0175] 年龄相关性黄斑变性

[0176] 年龄相关性黄斑变性 (AMD) 是指导致视网膜黄斑异常的疾病; 其在欧洲和美国为视力丧失的首要原因。在日本, 因为人口老化, 该疾病亦稳定增加。黄斑位于视网膜中心,

该区域中在光感受器细胞之间密布着视锥细胞。来自外界的光线被角膜和晶状体折射,然后汇集于黄斑上,尤其是中央凹。阅读文字的能力取决于此区域的功能。在年龄相关性黄斑变性中,黄斑(其为如上所述的重要区域)随着年龄的增长而变性,并造成视力缺损,主要的形式为影像失真(偏视)和中心暗点。

[0177] 中心地图样萎缩—“干”型晚期 AMD—是由视网膜下方的视网膜色素上皮层萎缩造成的,其通过眼睛中心部分的光感受器(视杆和视锥)丧失而导致视力丧失。新生血管性或渗出性 AMD—“湿”型晚期 AMD—由于脉络膜毛细血管层中穿过布鲁赫膜的异常血管生长(脉络膜新血管形成),最终导致黄斑下血液和蛋白质渗漏而造成视力丧失。若不经治疗,来自这些血管的出血、渗出和瘢痕形成最终造成对光感受器的不可逆损害以及快速的视力丧失。湿型年龄相关性黄斑变性为预后不良的疾病,其造成快速且严重的视力缺损。主要的病理状况为脉络膜新血管形成。

[0178] 年龄相关性黄斑变性(AMD)为发达国家中失明的主要原因之一。大分子 LUCENTIS®、AVASTIN®及MACUGEN®的获批已改善了可用于 AMD 患者的治疗选择。LUCENTIS®为一种 Fab,而 AVASTIN®为一种单克隆抗体。它们均结合血管内皮生长因子(VEGF)且可用于治疗 AMD;然而,只有少数的经治疗的患者经历视敏度的显著改善。

#### [0179] 脉络膜新血管形成

[0180] 脉络膜新血管形成(CNV)是指在眼睛的脉络膜层中生成新血管。CNV 可在布鲁赫膜(脉络膜的最里层)有缺陷的个体中迅速发生。其还与血管内皮生长因子(VEGF)过量有关。如同在湿型 AMD 中,CNV 也常与罕见的遗传性疾病弹性假黄瘤同时出现,而很少与较常见的视盘玻璃疣同时出现。CNV 也与极端近视或恶性近视性变性有关,其中脉络膜新血管形成主要在视网膜(特异地)黄斑组织内出现被称为漆样裂纹的裂纹时发生。

[0181] CNV 可使中心视力突然恶化,在几个星期内明显可见。其他可发生的症状包括视物变形症及颜色混乱。新血管出血可加速 CNV 症状发作。

[0182] CNV 可通过测量择优超锐度视野计量(Preferential Hyperacuity Perimeter)来检测。根据荧光素血管造影术,CNV 可被描述为典型的或隐匿性的。PHP 是应用静态和自动化视野测量原理来检测视野中的缺陷的专业视野计量。PHP 依赖超锐度的概念来测量中心与近中心视野的细微差别,而非测量周围视野。超锐度是辨别目标物的细微错位的能力。中央凹中的超锐度,或游标敏锐度的阈值为 3 至 6 弧秒。因此,超锐度的阈值比最佳分辨物体所需的值(中央凹中为 30 到 60 弧秒)低约 10 倍。

[0183] 除了其他眼科疾病外,脉络膜新血管形成(CNV)还常在黄斑变性中发生且与脉络膜内皮细胞的增殖、细胞外基质的过度产生及纤维血管视网膜下膜的形成相关。视网膜色素上皮细胞增殖和血管生成因子的产生似乎影响脉络膜新血管形成。

[0184] 现行视网膜学中的医护标准为在玻璃体内注射抗 VEGF 药物以控制新血管形成及减少视网膜色素上皮下的流体面积。这些药物通常被称为 AVASTIN® 及 LUCENTIS®,虽然其有效性已显示显著改善具有 CNV 的视力预后,但这些新生血管区域的复发率仍然很高。根据美国眼科学会,以及临床报告的更进一步支持,患有 CNV 的个体应该意识到他们的对侧眼处于发展出 CNV 的更大风险中(25%)。

[0185] 在“湿型”(亦称为“新生血管性”)年龄相关性黄斑变性中,CNV 用光动力疗法结合光敏药物如维替泊芬进行治疗。维替泊芬(一种苯并卟啉衍生物)是一种静脉内亲脂性

光敏药物,具有690nm的吸收峰。该药物最早在2000年4月12日由食品药品管理局(FDA)批准,在2000年7月18日获准列入到美国药典中,当与眼部光动力疗法(见§80.2,“光动力疗法”)一起使用,由医师服务通过静脉内提供时符合Medicare的药物定义。对年龄相关性黄斑变性患者而言,维替泊芬仅涵盖在初访时通过荧光素血管造影术(CPT代码92235)确定的、具有主要为典型的中央凹下脉络膜新生血管(CNV)病变(其中典型CNV区占整个病变区的>50%)的新生血管性年龄相关性黄斑变性的诊断(ICD-9-CM362.52)。后续的随访将需要在治疗前进行荧光素血管造影术。OPT加维替泊芬涵盖上述适应症且仍不涵盖所有其他与AMD相关的适应症(见§80.2)。用于非AMD病况的OPT加维替泊芬通过个体立约者自由裁量而有资格涵盖。维替泊芬通过静脉内给药。然后,由激光在眼睛内活化。该药物破坏新血管,并通过形成血栓来防止任何新血管形成。

[0186] 抗VEGF药物,如培加尼布和雷珠单抗(ranibizumab),也用于治疗CNV。抗VEGF结合VEGF并使其失活。

[0187] CNV是指脉络膜血管的异位生长,穿透布鲁赫膜及视网膜色素上皮。在湿型年龄相关性黄斑变性中,出血及包含脂肪的血浆组分从早产儿血管丛的渗漏是神经视网膜的快速功能损伤的直接原因。CNV被认为是由主要包含巨噬细胞(其浸润以吞噬积累在视网膜下黄斑区的玻璃疣)的炎性细胞引起的。炎性细胞如巨噬细胞亦为产生血管生成因子如血管内皮生长因子(VEGF)的来源,且其起到增加炎症部位的新血管形成的作用。此过程被称为“炎性新血管形成”。同时,玻璃疣包含晚期的糖化终产物(AGE)及淀粉样蛋白 $\beta$ ,其为刺激VEGF生成的物质;这些物质会刺激已迁移的视网膜色素上皮以吞噬玻璃疣,造成VEGF分泌,而这被认为是CNV发展的另一种可能的机制。涉及CNV的疾病包括近视性脉络膜新血管形成和特发性脉络膜新血管形成,以及年龄相关性黄斑变性。涉及CNV的疾病的发展有时可归因于血管样条纹、损伤、葡萄膜炎等。已提出主要为视网膜下黄斑区中的布鲁赫膜和视网膜色素上皮的组织损伤以及随后的炎症与这些疾病和年龄相关性黄斑变性中的CNV发病机制有关。

[0188] 需要长时间眼睛暴露的医疗程序

[0189] 大多数眼睛操作、手术、程序和检查需要暴露在瞄准眼睛的直接亮光下,且在许多情况下,此暴露是长时间的;此处所公开的化合物可用于限制或以其他方式预防由这类暴露对眼睛所造成的不希望的损害。

[0190] 一些医疗程序旨在校正眼睛的结构缺陷。

[0191] 眼屈光手术涉及手术重塑角膜或白内障的各种方法(例如放射状角膜切开术采用以金刚石刀作出的轮辐形切口)。在某些情况下,使用准分子激光来重塑角膜曲率。在某些情况下,成功的眼屈光手术减轻或治愈常见的视力障碍,如近视、远视和散光,以及退化性疾病,如圆锥形角膜。其他类型的眼屈光手术包括角膜磨削术(削去角膜盘,快速冷冻,车磨(athe-ground),然后回复至其原来的能力(power))、自动板层角膜移植术(ALK)、激光辅助的原位角膜磨削术(LASIK)、intraLASIK、激光辅助的上皮下方角膜磨削术(LASEK,又称Epi-LASIK)、屈光性角膜切削术、激光热角膜成形术、传导性角膜成形术、角膜缘松解切口、散光角膜切开术、放射状角膜切开术、微型不对称放射状角膜切开术、六角状角膜切开术、表面角膜镜片术、角膜内环或环段植入体(角膜环(Intacs))、接触镜植入体、老花眼逆转术、前睫状巩膜切开术、老花眼激光逆转术、巩膜膨胀带及Karmra镶嵌。

[0192] 角膜手术包括但不限于角膜移植手术、穿透性角膜成形术、人工角膜手术、光性治疗性角膜切除术、翼状胬肉切除术、角膜染色术及骨齿人工角膜手术 (OOKP)。在某些情况下,角膜手术不需要激光。在其他情况下,角膜手术使用激光 (例如光性治疗性角膜切除术,其除去浅表角膜混浊及表面不平)。在某些情况下,在这些程序后给予患者深色眼镜以保护其眼睛避免亮光。

[0193] 一些程序旨在从眼睛移除有缺陷的组分或流体。

[0194] 白内障手术涉及手术移除晶状体并以塑料人工晶状体替换。通常,使用光来协助外科医生。

[0195] 促使多余的房水从眼睛逸出以降低眼内压的青光眼手术有多种类型。在某些情况下,这些医疗程序使用激光 (例如,激光小梁成形术应用激光束来烧灼位于靠近虹膜基底的小梁网区域,以增加流体流出;激光周边虹膜切开术应用激光束来选择性地烧灼出穿过虹膜接近其基底的孔等)。管道成形术是一种先进的、非穿透性程序,其设计成在简单的微创手术中使用微导管技术来增加通过眼睛的自然排液系统而排液。其他用于治疗青光眼的医疗程序包括激光、非穿透性手术、防护性滤过手术及西顿阀 (seton valve) 植入体。

[0196] 玻璃体视网膜手术包括玻璃体切割术 (例如前部玻璃体切割术及平坦部玻璃体切割术)。在某些情况下,玻璃体视网膜手术用于在白内障或角膜手术期间预防或治疗玻璃体损失、除去在例如无晶状体瞳孔阻滯性青光眼的病症中错位的玻璃体组织、通过切口去除玻璃体混浊及膜、视网膜脱离修复 (使用火针术、巩膜扣压术或激光光凝术、充气性视网膜粘结术、视网膜冷冻固定术或视网膜冷冻疗法)、黄斑孔修复、部分板层巩膜葡萄膜切割术、部分板层巩膜环脉络膜切割术、部分板层巩膜脉络膜切割术、后巩膜切割术、放射状视神经切开术及黄斑转位手术。泛视网膜光凝 (PRP) (这是一种经常用于治疗糖尿病性视网膜病的光凝激光疗法) 旨在治疗玻璃体出血、眼中从伤口的流血、视网膜撕裂、蛛网膜下出血或阻塞的血管。在一些情况下,一旦移除血液后以激光进行光凝而使不健康的血管收缩或将视网膜孔密封。

[0197] 一些医疗程序解决支持眼功能或眼睛外观的结构或特征。眼肌手术通常修正斜视,并包括以下内容:放松及弱化程序 (例如消退、肌切除术、肌切开术、肌腱切除术、肌腱切断术、拉紧等)、增强程序 (例如眼肌的切除、翻折、从其附着在眼球上的原始位置移动至更前面的位置等);转置和重新定位程序及可调整的缝合手术 (例如在手术后第一天内通过可缩短或拉长的缝线重新连接眼外肌以取得更好的眼睛对位的方法)。

[0198] 眼整形手术或眼成形术是处理眼睛和相关结构的重构的眼科学的亚专科,包括眼睑手术、泪小管阻塞的修复、眼眶骨折修复、眼内和眼周围肿瘤的去除及脸部回春程序,包括激光皮肤磨削术、眼睛拉提、眉毛拉提、脸部拉提、肉毒毒素 (Botox) 注射、超级除皮微晶磨皮 (ultrapeel microdermabrasion) 及脂肪抽吸。一些眼睛程序改善泪器,包括泪囊鼻腔吻合术、泪管泪囊吻合术、泪小管切开术、泪腺切割术、泪囊切割术和泪囊造口术。

[0199] 视觉周期调节化合物

[0200] 如在本说明书及所附权利要求书中所使用的,除非指出意思相反,否则下列术语具有以下所述的含义。

[0201] 如本文和所附权利要求书中所使用的单数形式“一种”、“和”以及“该 (所述)”包括复数的指代,除非上下文明确指明不是这样。因此,例如,所提及的“一种化合物”包括多

种这样的化合物，并且所提及的“该细胞”包括所提及的一种或多种细胞（或多个细胞）和本领域技术人员已知的其等同物，等等。此外，例如，提及“该方法”包括一种或多种本文所描述的类型的方法和 / 或步骤，和 / 或本领域技术人员在阅读本公开内容后将会明白的方法和 / 或步骤，等等。当在此对物理性质（例如分子量）或化学性质（例如化学式）使用范围时，意在包括范围的所有组合和子组合以及其中的具体实施方式。当提及数字或数值范围时使用的术语“约”是指所提及的数字或数值范围为在实验可变性范围内（或者在统计实验误差范围内）的近似值，因而该数字或数值范围可以在所述数字或数值范围的 1% 至 15% 之间变化。术语“包含”（以及相关的术语，例如“包括”或“具有”或“含有”）并非旨在排除，在其他某些实施方式中，例如，本文描述的物质、组合物、方法或过程等的实施方式可以“由所述特征组成”或“基本由所述特征组成”。

[0202] “氨基”是指  $-\text{NH}_2$  基团。

[0203] “氰基”是指  $-\text{CN}$  基团。

[0204] “硝基”是指  $-\text{NO}_2$  基团。

[0205] “氧杂”是指  $-\text{O}-$  基团。

[0206] “氧代”是指  $=\text{O}$  基团。

[0207] “硫代”是指  $=\text{S}$  基团。

[0208] “亚氨基”是指  $=\text{N}-\text{H}$  基团。

[0209] “肼基”是指  $=\text{N}-\text{NH}_2$  基团。

[0210] 烷基是指仅由碳和氢原子组成、不包含不饱和部分、具有 1 至 15 个碳原子的直链或支链烃链基团（例如， $\text{C}_1-\text{C}_{15}$  烷基）。在某些实施方式中，烷基包含 1-13 个碳原子（例如， $\text{C}_1-\text{C}_{13}$  烷基）。在某些实施方式中，烷基包含 1-8 个碳原子（例如， $\text{C}_1-\text{C}_8$  烷基）。在其他一些实施方式中，烷基包含 5-15 个碳原子（例如， $\text{C}_5-\text{C}_{15}$  烷基）。在其他一些实施方式中，烷基包含 5-8 个碳原子（例如， $\text{C}_5-\text{C}_8$  烷基）。烷基通过单键连接到分子的其余部分上，例如，甲基 (Me)、乙基 (Et)、正丙基、1- 甲基乙基（异丙基）、正丁基、正戊基、1, 1- 二甲基乙基（叔丁基）、3- 甲基己基、2- 甲基己基，等等。除非在说明书中另有特别说明，烷基任选地被如下取代基中的一种或多种所取代：卤代、氰基、硝基、氧代、硫代、三甲基硅烷基、 $-\text{OR}^a$ 、 $-\text{SR}^a$ 、 $-\text{OC}(\text{O})-\text{R}^a$ 、 $-\text{N}(\text{R}^a)_2$ 、 $-\text{C}(\text{O})\text{R}^a$ 、 $-\text{C}(\text{O})\text{OR}^a$ 、 $-\text{C}(\text{O})\text{N}(\text{R}^a)_2$ 、 $-\text{N}(\text{R}^a)\text{C}(\text{O})\text{OR}^a$ 、 $-\text{N}(\text{R}^a)\text{C}(\text{O})\text{R}^a$ 、 $-\text{N}(\text{R}^a)\text{S}(\text{O})_t\text{R}^a$ （其中， $t$  为 1 或 2）、 $-\text{S}(\text{O})_t\text{OR}^a$ （其中， $t$  为 1 或 2）和  $-\text{S}(\text{O})_t\text{N}(\text{R}^a)_2$ （其中， $t$  为 1 或 2），其中各  $\text{R}^a$  均独立地为氢、烷基、氟代烷基、碳环基、碳环基烷基、芳基、芳烷基、杂环基、杂环基烷基、杂芳基或杂芳基烷基。

[0211] “烯基”是指仅由碳和氢原子组成、包含至少一个双键并且具有 2-12 个碳原子的直链或支链烃链基团。在某些实施方式中，烯基包含 2-8 个碳原子。在其他一些实施方式中，烯基包含 2-4 个碳原子。烯基通过单键连接在分子的其余部分上，例如，乙烯基 (ethenyl)（即，乙烯基 (vinyl)）、丙-1- 烯基（即，烯丙基）、丁-1- 烯基、戊-1- 烯基、戊-1, 4- 二烯基等。除非在说明书中另有特别说明，烯基基团任选地被如下取代基中的一种或多种所取代：卤代、氰基、硝基、氧代、硫代、三甲基硅烷基、 $-\text{OR}^a$ 、 $-\text{SR}^a$ 、 $-\text{OC}(\text{O})-\text{R}^a$ 、 $-\text{N}(\text{R}^a)_2$ 、 $-\text{C}(\text{O})\text{R}^a$ 、 $-\text{C}(\text{O})\text{OR}^a$ 、 $-\text{C}(\text{O})\text{N}(\text{R}^a)_2$ 、 $-\text{N}(\text{R}^a)\text{C}(\text{O})\text{OR}^a$ 、 $-\text{N}(\text{R}^a)\text{C}(\text{O})\text{R}^a$ 、 $-\text{N}(\text{R}^a)\text{S}(\text{O})_t\text{R}^a$ （其中， $t$  为 1 或 2）、 $-\text{S}(\text{O})_t\text{OR}^a$ （其中， $t$  为 1 或 2）和  $-\text{S}(\text{O})_t\text{N}(\text{R}^a)_2$ （其中， $t$  为 1 或 2），其中各  $\text{R}^a$  均独立地为氢、烷基、氟代烷基、碳环基、碳环基烷基、芳基、芳烷基、杂环基、杂环基烷基、杂芳基或杂芳基烷基。

[0212] “炔基”是指仅由碳和氢原子组成、包含至少一个三键并且具有2-12个碳原子的直链或支链烃链基团。在某些实施方式中，炔基包含2-8个碳原子。在其他一些实施方式中，炔基具有2-4个碳原子。炔基通过单键连接在分子的其余部分上，例如，乙炔基、丙炔基、丁炔基、戊炔基、己炔基等。除非在说明书中另有特别说明，炔基任选地被选自如下取代基中的一种或多种所取代：卤代、氰基、硝基、氧代、硫代、三甲基硅烷基、-OR<sup>a</sup>、-SR<sup>a</sup>、-OC(0)-R<sup>a</sup>、-N(R<sup>a</sup>)<sub>2</sub>、-C(0)R<sup>a</sup>、-C(0)OR<sup>a</sup>、-C(0)N(R<sup>a</sup>)<sub>2</sub>、-N(R<sup>a</sup>)C(0)OR<sup>a</sup>、-N(R<sup>a</sup>)C(0)R<sup>a</sup>、-N(R<sup>a</sup>)S(0)<sub>t</sub>R<sup>a</sup>（其中，t为1或2）、-S(0)<sub>t</sub>OR<sup>a</sup>（其中，t为1或2）和-S(0)<sub>t</sub>N(R<sup>a</sup>)<sub>2</sub>（其中，t为1或2），其中各R<sup>a</sup>均独立地为氢、烷基、氟代烷基、碳环基、碳环基烷基、芳基、芳烷基、杂环基、杂环基烷基、杂芳基或杂芳基烷基。

[0213] “亚烷基”或“亚烷基链”是指将分子的其余部分连接到一个基团上、仅由碳和氢组成、不包含不饱和部分并且具有1-12个碳原子的直链或支链二价烃链，例如，亚甲基、亚乙基、亚丙基、亚正丁基等。亚烷基链通过单键连接在分子的其余部分上并通过单键连接在所述基团上。亚烷基链连接到分子的其余部分上以及连接到所述基团上的连接点可以是通过该亚烷基链中的一个碳或通过该链内的任意两个碳。除非在说明书中另有特别说明，亚烷基链任选地被如下取代基中的一种或多种所取代：卤代、氰基、硝基、芳基、环烷基、杂环基、杂芳基、氧代、硫代、三甲基硅烷基、-OR<sup>a</sup>、-SR<sup>a</sup>、-OC(0)-R<sup>a</sup>、-N(R<sup>a</sup>)<sub>2</sub>、-C(0)R<sup>a</sup>、-C(0)OR<sup>a</sup>、-C(0)N(R<sup>a</sup>)<sub>2</sub>、-N(R<sup>a</sup>)C(0)OR<sup>a</sup>、-N(R<sup>a</sup>)C(0)R<sup>a</sup>、-N(R<sup>a</sup>)S(0)<sub>t</sub>R<sup>a</sup>（其中，t为1或2）、-S(0)<sub>t</sub>OR<sup>a</sup>（其中，t为1或2）和-S(0)<sub>t</sub>N(R<sup>a</sup>)<sub>2</sub>（其中，t为1或2），其中各R<sup>a</sup>均独立地为氢、烷基、氟代烷基、碳环基、碳环基烷基、芳基、芳烷基、杂环基、杂环基烷基、杂芳基或杂芳基烷基。

[0214] “亚烯基”或“亚烯基链”是指将分子的其余部分连接到一个基团上、仅由碳和氢组成、包含至少一个双键并具有2-12个碳原子的直链或支链的二价烃链，例如，亚乙烯基、亚丙烯基、亚正丁烯基等。亚烯基链通过双键或单键连接在分子的其余部分上，并通过双键或单键连接到所述基团上。亚烯基链连接到分子的其余部分上以及连接到所述基团上的连接点可以是通过一个碳或该链内的任意两个碳。除非在说明书中另有特别说明，亚烯基链任选地被如下取代基中的一种或多种所取代：卤代、氰基、硝基、芳基、环烷基、杂环基、杂芳基、氧代、硫代、三甲基硅烷基、-OR<sup>a</sup>、-SR<sup>a</sup>、-OC(0)-R<sup>a</sup>、-N(R<sup>a</sup>)<sub>2</sub>、-C(0)R<sup>a</sup>、-C(0)OR<sup>a</sup>、-C(0)N(R<sup>a</sup>)<sub>2</sub>、-N(R<sup>a</sup>)C(0)OR<sup>a</sup>、-N(R<sup>a</sup>)C(0)R<sup>a</sup>、-N(R<sup>a</sup>)S(0)<sub>t</sub>R<sup>a</sup>（其中，t为1或2）、-S(0)<sub>t</sub>OR<sup>a</sup>（其中，t为1或2）和-S(0)<sub>t</sub>N(R<sup>a</sup>)<sub>2</sub>（其中，t为1或2），其中各R<sup>a</sup>均独立地为氢、烷基、氟代烷基、环烷基、环烷基烷基、芳基（任选地被一个或多个卤代基团所取代）、芳烷基、杂环基、杂环基烷基、杂芳基或杂芳基烷基，并且除非另有说明，否则上述每个取代基为未取代的。

[0215] “芳基”是指通过从环碳原子中除去氢原子而由芳香族单环或多环烃环体系衍生的基团。芳香族单环或多环烃环体系仅包含氢和6-18个碳原子的碳，其中，在环系中的至少一个环为完全不饱和的，即，其根据休克尔(Hückel)理论包含环状、离域的(4n+2)π-电子体系。芳基包括但不限于例如苯基、芴基和萘基的基团。除非在说明书中另有特别说明，术语“芳基”或前缀“芳-”（例如“芳烷基”）意在包括任选地被独立地选自如下基团的一种或多种取代基所取代的芳基：烷基、烯基、炔基、卤代、氟代烷基、氰基、硝基、任选地取代的芳基、任选地取代的芳烷基、任选地取代的芳烯基、任选地取代的芳炔基、任选地取代的碳环基、任选地取代的碳环基烷基、任选地取代的杂环基、任选地取代的杂环基烷基、任选地取代的杂芳基、任选地取代的杂芳基烷基、-R<sup>b</sup>-OR<sup>a</sup>、-R<sup>b</sup>-OC(0)-R<sup>a</sup>、-R<sup>b</sup>-N(R<sup>a</sup>)<sub>2</sub>、-R<sup>b</sup>-C(

0)  $R^a$ 、 $-R^b-C(O)OR^a$ 、 $-R^b-C(O)N(R^a)_2$ 、 $-R^b-O-R^c-C(O)N(R^a)_2$ 、 $-R^b-N(R^a)C(O)OR^a$ 、 $-R^b-N(R^a)C(O)R^a$ 、 $-R^b-N(R^a)S(O)_tR^a$  (其中,  $t$  为 1 或 2)、 $-R^b-S(O)_tOR^a$  (其中,  $t$  为 1 或 2) 以及  $-R^b-S(O)_tN(R^a)_2$  (其中,  $t$  为 1 或 2), 其中, 各  $R^a$  均独立地为氢、烷基、氟代烷基、环烷基、环烷基烷基、芳基 (任选地被一个或多个卤代基团取代)、芳烷基、杂环基、杂环基烷基、杂芳基或杂芳基烷基, 各  $R^b$  均独立地为直接键, 或者直链或支链亚烷基或亚烯基链, 且  $R^c$  为直链或支链亚烷基或亚烯基链, 并且其中, 除非另有说明, 否则上述每个取代基为未取代的。

[0216] “芳烷基”是指式  $-R^c-$  芳基的基团, 其中  $R^c$  为如上所定义的亚烷基链, 例如, 苄基、二苯基甲基等。芳烷基的亚烷基链部分如以上对于亚烷基链所述任选地被取代。芳烷基的芳基部分如以上对于芳基所述任选地被取代。

[0217] “芳烯基”是指式  $-R^d-$  芳基的基团, 其中,  $R^d$  为如上所定义的亚烯基链。芳烯基的芳基部分如以上对于芳基所述任选地被取代。芳烯基的亚烯基链部分如以上对于亚烯基所述任选地被取代。

[0218] “芳炔基”是指式  $-R^e-$  芳基的基团, 其中,  $R^e$  为如上所定义的亚炔基链。芳炔基的芳基部分如以上对于芳基所述任选地被取代。芳炔基的亚炔基链部分如以上对于亚炔基链所述任选地被取代。

[0219] “碳环基”是指仅由碳和氢原子组成的稳定的非芳香族单环或多环烃基, 其可包括稠环或桥环体系、含有 3-15 个碳原子的。在某些实施方式中, 碳环基包含 3-10 个碳原子。在其他一些实施方式中, 碳环基包含 5-7 个碳原子。碳环基通过单键连接在分子的其余部分上。碳环基可以是饱和的 (即, 仅包含 C-C 单键) 或者是不饱和的 (即, 包含一个或多个双键或三键)。完全饱和的碳环基也称作“环烷基”。单环环烷基的实例包括, 例如, 环丙基、环丁基、环戊基、环己基、环庚基和环辛基。不饱和的碳环基也称作“环烯基”。单环环烯基的实例包括, 例如环戊烯基、环己烯基、环庚烯基和环辛烯基。多环碳环基团包括, 例如, 金刚烷基、降冰片烷基 (即, 双环 [2.2.1] 庚烷基)、降冰片烯基、十氢化萘基、7,7-二甲基-双环 [2.2.1] 庚烷基等。除非在说明书中另有特别说明, 术语“碳环基”意在包括任选地被独立地选自如下基团中的一种或多种取代基所取代的碳环基: 烷基、烯基、炔基、卤代、氟代烷基、氧代、硫代、氰基、硝基、任选地取代的芳基、任选地取代的芳烷基、任选地取代的芳烯基、任选地取代的芳炔基、任选地取代的碳环基、任选地取代的碳环基烷基、任选地取代的杂环基、任选地取代的杂环基烷基、任选地取代的杂芳基、任选地取代的杂芳基烷基、 $-R^b-OR^a$ 、 $-R^b-SR^a$ 、 $-R^b-OC(O)-R^a$ 、 $-R^b-N(R^a)_2$ 、 $-R^b-C(O)R^a$ 、 $-R^b-C(O)OR^a$ 、 $-R^b-C(O)N(R^a)_2$ 、 $-R^b-O-R^c-C(O)N(R^a)_2$ 、 $-R^b-N(R^a)C(O)OR^a$ 、 $-R^b-N(R^a)C(O)R^a$ 、 $-R^b-N(R^a)S(O)_tR^a$  (其中,  $t$  为 1 或 2)、 $-R^b-S(O)_tOR^a$  (其中,  $t$  为 1 或 2) 以及  $-R^b-S(O)_tN(R^a)_2$  (其中,  $t$  为 1 或 2), 其中, 各  $R^a$  均独立地为氢、烷基、氟代烷基、环烷基、环烷基烷基、芳基、芳烷基、杂环基、杂环基烷基、杂芳基或杂芳基烷基, 各  $R^b$  均独立地为直接键或者直链或支链亚烷基或亚烯基链, 且  $R^c$  为直链或支链亚烷基或亚烯基链, 并且除非另有说明, 上述每个取代基为未取代的。

[0220] “碳环基烷基”是指式  $-R^c-$  碳环基的基团, 其中,  $R^c$  为如上所定义的亚烷基链。亚烷基链和碳环基如上所定义地任选地被取代。

[0221] “卤代”或“卤素”是指溴、氯、氟或碘取代基。

[0222] “氟代烷基”是指被一个或多个如上所定义的氟基所取代的如上所定义的烷基, 例如, 三氟甲基、二氟甲基、2,2,2-三氟乙基、1-氟甲基-2-氟乙基等。氟代烷基的烷基部分

如以上对于烷基所定义地可任选地被取代。

[0223] “杂环基”是指包含 2-12 个碳原子和 1-6 个选自氮、氧和硫的杂原子的稳定的 3 元至 18 元非芳香环基团。除非在说明书中另有特别说明,杂环基为单环、双环、三环或四环环系,其可包括稠环或桥环体系。在杂环基中的杂原子可任选地被氧化。如果存在一个或多个氮原子,其任选地被季铵化。杂环基为部分或者完全饱和的。杂环基通过环上的任意原子可连接在分子的其余部分上。这样的杂环基的实例包括但不限于,二氧杂环戊烷基、噻吩基 [1, 3] 二噻烷基 (thienyl[1, 3]dithianyl)、十氢异喹啉基、咪唑啉基、咪唑烷基、异噻唑烷基、异噁唑烷基、吗啉基、八氢吲哚基、八氢异吲哚基、2- 氧代哌嗪基、2- 氧代哌啶基、2- 氧代吡咯烷基、噁唑烷基、哌啶基、哌嗪基、4- 哌啶酮基、吡咯烷基、吡唑烷基、奎宁环基、噻唑烷基、四氢呋喃基、三噻烷基、四氢吡喃基、硫代吗啉基 (thiomorpholiny1)、硫杂吗啉基 (thiamorpholiny1)、1- 氧代- 硫代吗啉基和 1, 1- 二氧代- 硫代吗啉基。除非在说明书中另有特别说明,术语“杂环基”意在包括任选地被选自如下基团的一种或多种取代基所取代的如上所定义的杂环基:烷基、烯基、炔基、卤代、氟代烷基、氧代、硫代、氰基、硝基、任选地取代的芳基、任选地取代的芳烷基、任选地取代的芳烯基、任选地取代的芳炔基、任选地取代的碳环基、任选地取代的碳环基烷基、任选地取代的杂环基、任选地取代的杂环基烷基、任选地取代的杂芳基、任选地取代的杂芳基烷基、-R<sup>b</sup>-OR<sup>a</sup>、-R<sup>b</sup>-SR<sup>a</sup>、-R<sup>b</sup>-OC(0)-R<sup>a</sup>、-R<sup>b</sup>-N(R<sup>a</sup>)<sub>2</sub>、-R<sup>b</sup>-C(0)R<sup>a</sup>、-R<sup>b</sup>-C(0)OR<sup>a</sup>、-R<sup>b</sup>-C(0)N(R<sup>a</sup>)<sub>2</sub>、-R<sup>b</sup>-O-R<sup>c</sup>-C(0)N(R<sup>a</sup>)<sub>2</sub>、-R<sup>b</sup>-N(R<sup>a</sup>)C(0)OR<sup>a</sup>、-R<sup>b</sup>-N(R<sup>a</sup>)C(0)R<sup>a</sup>、-R<sup>b</sup>-N(R<sup>a</sup>)S(0)<sub>t</sub>R<sup>a</sup> (其中, t 为 1 或 2)、-R<sup>b</sup>-S(0)<sub>t</sub>OR<sup>a</sup> (其中, t 为 1 或 2) 和 -R<sup>b</sup>-S(0)<sub>t</sub>N(R<sup>a</sup>)<sub>2</sub> (其中, t 为 1 为 2),其中,各 R<sup>a</sup> 均独立地为氢、烷基、氟代烷基、环烷基、环烷基烷基、芳基、芳烷基、杂环基、杂环基烷基、杂芳基或杂芳基烷基,各 R<sup>b</sup> 独立地为直接键或者直链或支链亚烷基或亚烯基链,并且 R<sup>c</sup> 为直链或支链亚烷基或亚烯基链,并且除非另有说明,上述各个取代基为未取代的。

[0224] “N- 杂环基”或“N- 连接的杂环基”是指包含至少一个氮的如上所定义的杂环基,并且其中杂环基连接到分子的其余部分上的连接点是通过杂环基中的氮原子。N- 杂环基如以上对于杂环基所述任选地被取代。这样的 N- 杂环基基团的实例包括但不限于,1- 吗啉基、1- 哌啶基、1- 哌嗪基、1- 吡咯烷基、吡唑烷基、咪唑啉基和咪唑烷基。

[0225] “C- 杂环基”或“C- 连接的杂环基”是指包含至少一个杂原子的如上所定义的杂环基,并且其中杂环基连接到分子的其余部分上的连接点是通过杂环基中的碳原子。C- 杂环基如以上对于杂环基所述任选地被取代。这样的 C- 杂环基的实例包括但不限于,2- 吗啉基、2- 或 3- 或 4- 哌啶基、2- 哌嗪基、2- 或 3- 吡咯烷基等。

[0226] “杂环基烷基”是指式 -R<sup>c</sup>- 杂环基的基团,其中, R<sup>c</sup> 为如上所定义的亚烷基链。如果杂环基为含氮的杂环基,则杂环基任选地在氮原子上连接到烷基基团上。杂环基烷基基团的亚烷基链如以上对于亚烷基链所定义地任选地被取代。杂环基烷基基团的杂环基部分如以上对于杂环基所定义地任选地被取代。

[0227] “杂芳基”是指由包含 2-17 个碳原子和 1-6 个选自氮、氧和硫的杂原子的 3 元至 18 元芳香环基团所衍生的基团。如本文所使用的,杂芳基可为单环、双环、三环或四环环系,其中,环系中的至少一个环为完全不饱和的,即,其根据休克尔理论包含环状、离域的 (4n+2)  $\pi$  - 电子体系。杂芳基包括稠环或桥环体系。杂芳基基团中的杂原子任选地被氧化。如果存在一个或多个氮原子,其任选地被季铵化。杂芳基通过环上任意原子连接到

分子的其余部分上。杂芳基的实例包括但不限于，氮杂䓬基 (azepinyl)、吖啶基、苯并咪唑基、苯并吲哚基、1, 3- 苯并二氧杂环戊基、苯并呋喃基、苯并噁唑基、苯并 [d] 嘧唑基、苯并噻二唑基、苯并 [b] [1, 4] 二氧杂䓬基、苯并 [b] [1, 4] 噁嗪基、1, 4- 苯并二噁烷基、苯并萘并呋喃基、苯并噁唑基、苯并二氧杂环戊基、苯并二氧杂环己基、苯并吡喃基、苯并吡喃酮基、苯并呋喃基、苯并呋喃酮基、苯并噻吩基 (benzothiophenyl)、苯并噻吩并 [3, 2-d] 噻啶基、苯并三唑基、苯并 [4, 6] 咪唑并 [1, 2-a] 吡啶基、咔唑基、噌啉基、环戊并 [d] 噻啶基、6, 7- 二氢 -5H- 环戊并 [4, 5] 噢吩并 [2, 3-d] 噢啶基、5, 6- 二氢苯并 [h] 噩唑啉基、5, 6- 二氢苯并 [h] 噌啉基、6, 7- 二氢 -5H- 苯并 [6, 7] 环庚并 [1, 2-c] 哒嗪基、二苯并呋喃基、二苯并噻吩基、呋喃基、呋喃酮基、呋喃并 [3, 2-c] 吡啶基、5, 6, 7, 8, 9, 10- 六氢环辛并 [d] 噢啶基、5, 6, 7, 8, 9, 10- 六氢环辛并 [d] 吡啶基、异噁唑基、咪唑基、吲唑基、吲哚基、异吲哚基、二氢吲哚基、异二氢吲哚基、异喹啉基、吲嗪基、异噁唑基、5, 8- 甲桥 -5, 6, 7, 8- 四氢喹唑啉基、萘啶基、1, 6- 萘啶酮基 (1, 6-naphthyridinonyl)、噁二唑基、2- 氧氮杂䓬基、噁唑基、环氧乙烷基、5, 6, 6a, 7, 8, 9, 10, 10a- 八氢苯并 [h] 噬唑啉基、1- 苯基 -1H- 吡咯基、吩嗪基、吩噁嗪基、吩噁嗪基、酞嗪基、蝶啶基、嘌呤基、吡咯基、吡唑基、吡唑并 [3, 4-d] 噢啶基、吡啶基、吡啶并 [3, 2-d] 噢啶基、吡啶并 [3, 4-d] 噢啶基、吡嗪基、噁啶基、哒嗪基、吡咯基、噓唑啉基、噓啉基、异噓啉基、四氢噓啉基、5, 6, 7, 8- 四氢噓啉基、5, 6, 7, 8- 四氢苯并 [4, 5] 噢吩并 [2, 3-d] 噢啶基、6, 7, 8, 9- 四氢 -5H- 环庚并 [4, 5] 噢吩并 [2, 3-d] 噢啶基、5, 6, 7, 8- 四氢吡啶并 [4, 5-c] 哒嗪基、噁唑基、噁二唑基、三唑基、四唑基、三嗪基、噁吩并 [2, 3-d] 噢啶基、噁吩并 [3, 2-d] 噢啶基、噁吩并 [2, 3-c] 吡啶基和噁吩基 (即，噁吩基 (thienyl))。除非在说明书中另有特别说明，术语“杂芳基”意在包括任选地被选自如下基团的一种或多种取代基所取代的如上所定义的杂芳基基团：烷基、烯基、炔基、卤代、氟代烷基、卤代烯基、卤代炔基、氧代、硫代、氰基、硝基、任选地取代的芳基、任选地取代的芳烷基、任选地取代的芳烯基、任选地取代的芳炔基、任选地取代的碳环基、任选地取代的碳环基烷基、任选地取代的杂环基、任选地取代的杂环基烷基、任选地取代的杂芳基、任选地取代的杂芳基烷基、 $-R^b-OR^a$ 、 $-R^b-SR^a$ 、 $-R^b-OC(O)-R^a$ 、 $-R^b-N(R^a)_2$ 、 $-R^b-C(O)R^a$ 、 $-R^b-C(O)OR^a$ 、 $-R^b-C(O)N(R^a)_2$ 、 $-R^b-O-R^c-C(O)N(R^a)_2$ 、 $-R^b-N(R^a)C(O)OR^a$ 、 $-R^b-N(R^a)C(O)R^a$ 、 $-R^b-N(R^a)S(O)_tR^a$  (其中， $t$  为 1 或 2)、 $-R^b-S(O)_tOR^a$  (其中， $t$  为 1 或 2) 和  $-R^b-S(O)_tN(R^a)_2$  (其中， $t$  为 1 为 2)，其中，各  $R^a$  均独立地为氢、烷基、氟代烷基、环烷基、环烷基烷基、芳基、芳烷基、杂环基、杂环基烷基、杂芳基或杂芳基烷基，各  $R^b$  均独立地为直接键或者直链或支链亚烷基或亚烯基链，且  $R^c$  为直链或支链亚烷基或亚烯基链，并且除非另有说明，上述每个取代基为未取代的。

[0228] “N- 杂芳基”是指包含至少一个氮的如上所定义的杂芳基基团，并且其中杂芳基基团连接到分子的其余部分上的连接点是通过杂芳基基团中的氮原子。N- 杂芳基基团如以上对于杂芳基基团所述任选地被取代。

[0229] “C- 杂芳基”是指如上所定义的杂芳基，并且其中杂芳基基团连接到分子的其余部分上的连接点是通过杂芳基基团中的碳原子。C- 杂芳基如以上对于杂芳基基团所述任选地被取代。

[0230] “杂芳基烷基”是指式  $-R^c-$  杂芳基的基团，其中， $R^c$  为如上所定义的亚烷基链。如

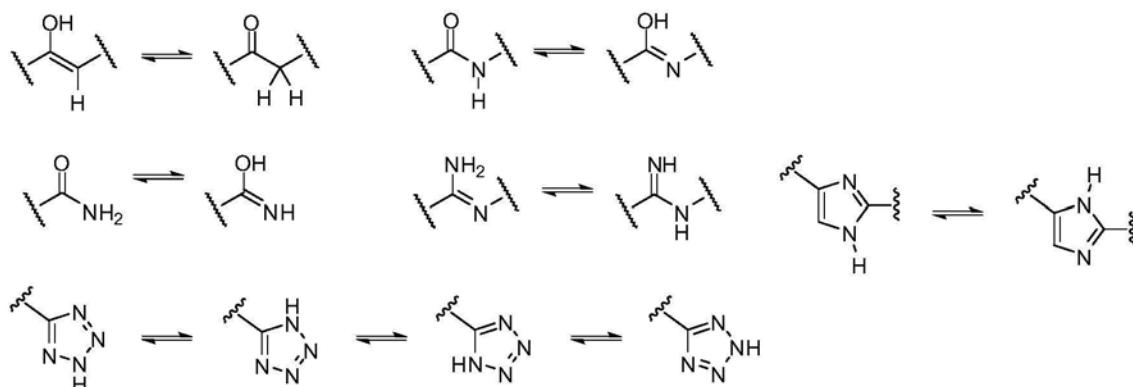
果杂芳基为含氮杂芳基，则该杂芳基任选地在氮原子处连接到烷基基团上。杂芳基烷基基团的亚烷基链如以上对于亚烷基链所定义地任选地被取代。杂芳基烷基基团的杂芳基部分如以上对于杂芳基基团所定义地任选地被取代。

[0231] 所述化合物或其药学上可接受的盐可包含一个或多个非对称中心，因而可以产生对映异构体、非对映体和其他立体异构体形式，其根据绝对立体化学可被定义为 (R)- 或 (S)-，或者对于氨基酸被定义为 (D)- 或 (L)-。当本文描述的化合物包含烯烃双键或其他几何非对称中心时，并且除非另有特别说明，化合物意欲包括 E 和 Z 几何异构体（例如，顺式或反式）。同样地，也意欲包括所有可能的异构体和它们的外消旋及光学纯形式，以及所有互变异构体形式。

[0232] “立体异构体”是指由通过相同键结合的相同原子组成但具有不同三维结构的化合物，这些结构不可互换。因此考虑了各种立体异构体及其混合物并且包括“对映异构体”，对映异构体是指其分子互为不可重叠 (nonsuperimposeable) 的镜像的两种立体异构体。

[0233] 本文提出的化合物可作为互变异构体存在。“互变异构体”是指质子从分子的一个原子转移到同一分子的另一个原子，伴随有相邻双键的异构化。在可能发生互变异构化的键合排列中，将存在互变异构体的化学平衡。本文公开的化合物的所有互变异构形式都被考虑在内。互变异构体的确切比例取决于几个因素，包括温度、溶剂和 pH。互变异构互变的一些实例包括：

[0234]



[0235] “任选的”或“任选地”是指在其后描述的事件或情况可以发生或者可以不发生，并且该描述包括当事件或情况发生时的情形和不发生时的情形。例如，“任选地取代的芳基”是指芳基可以被取代或者可以不被取代，并且该描述既包括被取代的芳基也包括没有取代的芳基。

[0236] “药学上可接受的盐”既包括酸加成盐也包括碱加成盐。本文描述的任意一种取代的杂环胺衍生物化合物的药学上可接受的盐意在包括任意的和所有的药学上合适的盐形式。本文所述化合物的优选的药学上可接受的盐为药学上可接受的酸加成盐和药学上可接受的碱加成盐。

[0237] “药学上可接受的酸加成盐”是指保留了生物有效性和游离碱的性质的那些盐，其在生物学上或其他方面不是不合需要的，并且其是用例如盐酸、氢溴酸、硫酸、硝酸、磷酸、氢碘酸、氢氟酸、亚磷酸等无机酸形成的。也包括用如下有机酸形成的盐：例如脂肪族单羧酸和二羧酸、苯基取代的链烷酸、羟基链烷酸、链烷二酸、芳族酸、脂肪族和芳香族磺酸等，并且包括例如醋酸、三氟醋酸、丙酸、羟基乙酸、丙酮酸、草酸、马来酸、丙二酸、

琥珀酸、富马酸、酒石酸、柠檬酸、苯甲酸、肉桂酸、扁桃酸、甲磺酸、乙磺酸、对甲苯磺酸、水杨酸等。因此,示例性的盐包括硫酸盐、焦硫酸盐、硫酸氢盐、亚硫酸盐、亚硫酸氢盐、硝酸盐、磷酸盐、磷酸单氢盐、磷酸二氢盐、偏磷酸盐、焦磷酸盐、氯化物、溴化物、碘化物、醋酸盐、三氟醋酸盐、丙酸盐、辛酸盐、异丁酸盐、草酸盐、丙二酸盐、琥珀酸盐、辛二酸盐、癸二酸盐、富马酸盐、马来酸盐、扁桃酸盐、苯甲酸盐、氯苯甲酸盐、甲基苯甲酸盐、二硝基苯甲酸盐、邻苯二甲酸盐、苯磺酸盐、甲苯磺酸盐、苯基醋酸盐、柠檬酸盐、乳酸盐、苹果酸盐、酒石酸盐、甲磺酸盐等。还考虑到氨基酸的盐如精氨酸盐、葡糖酸盐和半乳糖醛酸盐(参见,例如,Berge S. M. 等,"Pharmaceutical Salts,"Journal of Pharmaceutical Science, 66:1-19(1997),其通过引用整体并入本文)。根据本领域熟练技术人员所熟知的方法和技术,通过使游离碱形式与足量的所需酸接触以产生盐,可制备碱性化合物的酸加成盐。

[0238] "药学上可接受的碱加成盐"是指保留生物有效性和游离酸的性质的那些盐,其在生物学上或其他方面不是不合需要的。这些盐是通过向游离酸中加入无机碱或有机碱而制备的。药学上可接受的碱加成盐可以用金属或胺(如碱金属和碱土金属或有机胺)来形成。源于无机碱的盐包括但不限于,钠、钾、锂、铵、钙、镁、铁、锌、铜、锰、铝的盐等。源于有机碱的盐包括但不限于下列有机碱的盐:伯胺、仲胺和叔胺;取代的胺(包括天然存在的取代的胺);环胺和碱离子交换树脂,例如,异丙胺、三甲胺、二乙胺、三乙胺、三丙胺、乙醇胺、二乙醇胺、2-二甲基氨基乙醇、2-二乙基氨基乙醇、二环己基胺、赖氨酸、精氨酸、组氨酸、咖啡因、普鲁卡因、N,N-二苄基乙二胺、氯普鲁卡因、海巴明(hydramine)、胆碱、甜菜碱、乙二胺、亚乙基二苯胺、N-甲基葡萄糖胺、葡萄糖胺、甲基葡萄糖胺、可可碱(theobromine)、嘌呤、哌嗪、哌啶、N-乙基哌啶、聚胺树脂等。参见Berge等,同上。

[0239] 本文使用的"治疗"或"处理"或"减轻"或"改善"在本文中可以互换使用。这些术语是指用于获得包括但不限于治疗益处和/或预防益处的有益或所需结果的方法。"治疗益处"意指使正在治疗的潜在病症的消除或改善。同样地,治疗益处是通过与潜在病症相关的一种或多种生理学症状的消除或改善来实现的,从而在患者身上观察到改善,即使该患者仍然遭受潜在病症的折磨。至于预防益处,可以将所述组合物施用于具有发展成特定疾病的风险的患者,或者报告疾病的一种或多种生理学症状的患者,即使可能还没有作出该疾病的诊断。

[0240] "前药"是指在生理条件下或通过溶剂分解可以转化为本文描述的生物活性化合物的化合物。因此,术语"前药"是指药学上可接受的生物活性化合物的前体。当施用于受试者时,前药可以是无活性的,但是在体内转化为活性化合物,例如,通过水解。前药化合物通常在哺乳动物生物体内提供溶解性、组织相容性或延迟释放的优点(参见,例如,Bundgaard, H., Design of Prodrugs(1985), pp. 7-9, 21-24(Elsevier, Amsterdam))。

[0241] 在Higuchi, T. 等,"Pro drugs as Novel Delivery Systems,"A. C. S. Symposium Series, Vol. 14 和 Bioreversible Carriers in Drug Design, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987 中均提供了对前药的论述,其全部内容均通过引用并入本文。

[0242] 术语"前药"也意在包括任何共价键合的载体,当将这样的前药施用于哺乳动物受试者时,其在体内释放活性化合物。本文所述的活性化合物的前药可通过以下方式修饰

存在于活性化合物中的官能团而制备：该方式使得该修饰以常规操作或者在体内被切割为母体活性化合物。前药包括其中羟基、氨基或巯基键合到任一基团上的化合物，当将活性化合物的前药施用于哺乳动物受试者时，该基团裂解以分别形成游离羟基、游离氨基或游离巯基。前药的实例包括但不限于，活性化合物中的醇或胺官能团的乙酸酯、甲酸酯和苯甲酸酯衍生物等。

[0243] 组合物及施用模式

[0244] 在一些实施方式中，本文所述的化合物当与可接受的载体或赋形剂组合时配制成为药学上可接受的组合物。

[0245] 因此，在一些实施方式中，组合物除活性成分外，还包含可接受的赋形剂、载体、缓冲剂、稳定剂或其他本领域中已知的供在施用于患者的组合物中使用的物质。这类物质为非毒性的且不干扰活性成分的效力。载体或其他物质的确切性质取决于施用途径。

[0246] 可接受的载体及其制剂描述于且通常描述于，例如 Remington' pharmaceutical Sciences (第 18 版, A. Gennaro 编, Mack Publishing Co., Easton, PA1990) 中。

[0247] 组合物被配制成为与心目中特定的施用途径相容。因此，组合物包含适合通过各种途径施用的载体、稀释剂或赋形剂。

[0248] 待施用的组合物的“治疗有效量”是预防、改善或治疗疾病或病症所需的最低量。组合物任选地与目前用于预防或治疗所讨论的病症的一种或多种试剂一起配制。这类其他试剂的有效量取决于该制剂中存在的化合物的量、病症或治疗的类型以及上文讨论的其他因素。这些通常以如上文所使用的相同剂量和施用途径使用，或按上文所使用的剂量的约 1-99% 使用。一般而言，疾病或病症的减轻或治疗涉及一种或多种与该疾病或病症相关的症状或医疗问题的减轻。

[0249] 本文所述的化合物以任何适合有效达到眼睛所需的治疗效果的方式施用。因此，施用方法包括但不限于局部、眼内（包括玻璃体内）、经皮、口服、静脉内、结膜下、视网膜下或腹膜施用途径。

[0250] 该化合物可采用的施用技术和方法是本领域中所熟知的且描述于本文中，例如在 Goodman 和 Gilman, The Pharmacological Basis of Therapeutics, 现行版本; Pergamon 和 Remington' s, Pharmaceutical Sciences (现行版本), Mack Publishing Co., Easton, Pa 中所讨论的。在某些实施方式中，本文所述的化合物及组合物通过口服施用。

[0251] 用于口服施用的液体制剂剂型可以是水性悬浮液，例如药学上可接受的水性口服分散液、乳剂、溶液、酏剂、凝胶及糖浆。参见，例如 Singh 等人，Encyclopedia of Pharmaceutical Technology, 2nd Ed., pp. 754-757 (2002)。除了该化合物外，液体剂型任选地包含适合于口服的药学上可接受的载体或赋形剂，及任选地，一种或多种添加剂，例如：(a) 崩解剂；(b) 分散剂；(c) 润湿剂；(d) 防腐剂；(e) 黏度增强剂；(f) 甜味剂；和 / 或 (g) 调味剂。在一些实施方式中，该水性分散液进一步包含晶体形成抑制剂。

[0252] 在一个实施方式中，可将乳化剂和 / 或悬浮剂以及稀释剂（例如水、乙醇、丙二醇、甘油及其各种组合）添加入组合物中。

[0253] 可加入水（例如 5%），作为模拟长期储存的手段，以确定例如该制剂随着时间推移的贮存期或稳定性的特性。无水组合物及剂型可使用无水或含低水分的成分及低水分或低湿度条件来制备。若预期在制造、包装和 / 或贮存期间会大量接触水分和 / 或湿度，则可

将含有乳糖的组合物和剂型制成无水的。可制备和贮存无水组合物,以使得保持其无水性质。因此,可使用已知可防止暴露于水的材料来包装无水组合物,以使其能够包含在合适的处方试剂盒中。

[0254] 在额外或备选的实施方式中,该组合物可以为片剂、胶囊、丸剂、粉剂、持续释放制剂、溶液、悬浮液或乳剂的形式。

[0255] 用于口服施用的固体剂型包括,例如,但不限于胶囊、片剂、丸剂、粉剂和颗粒。

[0256] 在这类固体剂型中,本文所公开的组合物可与下列物质混合:至少一种惰性的、药学上可接受的赋形剂或载体,例如柠檬酸钠或磷酸二钙,和/或a)填充剂或增量剂,例如淀粉、乳糖、蔗糖、葡萄糖、甘露醇及硅酸;b)黏合剂,例如羧甲基纤维素、藻酸盐、明胶、聚乙烯吡咯烷酮、蔗糖及阿拉伯胶;c)湿润剂,例如甘油;d)崩解剂,例如琼脂、碳酸钙、马铃薯或木薯淀粉、藻酸、某些硅酸盐及碳酸钠;e)溶液阻滞剂,例如石蜡;f)吸收促进剂,例如季铵化合物;g)润湿剂,例如鲸蜡醇及单硬脂酸甘油脂;h)吸收剂,例如高岭土和膨润土黏土;及i)润滑剂,例如滑石、硬脂酸钙、硬脂酸镁、固体聚乙二醇、月桂基硫酸钠及其混合物。在胶囊、片剂和丸剂的情况下,该剂型也可包含缓冲剂。

[0257] 类似类型的固体组合物也可用作软和硬填充明胶胶囊中的填充剂,其中使用例赋形剂(如乳糖或奶糖以及高分子量聚乙二醇等)。活性组分也可以是适当时具有一种或多种上述赋形剂的微包封形式。在制备本文中所公开的用于口服的剂量单位形式的药学制剂时,选定的化合物可与固态粉末成分(例如乳糖、蔗糖、山梨糖醇、甘露糖醇、淀粉、支链淀粉、纤维素衍生物、明胶或另外合适的成分)以及崩解剂和润滑剂(如硬脂酸镁、硬脂酸钙、硬脂酰富马酸钠及聚乙二醇蜡)混合。然后将该混合物加工成颗粒或压制成片剂。

[0258] 组合物可以是适合于单次施用准确剂量的单位剂型。在进一步或另外的实施方式中,化合物的量在约0.001至约1000mg/kg体重/天的范围内。在进一步或另外的实施方式中,化合物的量在约0.5至约50mg/kg/天的范围内。在进一步或另外的实施方式中,化合物的量为约0.001至约7g/天。在进一步或另外的实施方式中,化合物的量为约0.002至约6g/天。在进一步或另外的实施方式中,化合物的量为约0.005至约5g/天。在进一步或另外的实施方式中,化合物的量为约0.01至约5g/天。在进一步或另外的实施方式中,化合物的量为约0.05至约2.5g/天。在进一步或另外的实施方式中,化合物的量为约0.1至约1g/天。在一些实施方式中,低于上述范围的下限的剂量水平可能绰绰有余。在其他实施方式中,高于上述范围的上限的剂量水平可能是必要的。

[0259] 在一个方面,(R)-3-氨基-1-(3-(环己基甲氧基)苯基)丙-1-醇的每日剂量为约4mg至约100mg。在另一个方面,(R)-3-氨基-1-(3-(环己基甲氧基)苯基)丙-1-醇的每日剂量为约2mg、约5mg、约7mg、约10mg、约15mg、约20mg、约40mg、约60mg、约75mg或约100mg。

[0260] 在一些实施方式中,用于口服递送的组合物包含至少约1、5、10、20、30、40、50、60、70、80、90、95、99、99.5、99.9或99.99%的本文所述化合物。在其他实施方式中,用于口服递送的组合物包含不超过约2、5、10、20、30、40、50、60、70、80、90、95、99、99.5或100%的本文所述化合物。在一些实施方式中,组合物含有约1-100%、约10-100%、约20-100%、约50-100%、约80-100%、约90-100%、约95-100%或约99-100%的本文所述化合物。在

一些实施方式中,组合物含有约 1-90%、约 10-90%、约 20-90%、约 50-90% 或约 80-90% 的本文所述化合物。在一些实施方式中,组合物含有约 1-75%、约 10-75%、约 20-75% 或约 50-75% 的本文所述化合物。在一些实施方式中,组合物含有约 1-50%、约 10-50%、约 20-50%、约 30-50% 或约 40-50% 的本文所述化合物。在一些实施方式中,组合物含有约 1-40%、约 10-40%、约 20-40% 或约 30-40% 的本文所述化合物。在一些实施方式中,组合物含有约 1-30%、约 10-30% 或约 20-30% 的本文所述化合物。在一些实施方式中,组合物含有约 1-20% 或约 10-20% 的本文所述化合物。在一些实施方式中,组合物含有约 1-10% 的本文所述化合物。

[0261] 治疗方法

[0262] 本申请中提供了通过向患者施用治疗有效量的本文所提供的组合物来治疗患者的糖尿病性视网膜病(缓解一种或多种症状,或停止一种或多种症状)的方法。该治疗可改善患者的病况并且可通过确定是否发生下列一种或多种因素来评估:黄斑水肿减少或视敏度增加。本文所述的化合物也可用于治疗糖尿病性视网膜病的药物中。

[0263] “患者”为表现出本文所述的疾病或病症的一种或多种临床显现和/或症状的哺乳动物。患者的非限制性实例包括但不限于人类或非人类动物,例如灵长类动物、啮齿动物、牛、马、猪、绵羊等。在某些情况中,患者可能无症状,但仍具有该疾病或病症的临床显现。在一个实施方式中,待治疗的患者是人。

[0264] 根据患者的健康、疾病或病况的进展及治疗的疗效,可施用本文所提供的组合物一次或多次。在整个治疗过程中可以进行疗法和治疗的调整。

[0265] 糖尿病性视网膜病的迹象和症状包括但不限于下列的一项或多项:血管的变化;视网膜肿胀(黄斑水肿);视网膜上的灰白色沉积物;受损的神经组织;渗漏血管的视觉外观;中心或周边视力丧失;暂时或永久性视力丧失;斑点、模糊、朦胧或复视;眼痛;飞蚊症;色觉受损;视力丧失;中心视力中的暗点或盲点;静脉扩张和视网膜内微血管异常;神经病;视力波动及进行性恶化;黄斑水肿;黄斑缺血;牵引性视网膜脱离;内皮细胞增殖;闪光幻觉;虹膜发红或虹膜新血管生成;微动脉瘤;硬性渗出物;出血;及棉絮斑;是糖尿病性视网膜病的症状。

[0266] 在一个实施方式中,以本文所述化合物治疗 DR 可阻滞异常血管的形成、减缓从血管的渗漏、减少视网膜肿胀、防止视网膜脱离、防止或减缓失明和/或减少视力丧失。

[0267] 将在这类方法中施用的化合物通过任何合适的方式(如本文所述及本领域已知的方式)施用。

[0268] 对于疾病的预防或治疗,化合物的适当剂量将部分取决于待治疗的患者、疾病的严重程度和病程、施用该化合物是用于预防还是治疗目的、先前疗法、患者的临床病史和对该化合物的反应,以及主治医师的裁量。该化合物适当地一次或经一系列治疗施用于患者。

[0269] 该组合物可以与剂量制剂相容的方式并以治疗有效量来施用。欲施用的量取决于待治疗的受试者、患者的免疫系统利用该活性成分的能力。需要施用的活性成分的精确量取决于医生的判断并且是每个个体所特有的。初次施用及加强注射的合适方案也可改变。根据疾病的类型和严重程度,施用于患者的初始候选剂量为约 0.1  $\mu$ g/kg 至约 150mg/kg 的化合物,无论是例如通过一次或多次分开的施用,还是通过连续输注。其他初始剂量包括但不限于约 0.25  $\mu$ g/kg、约 0.5  $\mu$ g/kg、约 1  $\mu$ g/kg、约 10  $\mu$ g/kg、约 50  $\mu$ g/kg、约 100  $\mu$ g/kg、

约 250  $\mu$  g/kg、约 500  $\mu$  g/kg、约 750  $\mu$  g/kg、约 1mg/kg、约 5mg/kg、约 10mg/kg、约 15mg/kg、约 20mg/kg、约 25mg/kg、约 50mg/kg、约 75mg/kg、约 100mg/kg、约 125mg/kg、约 150mg/kg 或更多。之后，根据上述因素，典型的每日剂量可以是约 0.1  $\mu$  g/kg 至约 150mg/kg 或更多。对于在数天或更长时间内的反复施用，根据状况，治疗可持续到发生所需的疾病症状抑制。然而，其他剂量方案可能是有用的。剂量可以每天、每隔一天、每周、每月或每隔一月施用一次。此外，化合物的剂量可以每周施用两次、每周、每两周、每三周、每四周、每六周、每八周、每十二周施用一次，或以其中周数的任何组合施用。也考虑给药周期，例如每周施用化合物一次或两次，共 4 周，之后两周不给予治疗。也考虑额外的给药周期，包括，例如本文所述的剂量及每周周期的不同组合。治疗期间可评估一种或多种症状并相应地调整剂量。剂量可通过口服和 / 或玻璃体内施用。

[0270] 组合物可根据欲治疗的病况而单独施用或与第二治疗联合同时或顺次施用。当施用两种或更多种组合物或组合物和治疗时，这些组合物或组合物 / 治疗可联合施用（顺次或同时）。组合物可以单一剂量或多剂量施用。

[0271] 术语“单位剂量”当用于组合物时，是指适合作为人用单位剂量的物理上分离的单位，各单位含有预定量的活性物质，该预定量经计算为与所需稀释剂（即，载体或赋形剂）结合产生所需的治疗效果。

[0272] 根据疾病的类型和严重程度，用于施用于患者的初始候选剂量为约 0.1  $\mu$  g/kg 至约 150mg/kg 的化合物，无论是例如通过一次或多次分开的施用，还是通过连续输注。其他初始剂量包括但不限于约 0.25  $\mu$  g/kg、约 0.5  $\mu$  g/kg、约 1  $\mu$  g/kg、约 10  $\mu$  g/kg、约 50  $\mu$  g/kg、约 100  $\mu$  g/kg、约 250  $\mu$  g/kg、约 500  $\mu$  g/kg、约 750  $\mu$  g/kg、约 1mg/kg、约 5mg/kg、约 10mg/kg、约 15mg/kg、约 20mg/kg、约 25mg/kg、约 50mg/kg、约 75mg/kg、约 100mg/kg、约 125mg/kg、约 150mg/kg 或更多。之后，根据上述因素，典型的每日剂量可在约 0.1  $\mu$  g/kg 至约 150mg/kg 或更多的范围内。对于在数天或更长时间内的反复施用，根据状况，治疗可持续至发生所需的疾病症状抑制。然而，其他剂量方案可能是有用的。

[0273] 在一个实施方式中，如本文所述的患有年龄相关性黄斑变性、脉络膜新血管形成和 / 或糖尿病性视网膜病的患者的治疗包括至少一种本文所述症状的改善。改善包括，例如本文所描述的一种或多种迹象或症状的 2%、5%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95% 或 100% 的改善。组合物可以有效产生一些期望疗效的治疗有效量，以适用于任何医学治疗的合理的利益 / 风险比施用于患者。为了将本发明组合物施用于人类患者，该组合物可通过本领域普通技术人员已知的方法配制。

[0274] 本文所使用的术语“治疗”是指治疗性治疗及预防措施两者。需要治疗者包括那些已经患有病症以及那些要防止病症恶化的患者。在一个实施方式中，如本文所述的糖尿病性视网膜病患者的治疗是指一种或多种迹象或症状未恶化或进展。在另一个实施方式中，如本文所述的年龄相关性黄斑变性和 / 或脉络膜新血管形成患者的治疗是指一种或多种迹象或症状未恶化或进展。如本文所使用的“预防”是指糖尿病性视网膜病、年龄相关性黄斑变性和 / 或脉络膜新血管形成的症状发作的预防、防止，其一种或多种迹象或症状的进展的防止。如本文所使用的“抑制”、“治疗”和“处理”是指，例如症状停滞、存活延长、症状的部分或完全改善。

[0275] “施用”在本文中定义为以导致组合物在患者身体中的方式向患者提供组合物的方式。这样的施用可通过任何途径进行,包括但不限于本文所述或本领域中所熟知的施用模式。“共同施用”是指彼此在相对较短的期间内施用;这样的期间可少于2周、少于7天、少于1天,甚至可以同时施用。

[0276] 该组合物中的活性成分的实际剂量水平可以改变,以取得对于特定患者、组合物及施用模式有效达到所需的治疗反应,且对患者没有毒性的活性成分量。所选择的剂量水平将取决于多种因素,包括所使用的特定化合物的活性,施用途径,施用时间,所使用的特定化合物的排泄速率,治疗的持续时间,与所使用的特定化合物联合使用的其他药物、化合物和/或物质,接受治疗的患者的年龄、性别、体重、状况、一般健康和先前医疗史,及医学领域中所熟知的类似因素。

[0277] 在一个实施方式中,该化合物可以单一剂量施用,每天一次。在其他实施方式中,该化合物可以多剂量施用,多于每天一次。在其他实施方式中,该化合物可以每天施用两次。在其他实施方式中,该化合物可以每天施用三次。在其他实施方式中,该化合物可以每天施用四次。在其他实施方式中,该化合物可以每天施用多于四次。

[0278] 当患者经历疾病的部分或完全缓解,或疾病迹象或症状的减少(具体地包括但不限于存活期延长)时,实现应答。根据预后因素(包括复发次数、病期以及其他因素)预期的无进展存活时间可以数个月至数年测量。延长的存活期包括但不限于至少1个月(mo)、约为至少2个月(mos.)、约为至少3个月、约为至少4个月、约为至少6个月、约为至少1年、约为至少2年、约为至少3年或者更久的时间。总存活期也可以数个月至数年测量。患者的症状可以保持停滞或可能减少。

[0279] 具有本领域一般技术的医师或兽医可以容易地决定并开出所需的组合物的有效量( $ED_{50}$ )。例如,医师或兽医可在组合物中所使用的化合物剂量处于低于欲达到理想疗效所需的水平开始,再逐渐增加剂量,直到达到所需的效果。或者,剂量可以保持不变。

[0280] 这类成分的毒性和疗效可通过标准的制药程序在细胞培养或实验动物中测定,例如用于测定 $LD_{50}$ (使群体中的50%致死的剂量)和 $ED_{50}$ (在群体的50%中治疗有效的剂量)的程序。毒性和疗效之间的剂量比为治疗指数且其可以表示为 $LD_{50}/ED_{50}$ 比。虽然可使用表现出毒性副作用的化合物,但应小心设计将这类化合物靶向至受影响组织的部位的递送系统,以使对健康细胞的潜在伤害最小化,从而减少副作用。

[0281] 本文还提供了通过施用含有本文所述化合物的组合物来治疗有需要的患者中的早产儿视网膜病(ROP)的方法。

[0282] 本申请中提供了治疗或预防早产儿视网膜病的方法,其包括向有需要的患者施用包含视觉周期调节剂(VCM)化合物的组合物(正如本文所述的那些)。

[0283] 在一个实施方式中,该化合物改变视觉周期。欲以这类方法治疗的患者为早产儿。

[0284] 在另一个实施方式中,该患者另外以补充氧治疗。

[0285] 在另一个实施方式中,该治疗局部施用于眼睛或全身施用。

[0286] 本申请中提供了如本文所述的视觉周期调节剂在配制用于治疗早产儿视网膜病的药物中的用途。本文所述的治疗可由医生施用和监测。施用途径、剂量和疗效的具体测量可由施用医生选择,并且可取决于例如疾病的严重性、患者的年龄、体重和性别等因素以及其他因素,例如患者的其他医疗问题。

[0287] 任何给定组合物的效力也可使用实验动物模型,例如本文所述的 ROP 大鼠模型来测定。当使用实验动物模型时,在观察到 ROP 的标记或症状减少时可评估治疗的效力。

[0288] 施用的量和频率也将部分取决于组合物本身、其稳定性和比活性,以及施用途径。与区域性 / 局部性施用的组合物相比,较大量的组合物通常必须全身施用。

[0289] 眼睛提供非常适合局部施用许多药物的组织或结构。眼内注射及口服施用也可能是有效的。剂量将可能取决于施用途径,并且对于全身施用,将在例如约 0.1mg/kg 体重至约 0mg/kg 体重之间变化,对于局部或眼内注射途径,将在 0.01mg 至 10mg 之间变化。其他剂量也在本发明的考量内。

[0290] 欲施用的组合物的“治疗有效量”将取决于这类考虑因素,并且是预防、改善或治疗疾病或病症所需的最低量。该组合物不必,但可任选地与一种或多种目前用于预防或治疗所讨论的病症的试剂一起配制。这类其他试剂的有效量取决于在制剂中存在的化合物的量、病症或治疗的类型以及上文中讨论的其他因素。这些通常以如上使用的相同剂量和施用途径使用,或为上述所使用的剂量的约 1 至 99%。一般来说,疾病或病症的减轻或治疗涉及与该疾病或病症相关的一种或多种症状或医疗问题的减轻。

[0291] 一般而言,在本文所描述的方法中,如果 (a) 可测量的症状(例如,血管异常)与治疗开始前所测量的相比减少(例如至少 10%), (b) 该疾病的进展中止(例如患者不恶化或脉管系统停止病理性生长),或 (c) 症状减轻或甚至改善(例如,测得的血管数目或弯曲减少),则该化合物被确定为“治疗上有效的”。治疗的效力可由普通医师判断,或如本文所述及本领域已知的来判断。

[0292] 如本文所公开的组合物也可以预防或治疗有效量施用。预防或治疗有效量是指,至少部分地达到所需效果,或使正在接受治疗的特定疾病或病症的发病延缓、进展受抑制或完全停止所需的量。当然,这样的量将取决于正在接受治疗的特定病况、该病况的严重性及个体患者的参数,包括年龄、身体状况、尺寸、重量和同时进行的治疗。这些因素为本领域普通技术人员所熟知且仅仅通过常规实验即可得到解决。一般而言,优选使用最大剂量,也就是说,根据充分的医学判断的最高安全剂量。然而,本领域普通技术人员将会理解,出于医疗原因、心理原因或几乎任何其他原因可施用较低剂量或可耐受的剂量。

[0293] 本文所使用的“改善由视杆介导的视网膜功能”是指由视杆介导的视网膜功能增加至少 10%、至少 20%、至少 30%、至少 40%、至少 50%、至少 60%、至少 70%、至少 80%、至少 90%、至少 95%、至少 99%、至少 1 倍、至少 2 倍、至少 5 倍、至少 10 倍、至少 50 倍、至少 100 倍、至少 1000 倍或更高。

[0294] “由视杆介导的视网膜功能”是指在工作的视网膜中的视杆细胞的功能,且可包括例如周边视力程度、低水平明视力、暗视 /“夜视力”及对周围运动的敏感性的临床终点。由视杆介导的视网膜功能可通过下述方法在体内进行评估,例如:光转导的视杆活化或光转导的去活化的视网膜电流扫描计测量;光漂白后暗电流的恢复;ERG a 波或 b 波的测量;恢复光转导的速度;或由视杆介导的反应幅度。用于测量由视杆介导的视网膜功能的方法为本领域所知和 / 或在本文中更详细地解释。

[0295] 治疗的效力可由施用临床医师监测。当疾病或病症为早产儿视网膜病时,可适用国际视网膜病或早产分类 (International Classification of Retinopathy or Prematurity, ICROP)。ICROP 使用一系列参数对疾病进行分类。这些参数包括将疾病定位在

区域中（第1、2及3区）、根据1-12时钟小时的疾病圆周范围（circumferential extent）、疾病的严重程度（第1-5期）及“外加疾病”的存在或不存在。

[0296] 这些区域位于视神经的中心。第1区为视网膜的后区，其定义为半径为从视神经延伸至到黄斑的距离的两倍的圆圈。第2区为内缘由第1区界定，外缘由半径（此半径被定义为从视神经至鼻侧锯齿缘的距离）界定的环形。第3区为视网膜剩余的颞侧新月体（temporal crescent）。

[0297] 疾病的圆周范围是以节段描述的，犹如眼睛的顶部是时钟面上的12。例如，人们可以报告在4至7点钟的3个时钟小时内有第一期疾病。

[0298] 分期描述在血管化的和无血管的视网膜间的交界处的检眼镜检查结果。第1期为隐约的分界线。第2期为提高的脊。第3期为视网膜外纤维血管组织。第4期为次全视网膜脱离（sub-total retinal detachment）。第5期为广泛视网膜脱离。

[0299] 此外，“外加疾病”可出现在任何阶段。“外加疾病”描述在后视网膜血管处所观察到的明显程度的血管扩张和弯曲。这反映通过视网膜的血流量的增加。

[0300] 相对于治疗前的分类，ICROP的任何改善均被认为是有效的治疗。类似地，当疾病预防为目标时，若已治疗的个体中的ROP的一种或多种迹象或症状相对于未接受这类治疗的类似个体的预期病程较不严重，则该治疗被认为是有效的。此疾病为已知的并且被表征的程度使得熟练的临床医师通常能够在没有治疗的情况下（例如根据之前的患者的知识）来预测将会出现的疾病程度。没有发展ROP（或者就此而言，任何其他涉及异常血管形成的视网膜疾病或病症）的一种或多种症状或经历其恶化均可被认为是对否则被预期发展或经历此类疾病的恶化的个体中的疾病的有效预防。类似地，相对于无治疗时所预期的疾病状态的任何改善均可被认为是有效的治疗。

[0301] 作为ICROP量度的备选方案，也可测量本领域技术人员所知的视网膜疾病的其他临幊上公认的标记来监测或确定如本文所述的视网膜疾病或病症的治疗或预防的效力。一般而言，视网膜疾病标记中至少10%的差异被认为是有意义的。

[0302] 本申请中提供了用于减少或抑制患者眼睛中的血管形成（例如，新血管形成）的方法。在此也提供了用于治疗与患者眼睛中的新血管形成相关的眼科疾病或病症的方法，其中该与新血管形成相关的眼科疾病或病症为视网膜新血管形成。另一个实施方式提供了用于治疗与患者眼睛中的新血管形成相关的眼科疾病或病症的方法，其中该与新血管形成相关的眼科疾病或病症为脉络膜新血管形成。另一个实施方式提供了用于治疗与患者眼睛中的新血管形成相关的眼科疾病或病症的方法，其中该与新血管形成相关的眼科疾病或病症选自镰状细胞性视网膜病、伊尔斯病、眼缺血综合征、颈动脉海绵窦瘤、家族性渗出性玻璃体视网膜病、高粘稠度综合征、特发性闭塞性小动脉炎、放射性视网膜病、视网膜静脉闭塞、视网膜动脉闭塞、视网膜栓塞、伯尔绍视网膜脉络膜病、视网膜血管炎、结节病、弓形体病、葡萄膜炎、脉络膜黑色素瘤、慢性视网膜脱离、色素失调症及色素性视网膜炎。另一个实施方式提供了用于治疗与患者眼睛中的新血管形成相关的眼科疾病或病症的方法，其中该与新血管形成相关的眼科疾病或病症为湿型年龄相关性黄斑变性。另一个实施方式提供了用于治疗与患者眼睛中的新血管形成相关的眼科疾病或病症的方法，其中该与新血管形成相关的眼科疾病或病症为新生血管性年龄相关性黄斑变性。

[0303] 本申请中提供了通过向患者施用治疗有效量的本文所提供的组合物来治疗患者

的新生血管性年龄相关性黄斑变性（例如湿型年龄相关性黄斑变性（AMD））或脉络膜新血管形成（CNV）的方法。本文所述的化合物也可在用于治疗黄斑变性（例如年龄相关性黄斑变性（AMD））或脉络膜新血管形成（CNV）的药物中使用。如本文所提供的，所有对年龄相关性黄斑变性的提及是指该疾病的新生血管性或湿型阶段。

[0304] 本申请中提供了通过向患者施用治疗有效量的本文所提供的组合物来治疗患者的年龄相关性黄斑变性（AMD）的方法。该治疗可改善患者的病况并可通过确定是否发生下列一种或多种因素来评估：玻璃疣；色素改变；渗出物变化（例如眼中出血、硬性渗出物、视网膜下/RPE下/视网膜内流体）；萎缩（初期及地图样）；视敏度急剧降低（2个水平或更多；例如20/20至20/80）；择优超锐度视野计量变化（preferential hyperacuity perimetry change）（对于湿型AMD）；视力模糊（非渗出性黄斑变性患者可能无症状或注意到中心视力逐渐丧失，而渗出性黄斑变性患者经常注意到视力丧失的快速发病）；中心暗点（视觉的阴影或缺失区域）；视力扭曲（即，视物变形症；直线格子显示为波浪形而部分格子可能出现空白。当患者在家中注视小百叶窗时往往首先注意到这一点）；难以分辨颜色（特别是深色与深色的分辨以及淡色与淡色的分辨）；暴露于亮光后视觉功能恢复缓慢；及对比敏感度丧失。本文描述了通过施用本文所述的化合物来治疗或预防AMD的方法。本文所述的化合物也可在用于治疗AMD的药物中使用。在一个实施方式中，将本文所述化合物之一施用于患者后，AMD的一种或多种迹象或症状得到改善。改善也包括一种或多种症状的停滞，以便其不恶化。

[0305] 涉及CNV的疾病的“治疗”是指涉及CNV的疾病，其中由上述疾病所引起的症状得到抑制或改善。涉及CNV的疾病的治疗也指抑制CNV进展及抑制由从异常新生血管的出血或血浆成分渗漏所造成的视网膜的功能障碍。

[0306] 本文所使用的“抑制CNV”除抑制新血管形成外，还指抑制视网膜中的炎症（抑制视网膜中炎性细胞的生长）及抑制炎性细胞产生血管生成因子。视网膜中的炎症反应可由损伤诱发，或由代谢分解产物累积（如玻璃疣）诱发。

[0307] 通过使用荧光素眼底血管造影术等检测新血管形成的大小（体积）可以确认CNV受到抑制。当施用本发明的试剂后新血管形成的体积减少时，认为CNV受到抑制。用于检测CNV的方法并不限于上述方法，CNV可通过已知方法，也可通过本文的实施例中所述的方法进行检测。

[0308] 随着涉及CNV的疾病进展，视力因图像失真、中心暗点等而受损。在这类视力受损的情况下，当施用本文所述的化合物后视敏度改善时，认为该化合物对患有这类涉及CNV的疾病的患者有用。本申请中提供了用于治疗脉络膜新血管形成的方法。该治疗可改善患者的病况且可以通过确定视敏度是否增加来评估。本文描述了通过施用本文所述的化合物来治疗或预防脉络膜新血管形成的方法。

[0309] 除了其他眼部疾病外，脉络膜新血管形成（CNV）还常发生在黄斑变性中，并且与脉络膜内皮细胞的增殖、细胞外基质的过度产生和纤维血管视网膜下膜的形成有关。视网膜色素上皮细胞增殖及血管生成因子的产生似乎影响脉络膜新血管形成。脉络膜新血管形成（CNV）为视网膜的视网膜色素上皮（RPE）层下方的异常血管的发展。这些血管突破布魯赫膜，破坏视网膜色素上皮，出血，最终导致黄斑瘢痕形成，这造成中心视力的深度丧失（盘状瘢痕形成）。

[0310] 在一个实施方式中,用本文所述化合物治疗 CNV 减少、减缓或抑制视网膜的视网膜色素上皮层下方的异常血管的发展,减缓或抑制布鲁赫膜的损坏,并减缓或抑制视网膜色素上皮的破裂,及减缓或抑制黄斑瘢痕形成。

[0311] 在许多与视网膜缺血相关的视网膜病(如镰状细胞性视网膜病、伊尔斯病、眼缺血综合征、颈动脉海绵窦瘘、家族性渗出性玻璃体视网膜病、高粘稠度综合征、特发性闭塞性小动脉炎、放射性视网膜病、视网膜静脉闭塞、视网膜动脉闭塞或视网膜栓塞)中会发展出视网膜新血管形成。视网膜新血管形成也可随着炎性疾病(如伯尔绍视网膜脉络膜病、视网膜血管炎、结节病、弓形体病或葡萄膜炎)或其他病况(如脉络膜黑色素瘤、慢性视网膜脱离、色素失调症及很少地在色素性视网膜炎中)而发生。

[0312] 几乎所有视网膜新血管形成的共同因素为视网膜缺血,其被认为释放出扩散性血管生成因子(如 VEGF)。新血管形成在视网膜内开始,然后破坏视网膜内界膜。新血管在视网膜内部生长,且在玻璃体已脱离(玻璃体脱离)后在玻璃体后表面上生长。新血管形成可从视盘或视网膜的表面发出。视网膜新血管形成常常进展成玻璃体视网膜新血管形成。虹膜新血管形成及新生血管性青光眼往往跟在视网膜新血管形成之后发生。

[0313] 治疗的效力通过常用于评估眼内新生血管性疾病的各种终点来测量。例如可评估视力丧失。视力丧失可通过例如但不限于下列方法来评估:根据从基线至所需时间点的最佳矫正视敏度(BCVA)的平均变化进行测量(例如,其中 BCVA 是基于早期治疗糖尿病性视网膜病研究(ETDRS)视力表,以及在 4 米的测试距离下的评估),测量与基线相比在所需时间点时视敏度丢失少于 15 个字母的受试者的比例,测量与基线相比在所需时间点时视敏度增加多于或等于 15 个字母的受试者的比例,测量在所需时间点时具有相当于 20/2000 或更糟的斯内伦视敏度的受试者的比例,测量 NEI 视觉功能问卷,测量在所需时间点时的 CNV 大小及 CNV 的渗漏量,例如通过荧光素血管造影术等。可进行眼部评估,例如,其包括但不限于,例如,进行眼睛检查、测量眼内压、评估视敏度、测量裂隙灯压力、评估眼内炎症等。

[0314] 本申请中提供了在需要让眼睛暴露于亮光、激光、导致瞳孔长期和/或过度放大,或其他方式使眼睛对光敏感的医疗程序中保护眼睛的方法,该方法包括向有需要的患者施用包含本文所述化合物的组合物。

[0315] 在一个实施方式中,该医疗程序为眼屈光手术、角膜手术、白内障手术、青光眼手术、管道成形术、玻璃体视网膜手术、泛视网膜光凝术、眼肌手术、眼整形手术、激光治疗或焦点或光栅激光光凝术(focal or grid laser photocoagulation)。在一个实施方式中,该医疗程序为眼屈光手术。在一个实施方式中,该医疗程序为角膜手术。在一个实施方式中,该医疗程序为白内障手术。在一个实施方式中,该医疗程序为青光眼手术。在一个实施方式中,该医疗程序为管道成形术。在一个实施方式中,该医疗程序为玻璃体视网膜手术。在一个实施方式中,该医疗程序为泛视网膜光凝术。在一个实施方式中,该医疗程序为眼肌手术。在一个实施方式中,该医疗程序为眼整形手术。在一个实施方式中,该医疗程序为激光疗法。在一个实施方式中,该医疗程序为焦点或光栅激光光凝术。

[0316] 在一个实施方式中,该组合物在医疗程序之前和之后通过口服施用于患者。

[0317] 在一个实施方式中,该组合物在医疗程序之前通过口服施用。在一个实施方式中,该组合物在医疗程序之前约 0.5h、1h、1.5h、2h、2.5h、3h、3.5h、4h、6h、12h 或 24h 施用。

[0318] 在一个实施方式中,该组合物在医疗程序之后施用。在一个实施方式中,该组合物

在医疗程序之后 1h、3h、6h、12h、24h 或 48h 施用。在一个实施方式中,该组合物在医疗程序之后 24h 施用。在一个实施方式中,该组合物在医疗程序之后 48h 施用。在一个实施方式中,该组合物在医疗程序之后 24h 和 48h 施用。

[0319] 在一个实施方式中,该组合物以化合物的单剂量形式施用。在一个实施方式中,该组合物包含约 2mg、5mg、10mg、15mg、20mg、25mg、30mg、35mg、40mg、45mg、50mg、60mg、70mg、80mg、90mg 或约 100mg。

[0320] 在这类方法中所要施用的化合物通过任何合适的方式如本文所述及本领域中已知的方式施用。

[0321] 对于疾病的预防或治疗,化合物的适当剂量将部分取决于欲治疗的患者、疾病的严重程度和病程、施用化合物是用于预防还是治疗目的、先前的疗法、患者的临床病史和对该化合物的反应,以及主治医生的裁量。该化合物适当地一次或在一系列的治疗中施用于患者。

[0322] 该组合物可以与剂量制剂相容的方式及以治疗有效量施用。施用量取决于欲治疗的受试者、患者的免疫系统利用活性成分的能力。需要施用的活性成分的精确量取决于医师的判断且为每个个体所特有的。合适的初次施用及加强注射的方案也是可变的。

[0323] 根据欲治疗的病况,组合物可单独施用或与第二治疗联合同时或顺次施用。当施用两种或更多种组合物,或组合物与治疗时,组合物或组合物 / 治疗可联合施用(顺次或同时)。组合物可以单一剂量或多剂量施用。

[0324] 本文所述的化合物可根据需要与本领域已知的和例如下文中更详细描述的一种或多种标准治疗性治疗联合施用。

#### [0325] 联合疗法

[0326] 糖尿病性视网膜病为作为其原因的糖尿病状况的后果,用于降低其发展的风险或延缓其发展的其他方法为:维持最佳的血糖水平;进行定期的、彻底的眼睛检查;遵循健康的饮食计划;食用不同种类的食物,且每餐食用适量的碳水化合物;定期运动;严格按照处方用药;食用低脂肪及低盐饮食,以将胆固醇和血压保持在正常水平;不抽烟;控制血压和胆固醇水平;及妊娠期间小心监测血压。

[0327] 应当理解,本文所描述的任何方法均可与一种或多种另外的疗法联合,该另外的疗法包括但不限于激光疗法(例如,焦点或光栅激光光凝术或焦点激光治疗或播散性(泛视网膜)激光光凝术或播散性激光治疗)、冷冻疗法、荧光素血管造影术、玻璃体切割术、皮质类固醇(例如,玻璃体内曲安奈德(triamcinolone acetonide))、抗血管内皮生长因子(VEGF)治疗(例如,培加尼布(Macugen;Pfizer, Inc., New York, USA)、雷珠单抗(Lucentis; Genentech, Inc., South San Francisco, California, USA)、贝伐珠单抗(Avastin; Genentech, Inc.)及 VEGF Trap-Eye(Regeneron Pharmaceuticals, Inc., Tarrytown, New York, USA)、用于持久性弥漫性糖尿病性黄斑水肿的玻璃体切割术、糖尿病性视网膜病处置中的药理性玻璃体溶解术、贝特类药物、肾素-血管紧张素系统(ras)阻滞剂、过氧化物酶体增殖物激活受体  $\gamma$ (PPAR- $\gamma$ )激动剂、抗蛋白激酶 C(芦布妥林)、胰岛细胞移植;治疗性寡核苷酸、生长激素和胰岛素生长因子(IGF)及系统性因子的控制。

[0328] 本文所使用的术语“共同施用”、“与…联合施用”及其语法等同词等意在包括将选定的化合物施用于单一患者,并且意在包括其中化合物通过相同或不同途径施用或在相同

或不同时间施用的治疗方案。在一些实施方式中,本文所述的化合物将与其他试剂共同施用。这些术语包括向患者施用两种或更多种化合物,从而使两种化合物同时存在于患者中。这些术语也包括向患者施用一种化合物及治疗(例如激光疗法),从而使两种化合物同时存在于患者中。这包括在分开的组合物中同时施用,在分开的组合物中在不同的时间施用,和/或在存有两种化合物的组合物中施用。因此,在一些实施方式中,该化合物及其他试剂/治疗在单一组合物中或在单一时间施用。在一些实施方式中,该化合物及其他试剂混合在单一组合物中。

[0329] 激光疗法

[0330] 从二十世纪六十年代起,激光光凝术已用于治疗非增生性糖尿病性视网膜病、黄斑水肿及增生性糖尿病性视网膜病。

[0331] 激光治疗通常针对受损的眼组织。一些激光直接通过“点焊”及密封渗漏区(光凝术)来治疗渗漏的血管。其他激光消除通过新血管形成而形成的异常血管。也可使用激光来破坏未涉及视觉的正常视网膜的外围部分。这样做是为了帮助维持视网膜中心部分的视力。

[0332] 常用来治疗显著糖尿病性眼病的两种类型的激光治疗为:

[0333] 焦点或光栅激光光凝术或焦点激光治疗

[0334] 此类型的激光能量直接针对患病区域,或以包含的网格状图案应用以破坏受损的眼组织并清除造成盲点及视力丧失的瘢痕。此激光治疗方法一般针对特定的、个别的血管。

[0335] 这是用于治疗糖尿病性黄斑水肿引起的黄斑病的主要视网膜病激光治疗方法。视网膜激光密封渗漏流体和血液的视网膜血管。其减少进一步的流体和血液渗漏并减少黄斑肿胀。视网膜激光也可能以某种方式刺激视网膜细胞将黄斑处的任何多余的流体“泵”出。激光仅针对于黄斑的某些部分;并未触及周边视网膜的其余部分。

[0336] 视网膜激光治疗的目的并非改善视力,而是防止其继续恶化。

[0337] 播散性(泛视网膜)激光光凝术或播散性激光治疗

[0338] 泛视网膜光凝术是对于增生性糖尿病性视网膜病的一线治疗。其向视网膜的最外(外围)区施加约1200至1800个激光能量小点,未触及内部。此激光治疗可收缩异常的血管。此治疗涉及激光照射大面积的视网膜,目的是将视网膜周边的缺血性视网膜细胞凝固或灼烧。

[0339] 在泛视网膜激光后,整个视网膜周边的缺血性细胞被瘢痕组织所取代。其减少会刺激异常新血管生长的化学物质的产生。播散性激光治疗通常在两个或更多个治疗期(session)中完成。

[0340] 激光手术通常有助于治疗糖尿病性视网膜病。为了减少黄斑水肿,激光聚焦在受损的视网膜上以密封渗漏的视网膜血管。对于异常的血管生长(新血管形成),将激光治疗施加在周边视网膜上。所产生的小激光瘢痕会减少异常的血管生长,并帮助将视网膜黏结至眼睛后方,从而防止视网膜脱离。激光手术可以大大地降低严重视力缺损的机会。

[0341] 冷疗法

[0342] 冷疗法(冷冻)可能有助于治疗糖尿病性视网膜病。若血液使玻璃体浑浊,则无法使用激光手术直到血液沉降或清除。在一些这样的病例中,视网膜冷冻疗法可以帮助收缩异常血管并将视网膜黏结至眼睛后方。

[0343] 荧光素血管造影术

[0344] 荧光素血管造影术已用作了解患糖尿病性视网膜病的眼睛的视网膜循环中临床病理学变化的研究工具。其也有助于对糖尿病性视网膜病进行分类并预测从基线荧光素血管造影术特征（尤其是毛细血管无灌注模式）的进展。

[0345] 其将鉴定黄斑周边渗漏的来源并引导黄斑水肿的激光治疗。荧光素血管造影术在增生性糖尿病性视网膜病的治疗中可能并非必要，但可能可用于评估视网膜缺血的迹象。在一些情况下，荧光素血管造影术可鉴定以其他方式看不到的新血管。

[0346] 在葡萄糖耐量降低的患者中，荧光素血管造影术可检测初期的视网膜微血管变化，在显现出糖尿病前指出血液-视网膜屏障的早期破裂。这些及其他研究证明荧光素血管造影术可在无临床视网膜病的糖尿病受试者中检测到明确的早期视网膜血管变化。

[0347] 然而，目前荧光素血管造影术在处置糖尿病性视网膜病中的常规使用应由临床经验来引导，因为几乎没有证据可提供坚实的指南。

[0348] 玻璃体切割术

[0349] 玻璃体切割术（从眼睛中间手术去除玻璃体凝胶）通常用于患有更晚期视网膜疾病的患者。该程序旨在防止视网膜的完全脱离。此程序常用于治疗不能清除的玻璃体出血、玻璃体黄斑牵引、视网膜外膜及视网膜脱离。

[0350] 在玻璃体切割手术期间，使用手术显微镜及小型手术器械来去除眼睛中伴随着异常血管的血液和瘢痕组织。去除玻璃体出血可使光线再次聚焦到视网膜上。

[0351] 玻璃体切割术通常通过去除引起出血的异常血管来防止进一步的玻璃体出血。瘢痕组织的去除有助于视网膜回复至其正常位置。玻璃体切割术后可在激光治疗之前进行或伴随激光治疗进行。

[0352] 若在玻璃体出血者中早期进行，玻璃体切割术可减少视力丧失，尤其是如果他们患有重度增生性视网膜病。

[0353] 传统的激光治疗在具有玻璃体出血的眼睛中或具有牵引性视网膜脱离和活动性进行性PDR的眼睛中可能会失败。早期玻璃体切割术已被证明改善增生性视网膜病及重度玻璃体出血患者的视力恢复。

[0354] 眼屈光手术

[0355] 眼屈光手术涉及手术重塑角膜或白内障的各种方法（例如放射状角膜切开术使用以金刚石刀作出的轮辐形切口）。在一些情况下，使用准分子激光来重塑角膜曲率。成功的眼屈光手术可以减轻或治愈常见的视力障碍，如近视、远视和散光，以及例如圆锥形角膜的退行性病症。其他类型的眼屈光手术包括角膜磨削术（削去角膜盘，快速冷冻，车磨，然后回复至其原来的能力）、自动板层角膜移植术（ALK）、激光辅助的原位角膜磨削术（LASIK）、intraLASIK、激光辅助的上皮下角膜磨削术（LASEK，又称Epi-LASIK）、屈光性角膜切削术、激光热角膜成形术、传导性角膜成形术、角膜缘松解切口、散光角膜切开术、放射状角膜切开术、微型不对称放射状角膜切开术、六角状角膜切开术、表面角膜镜片术、角膜内环或环段植入体（角膜环）、接触镜植入体、老花眼逆转术、前睫状巩膜切开术、老花眼激光逆转术、巩膜扩张带及Karmra镶嵌。

[0356] 角膜手术

[0357] 角膜手术的实例包括但不限于角膜移植手术、穿透性角膜成形术、人工角膜手术、

光性治疗性角膜切除术、翼状胬肉切除术、角膜染色术及骨齿人工角膜手术 (OOKP)。在一些情况下,角膜手术不需要激光。在其他情况下,角膜手术使用激光 (例如光性治疗性角膜切除术,其除去浅表角膜混浊及表面不平整)。在一些情况下,在这些程序后给予患者深色眼镜以保护其眼睛避免亮光。

[0358] 白内障和青光眼手术

[0359] 白内障手术涉及手术去除晶状体和以塑料人工晶状体替换。通常,使用光来协助外科医生。

[0360] 青光眼手术促进多余的房水从眼睛逸出以降低眼内压。在某些情况下,这些医疗程序使用激光 (例如,激光小梁成形术应用激光束来烧灼位于靠近虹膜基底的小梁网区域以增加流体流出;激光周边虹膜切开术应用激光束来选择性地烧灼出穿过虹膜接近其基底的孔等)。管道形成术是一种先进的、非穿透性程序,其设计成在简单的微创手术中使用微导管技术来增加通过眼睛的自然排液系统的排液。其他用于治疗青光眼的医疗程序包括激光、非穿透性手术、防护性滤过手术及西顿阀植入体。

[0361] 皮质类固醇 (玻璃体内曲安奈德)

[0362] 皮质类固醇降低血管通透性并减少血液视网膜屏障的破裂。其抑制 VEGF 基因转录和翻译以及白细胞向血管壁的粘附。其特别针对与血管通透性增加相关的并发症。

[0363] 玻璃体内曲安奈德 (IVTA) (4mg) 帮助降低糖尿病性视网膜病进展的风险。然而,该研究的结论是,在此时使用 IVTA 来降低视网膜病进展的可能性不能得到保证,因为与 IVTA 相关的青光眼和白内障的风险增加,并且因为用泛视网膜光凝术已经可以成功且安全地治疗 PDR。

[0364] 几项小规模的随机临床试验表明,对于治疗 PDR 和黄斑水肿而言,当与单独的激光光凝术相比较时,激光光凝术 (泛视网膜和黄斑) 与 IVTA 的组合与改善的最佳矫正视敏度和降低的黄斑中心厚度及总黄斑体积有关。另一方面,最近的研究表明,在具有共同存在的高危增生性糖尿病性视网膜病 (PDR) 和有临床意义的黄斑水肿的眼睛中,与作为这些患者的标准治疗的泛视网膜光凝术及黄斑光凝术相比,组合的 IVTA 与泛视网膜光凝术及黄斑光凝术治疗没有有益效果。

[0365] 抗血管内皮生长因子 (VEGF) 治疗

[0366] 目前有四种用于处置糖尿病性视网膜病的抗 VEGF 剂,包括培加尼布 (Macugen ;Pfizer, Inc., New York, USA)、雷珠单抗 (Lucentis ;Genentech, Inc., South San Francisco, California, USA)、贝伐珠单抗 (Avastin ;Genentech, Inc.) 及 VEGF Trap-Eye (Regeneron Pharmaceuticals, Inc., Tarrytown, New York, USA)。

[0367] 培加尼布为针对 VEGF-A 165 同种型的聚乙二醇化 RNA 适体。在 DME 患者中进行的玻璃体内培加尼布 II 期临床试验 (随访 36 周) 显示出更好的视敏度结果、降低的中心视网膜厚度以及降低的对其他光凝疗法的需求。对在基线处具有视网膜新血管形成的患者进行的相同研究的回顾性分析显示出在玻璃体内施用培加尼布后新血管形成的退化。最近在一项回顾性研究中,表明在玻璃体内反复施用培加尼布在糖尿病性黄斑水肿患者中产生最佳矫正视敏度的显著改善及黄斑中心平均厚度的减少。

[0368] 雷珠单抗为对人 VEGF-A 的所有同种型均具有特异性的重组人源化单克隆抗体片段。在 DME 患者中玻璃体内注射雷珠单抗的先导性研究表明了降低的中心凹厚度,以及维

持或改善的视敏度。最近,Nguyen 等人 (2009) 表明,在 6 个月期间在 DME 患者中反复玻璃体内注射雷珠单抗产生比焦点 / 光栅激光治疗显著更佳的视觉结果。糖尿病性视网膜病临床研究网 (2010a) 评估了与焦点 / 光栅激光组合的玻璃体内 0.5mg 雷珠单抗或 4mg 曲安奈德与单独的焦点 / 光栅激光对于治疗糖尿病性黄斑水肿的比较。Nguyen 等人 (2010) 在一项随机研究中显示,眼内注射雷珠单抗对糖尿病性黄斑水肿提供了至少 2 年的益处,而当与焦点或光栅激光治疗组合时,剩余的水肿量减少,控制水肿所需的注射的频率也降低。

[0369] VEGF Trap 是一种 115kDa 重组融合蛋白,其由与人 IgG1 的 Fc 结构域融合的人 VEGF 受体 1 和 2 的 VEGF 结合域组成。一项先导研究显示,在糖尿病性黄斑水肿患者中,单次玻璃体内 VEGF Trap-Eye 注射是良好耐受的并且是有效的。

[0370] 贝伐珠单抗为对 VEGF-A 的所有同种型都具有活性的全长重组人源化抗体。其被 FDA 批准作为用于转移性结直肠癌的辅助性全身治疗。数个研究报道了标示外 (off-label) 玻璃体内贝伐珠单抗 (IVB) 用于治疗糖尿病性黄斑水肿 (DME)、增生性糖尿病性视网膜病 (PDR) 的并发症及虹膜新血管形成的用途。数项研究表明了 IVB 注射在增生性糖尿病性视网膜病 (PDR) 患者中造成视网膜和虹膜新血管形成的显著退化及玻璃体出血的快速解决。此外,已表明 IVB 注射是高危增生性糖尿病性视网膜病 (PDR) 和新生血管性青光眼的治疗中对 PRP 的一种有效的辅助治疗。短期结果提示,IVB 不仅具有防止视网膜厚度增加的潜能,而且具有在白内障手术后降低具有糖尿病性黄斑水肿 (DME) 的眼睛的视网膜厚度的潜能。

[0371] 持久性弥漫性糖尿病性黄斑水肿的玻璃体切割术

[0372] 针对持久性弥漫性黄斑水肿 (DME) 去除黄斑前后玻璃状膜 (premacular posterior hyaloid) 的玻璃体切割术已获得快速的广泛接受。评估玻璃体切割术 (进行或不进行内界膜剥除) 疗效的大量系列研究已得到相互矛盾的结果。在一项试验中观察到,进行内界膜剥除的玻璃体切割术优于在先前对传统的激光治疗无反应的持久性弥漫性糖尿病性黄斑水肿 (DME) 眼睛中的观察结果,并且正面地影响距离和阅读视敏度以及水肿形态。其他研究表明,进行及不进行内界膜剥除的玻璃体切割术可以对具有激光光凝术难治性的弥漫性非牵引性无反应糖尿病性黄斑水肿 (DME) 的眼睛提供解剖和视觉益处。

[0373] 其他的研究显示,玻璃体切割术对糖尿病性黄斑水肿 (DME) 在视敏度和黄斑厚度方面的益处局限于显现出黄斑牵引迹象的患者,无论是在临幊上和 / 或在光学相干断层扫描上。

[0374] 糖尿病性视网膜病处置中的药理性玻璃体溶解术

[0375] 在示范期间,观察到玻璃体内微纤溶酶 (microplasmin) 注射并引起玻璃体后部脱离 (PVD) 及玻璃体液化的组合会增加玻璃体内的氧张力。另一方面,透明质酸酶诱导的玻璃体液化而不引起玻璃体后部脱离 (PVD) 则无法增加玻璃体内的氧张力。此外,当用微纤溶酶治疗的动物暴露于 100% 氧时,与对照或透明质酸酶治疗的眼睛相比,中部玻璃体腔中的氧水平加速增加。这些发现表明,外科玻璃体切割术对增加玻璃体腔内的氧张力的有益效果可以用 PVD 及玻璃体液化的酶诱导来重现,但无手术的时间、风险和费用。在 2009 年,表明了玻璃体内注射自体纤溶酶但不进行玻璃体切割术诱导了完全 PVD 并有效减少因难治性弥漫性糖尿病黄斑水肿造成的黄斑增厚并改善了视敏度。因此,在不进行玻璃体切割术的情况下创伤性药理性分离玻璃体后皮质,使内界膜与玻璃体后部之间被干净地分

开,这可以降低术中医源性损伤如视网膜撕裂和神经纤维损害以及术后后遗症的风险。

[0376] 贝特类药物

[0377] 贝特类药物为在血脂异常的治疗中广泛开具的降脂药。其主要临床效果(由过氧化物酶体增殖物激活受体 $\alpha$ 的活化所介导)为总胆固醇及低密度脂蛋白胆固醇水平的适度降低、甘油三脂的显著降低以及高密度脂蛋白胆固醇的增加。糖尿病的非诺贝特干预及事件减少(FIELD)研究表明,用非诺贝特进行的长期降脂疗法降低了2型糖尿病患者中糖尿病性视网膜病的进展以及对激光治疗的需求,虽然此效果的机制似乎与脂质的血浆浓度无关。最近,ACCORD研究小组(2010)表明,用于加强的血脂异常疗法的非诺贝特降低了2型糖尿病患者中的糖尿病性视网膜病的进展速度。

[0378] 肾素-血管紧张素系统(RAS)阻滞剂

[0379] 一些研究表明,RAS阻滞剂可降低糖尿病性视网膜病的负担。赖诺普利在胰岛素依赖性糖尿病中的Eurodiab对照试验(EUCLID)的结果表明,用血管紧张素转换酶抑制剂赖诺普利阻滞肾素-血管紧张素系统减少了1型糖尿病患者中视网膜病的发病率及进展。

[0380] 过氧化物酶体增殖物激活受体 $\gamma$ (PPAR $\gamma$ )激动剂

[0381] PPAR $\gamma$ 激动剂罗格列酮抑制在实验性糖尿病大鼠中所观察到的视网膜白细胞停滞及视网膜渗漏。此外,小鼠中内源性PPAR $\gamma$ 的表达减少导致糖尿病小鼠中的视网膜白细胞停滞和视网膜渗漏的恶化。马来酸罗格列酮(Avandia;GlaxoSmithKline, North Carolina, USA)是用于改善糖尿病患者的血糖控制的口服药物。此药物活化PPAR $\gamma$ 并导致脂肪和其他组织中的胰岛素致敏,具有潜在的抗血管生成活性。

[0382] 抗蛋白激酶C(芦布妥林)

[0383] PKC介导糖尿病的数种眼部并发症。其被VEGF活化并且是糖尿病性视网膜病疗法的潜在靶标。

[0384] 芦布妥林(RBX)(一种口服PKC $\beta$ 抑制剂)是具有足够的生物利用度以允许每日口服一次的选择性抑制剂。在蛋白激酶C $\beta$ 抑制剂-糖尿病性视网膜病研究2(PKC-DRS2)中,口服RBX(每日32mg)减少了持续的中度视力丧失、对激光治疗黄斑水肿的需求以及黄斑水肿的进展,同时增加了非增生性视网膜病患者的视力改善的发生。

[0385] 胰岛细胞移植

[0386] 最近的研究表明,使用加强的胰岛分离、不含糖皮质激素的免疫抑制以及提供超过10,000胰岛当量/kg体重的足够的胰岛质量可观察到改善的胰岛移植结果。这些改善已导致对1型糖尿病受试者的益处,包括长期的C肽分泌、改善的血糖控制及减少的低血糖发作。

[0387] 治疗性寡核苷酸

[0388] 寡核苷酸代表一种靶向疾病过程中的特定连接的新治疗实体。寡核苷酸治疗剂有两种主要类别:反义寡核苷酸,包括短干扰RNA(siRNA),以及寡核苷酸适体。

[0389] 反义寡核苷酸是设计用来结合特定信使RNA(mRNA)的新治疗剂,其造成编码靶蛋白质的信息的降解,从而致使与目标疾病相关的特定蛋白质的产生的减少。通过玻璃体内注射进行的反义寡核苷酸递送是治疗视网膜疾病的一种合理策略。药物递送反义及其他寡核苷酸的备选选择已在研究中,包括眶周施用、离子电渗疗法及持续释放制剂。

[0390] 生长激素和胰岛素生长因子(IGF)

[0391] 生长激素及胰岛素生长因子 (IGF) 调节视网膜内皮前体细胞的功能并响应于低氧而驱动视网膜血管生成 ; IGF 1 也可破坏血液 - 视网膜屏障并增加视网膜血管通透性。

[0392] 玻璃体内透明质酸酶

[0393] 玻璃体内绵羊透明质酸酶注射可有效清除玻璃体出血。数个人类病例系列表明, 自体纤溶酶的玻璃体内注射是用于治疗糖尿病性黄斑水肿和增生性糖尿病性视网膜病的玻璃体手术的一种安全且有效的辅助手段。

[0394] 系统性因素的控制 :

[0395] 糖尿病性视网膜病的基本预防涉及严格的血糖、脂质和血压控制。为了预防糖尿病性视网膜病而应控制的一些系统性因素详细说明如下。

[0396] 血糖控制

[0397] 高血糖触动一连串事件, 最终导致糖尿病性视网膜病的发展。因此, 可用于减缓糖尿病性视网膜病进展的一种治疗是血糖控制。血糖控制可降低 1 型和 2 型糖尿病中糖尿病性视网膜病的发展和进展的风险。

[0398] 血压控制

[0399] 高血压通过增加血流量和血管内皮细胞的机械损伤 (拉伸)、刺激 VEGF 的释放而加重糖尿病性视网膜病。在 2 型糖尿病患者中, 严格的血压控制可使视网膜病进展风险减少约三分之一、视力丧失减少一半并且对激光治疗的需求减少三分之一。血压控制也可减少糖尿病性视网膜病的发病率和进展。

[0400] 血清脂质控制

[0401] 血脂异常在糖尿病性视网膜病的发病机制中起作用。视网膜病的严重程度与逐渐增加的甘油三酯有关, 而与 HDL 胆固醇为负相关。羟基甲基戊二酰辅酶 A (HMG CoA) 抑制剂可用于处置血脂异常患者中的糖尿病性视网膜病 (DR) 及糖尿病性黄斑水肿 (DMO)。

[0402] 实施例

[0403] 通过参考以下非限制性实施例可以更好地理解本申请。下列实施例的呈现是为了更充分地说明代表性实施方式, 但绝不应解释为限制本申请的广泛范围。

[0404] 术语 ACU-4429 是指化合物 (R)-3-氨基-1-(3-(环己基甲氧基) 苯基) 丙-1-醇。术语 ACU-4935 是指化合物 (R)-3-氨基-1-(3-(2-丙基戊氧基) 苯基) 丙-1-醇。

[0405] 实施例 1: 糖尿病性视网膜病的接受的动物模型

[0406] 小鼠、大鼠、仓鼠、狗、猫和猴子是用于研究糖尿病性视网膜病的一些常见的动物模型。

[0407] 动物实验在了解视网膜病的发病机制中是关键的, 因为系统性结构、功能及生化研究不能在人类受试者中进行。动物实验在开发辅助治疗策略的尝试中极为重要。糖尿病中的特征性视网膜病变已在实验性糖尿病或半乳糖喂养的动物中得到成功再现。

[0408] 从细胞培养试验和动物研究中取得的数据可用于调配一系列在人体中使用的剂量。这类化合物的剂量优选落在没有或几乎没有毒性的循环浓度 (包括 ED50) 的范围内。根据所使用的剂型及所采用的施用途径, 该剂量可在该范围内变化。对于在本发明方法中所使用的任何化合物, 治疗上有效的剂量可从细胞培养试验中初步估计。如同在细胞培养中所确定的那样, 可在动物模型中对剂量进行调配, 以取得包括 IC50 (即, 实现半数最大抑制的试验化合物的浓度) 的循环血浆浓度安排。血浆中的水平可以例如通过高效液相色谱

法进行测量。这类信息可用于更准确地确定在人体中有用的剂量。

[0409] 用于研究糖尿病性视网膜病的一些常见的动物模型及来源和相关文本详述于下：

[0410] 小鼠

[0411] 可用于测试化合物在小鼠中的治疗效力的方案包括例如在 Elia Duh 的 Diabetic Retinopathy, Springer, Humana Press, 2009 ;Kern 等人 (Arch ophthalmol. 1996 ; 114(8) :986–990) ;Feit-Leichman 等人 (Investigative Ophthalmology&Visual Science, 46(11) :4281–4287, 2005 年 11 月) 中描述的那些方案。

[0412] 大鼠

[0413] 可用于测试化合物在大鼠中的治疗效力的方案包括例如在 Elia Duh 的 Diabetic Retinopathy, Springer, Humana Press, 2009 ;Sima 等人 (Current Eye Research, 1985, Vol. 4(10) 第 1087–1092 页) ;Kato 等人 (Journal of Diabetes and Its Complications, 第 17 卷 (6) :374–379, 2003 年 11 月) ;Sima 等人 (Metabolism, 32(7, Suppl. 1) :136–140, 1983 年 7 月) ;Lu 等人 (Journal of phthalmology, 47(1) :28–35, 2003) ; 及 Deng 等人 (International Journal of Diabetes, 第 6 卷 (第 1 期), 1998) 中描述的那些方案。

[0414] 仓鼠和其他啮齿类动物

[0415] 可用于测试化合物在仓鼠和其他啮齿类动物中的治疗效力的方案包括例如在 Elia Duh 的 Diabetic Retinopathy, Springer, Human Press, 2009 中描述的那些方案。

[0416] 狗

[0417] 可用于测试化合物在狗中的治疗效力的方案包括例如在 Elia Duh 的 Diabetic Retinopathy, Springer, Humana Press, 2009 ;Engerman 等人 (Arch Ophthalmol. 1995 ; 113(3) :355–358) ; 及 Kador 等人 (Arch Ophthalmol. 1990 ;108(9) :1301–1309) 中描述的那些方案。

[0418] 猫

[0419] 可用于测试化合物在猫中的治疗效力的方案包括例如在 Elia Duh 的 Diabetic Retinopathy, Springer, Humana Press, 2009 ;Mansour 等人 (Investigative Ophthalmology&Visual Science, Vol. 31, No. 3, 1990 年 3 月) ; 及 Henson 和 O'Brien (ILAR Journal 第 47 卷 (3) :234–242) 中描述的那些方案。

[0420] 猴子 / 灵长类动物

[0421] 可用于测试化合物在猴子和灵长类动物中的治疗效力的方案包括例如在 Kim 等人 (Invest Ophthalmol Vis Sci. 2004 ;45:4543–4553) ;Akimba:A Novel Murine Model for Diabetic Retinopathy ([www.Bio-link.com](http://www.Bio-link.com)) ; 和 Elia Duh 的 Diabetic Retinopathy, Springer, Humana Press, 2009 中描述的那些方案。

[0422] 实施例 2 : 化合物在糖尿病性视网膜病治疗中的用途

[0423] 启动单中心、开放标签、剂量递增先导研究来评估本文所述化合物的口服在中心累及的、有临床意义的糖尿病性黄斑水肿 (DME) 患者中的生物活性并报告任何相关的不良事件。患有涉及黄斑中心的 DME 且被研究的眼睛的最佳矫正视敏度 (BCVA) 介于 20/63 至 20/400 之间的患者入选。

[0424] 将符合条件的患者以 1:1 的比例随机分配以接受施用化合物的每日口服剂量 (2mg、5mg、7mg、10mg 或 20mg)，直到第 24 个月。主要终点为眼睛及全身不良事件的频率和严重程度。次要终点为：1) 使用开始测试距离为 2 米的标准化屈光及测试方案，用早期治疗糖尿病性视网膜病研究 (ETDRS) 表评估的最佳矫正视力评估，及 2) 通过光学相干断层扫描测量的视网膜厚度。评估的医生不知道患者的治疗分配；施用剂量的医生知道患者的有关测试或无效治疗 (sham treatment) 的治疗分配，但不知道化合物的剂量。每个研究地点的其他工作人员、患者和中央阅读中心的工作人员都不知道患者的治疗分配。

[0425] 在所有患者中根据治疗意向进行效力分析，其中对于缺失数据使用末次观察转入方法 (last-observation-carried-forward method)。对于所有成对比较，针对视敏度的基线得分 (<55 个字母相对于 ≥ 55 个字母) 调整统计模型。使用 Cochran 卡方检验进行二分法终点的组间比较。使用方差分析模型分析相对于基线视敏度的变化。对于病变特点的终点，使用调整基线值的协方差分析模型。使用 Hochberg-Bonferroni 多重比较程序来调整用于主要终点的两个成对治疗比较。安全性分析包括所有经治疗的患者。

[0426] 对 DME 患者而言，预期化合物是耐受性良好的疗法。在患有中心累及的、有临床意义的 DME 的患者中，该化合物将具有维持或改善最佳矫正视敏度及降低视网膜厚度的潜力。

[0427] 实施例 3：锰增强磁共振成像 (MEMRI) 方案

[0428] 在实验期开始前，将大鼠保持在规律的实验室照明下 (12 小时光照, 12 小时黑暗)——光暴露、漂白、暗适应将随小组 (cohort) 而改变 (见下文)。

[0429] 根据以下的组分配，通过口饲向动物给药。在实验期的每一周对大鼠进行称重。

[0430] 在光漂白前 10–30 分钟通过施用 1 滴托吡卡胺 (0.5%) 来放大瞳孔。在 MRI 成像前通过暴露于 5000 勒克司下 4 小时来对动物进行光漂白 10 分钟。

[0431] 漂白后，成像期开始前 4 小时立即对大鼠进行注射。MEMRI 信号反映实验期间活性依赖性通道的表达状态。

[0432] 在清醒大鼠的右下腹部中腹膜内注射 MnCl<sub>2</sub>。

[0433] 使用 20mg/mL 储备溶液以 60mg/kg 注射 MnCl<sub>2</sub>。

[0434] 标记每一只经注射的大鼠并在笔记本上记录注射、漂白时间、成像的开始和结束，以及光照条件。

[0435] 在注射和运送至成像中心进行实践实验之间的 4 小时期间，将除第 4 组之外的所有组的大鼠保持在黑暗 (IOP 房间) 中。在注射 MnCl<sub>2</sub> 和 MRI 成像之间的 4 小时期间，使第 4 组的动物暴露于光。其他遵循针对各小组所述的光 – 暗 – 漂白周期。

[0436] 经由 IACUC 批准的途径将大鼠运送至成像中心，对于每个小组严格遵循光 – 暗周期。

[0437] 对每只大鼠的双眼，或者根据特定实验的要求对单侧进行成像。

[0438] MRI 参数包括：

[0439] 采用内径 12mm 的线性表面线圈，使用快照 FLASH 反转复原 (IR) 成像序列来获取在轴向和矢状面上平分视网膜的单一成像层片。成像参数为 TR/TE = 1000/2.7ms，反转时间 (TI) 为 125ms，扫描宽度 = 73.5kHz，获取数 = 120；层片厚度 = 0.7mm，视野 = 12mm × 12mm，采用 256 × 256 数据矩阵，产生 47 微米的面内分辨率。每只动物的近似扫描时

间为约 16 分钟。

[0440] 一只眼睛成像（包括设置和侦察成像）所需的总时间约为 1 小时。若动物移动，则重新成像。

[0441] (T1 映像)：在方案产生期间确定为最佳

[0442] 采用内径 12mm 的线性表面线圈，使用快照 FLASH 反转复原 (IR) 成像序列来获取在轴向和矢状面上平分视网膜的单一成像层片。成像参数为  $TR/TE = 2000/2.7\text{ms}$ ，扫描宽度 = 73.5kHz，获取数 = 32；层片厚度 = 0.7mm，视野 = 12mm × 12mm，采用  $192 \times 192$  数据矩阵（补零至  $256 \times 256$ ），产生 47 微米的标称面内分辨率。使用在 6 个反转时间 [T1 = 50、150、300、400、900、1800ms] 获取的信号来取得 T1 映像。

[0443] 等到动物已从麻醉中苏醒时再运送。当动物从麻醉中醒来时，成像后使用热灯来帮助维持体温。

[0444] 小组

[0445]

组别	药物治疗	漂白后的光治疗	动物数目
1	ACU-4429 (1 mg/kg/天)	暗适应	5
2	ACU-4429 (10 mg/kg/天)	暗适应	5
3	ACU-4429 载体物	暗适应	5
4	ACU-4429 载体物	明适应	5
5	视黄基乙酰胺 (200 mg/kg)	暗适应	5
6	视黄基乙酰胺载体物	暗适应	5
<b>合计</b>			<b>30</b>

[0446] 研究设计

[0447] 第 1-3 组的时间轴示于图 1 中。

[0448] 第 4 组的时间轴示于图 2 中。

[0449] 第 5-6 组的时间轴示于图 3 中。

[0450] 单剂量研究

[0451] 本研究的目的是确定单一（高）剂量的 ACU-4429 是否减少光漂白后的暗适应后视网膜阳离子活性 ( $Mn^{2+}$  摄入) 的回复。对第 1-4 组进行给药 (ACU-4429 相对于载体物) 并将其保持在室内光线下 2 小时。在光漂白前 18 小时对第 5 和 6 组进行给药 (视黄基乙酰胺相对于载体物)。动物将会瞳孔放大并暴露于适度的漂白白光 (5,000 勒克司的漫射性白色荧光) 10 分钟。光漂白后立即对动物腹膜内 (IP) 注射  $Mn^{2+}$ ，接着暗适应 4 小时 (在成像队列中动物将保持暗适应)。第 3 组的动物将被留在环境室内光下以作为光对照 (预期为，用视黄基乙酰胺及 ACU-4429 治疗的视网膜的行为如同它们是经光适应的)。MRI 成

像（每只动物 30 分钟到 1 小时）在注射 Mn<sup>2+</sup>（腹膜内）后 4 小时时进行，并且在成像之前在安置动物的相同的光照条件下进行。动物的给药将交错进行，以确保所有动物从给药到成像的时间是相同的。

**[0452] 多剂量研究**

**[0453]** 本研究的目的是测试在正常的循环光下随着时间推移重复 ACU-4429 (10mg/kg/天) 治疗是否减少暗适应后视网膜阳离子活性 (Mn<sup>2+</sup> 摄入) 的回复。三组：第 1 组：5mg/kg 的 ACU-4429，每天两次 (10mg/kg/天)；第 2 组：载体物 (经暗适应的)；第 3 组：载体物 (室内光线)。所有给药都将在正常循环光暴露 (约 100 勒克司的漫射性白色荧光 12 小时) 条件下，在开灯和关灯时进行 6 天。在第 7 天早晨给药后立即向所有动物的双眼施用 1 滴硫酸阿托品 (1%) 以使瞳孔放大。施用最后一个剂量后 6 小时，以及在正常光线下至少 6 小时后，向第 1 组 (ACU-4429) 和第 2 组 (经暗适应的) 腹膜内注射 Mn<sup>2+</sup>，接着暗适应 4 小时 (在成像队列中动物将保持暗适应) 并在黑暗中成像 (每只动物 30 分钟到 1 小时)。第 3 组 (室内光线) 在最后一个剂量后 6 小时腹膜内注射 Mn<sup>2+</sup>，并保持在正常室内光线下超过 4 小时，直到成像。第 3 组的成像在正常光线下进行。

**[0454] 实施例 4：大鼠中由氧诱导的视网膜病的减少**

**[0455]** 目的：在具有由氧诱导的视网膜病 (OIR) 的大鼠（人类早产儿视网膜病 (ROP) 的常用模型）中评价测试化合物。OIR 和 ROP 二者的特征是异常的视网膜脉管系统及持续的神经视网膜功能障碍。

**[0456]** 方法：通过将 4 窝 Sprague-Dawley 幼鼠 (N = 24) 从出生日 (P0) 开始至 P14 暴露于 50% 和 10% 氧气的交替周期来诱导 OIR。光周期为 12 小时光照 (10-30 勒克司) 和 12 小时黑暗；光到暗的转变与每次氧气交替一致。从 P7 开始 15 天，在此转变的 1 小时内，向第一窝和第四窝幼鼠口服施用 6mg/kg 的临床开发候选物；第二窝和第三窝幼鼠只接受载体物。在 P2-22，当典型地观察到明显的视网膜血管异常时，记录视网膜电流图并评估感受器及感受器后功能。通过 ANOVA 评估治疗效果。

**[0457]** 评估：评估通过用临床开发候选物治疗而改变的最大视杆响应及光转导的放大常数。此外，通过双闪光方案评估光转导去活化的时间常数。亦评估感受器后敏感性 (log s) 及最大暗视 b 波振幅。可以评估用临床开发候选物治疗后光感受器响应的变化及源自内层视网膜的响应。内层视网膜由视网膜脉管系统供给；在这样的治疗后使用眼底照片的定量图像分析来确定与 OIR 相关的血管异常程度。用临床开发候选物治疗的动物中，血管异常程度预计会降低。

**[0458] 实施例 5：ROP 大鼠模型中的视觉周期调节和视杆功能**

**[0459]** ROP 的大鼠模型提供一种方便的体内系统，在其中可研究和操纵光感受器与视网膜脉管系统的关系。

**[0460]** OIR 和 ROP 二者的特征是神经视网膜的持续功能障碍及异常的视网膜脉管系统。在具有由氧诱导的视网膜病 (OIR) 的大鼠中研究临床开发候选物 (视觉周期调节剂 (VCM)) 的全身性效果。

**[0461]** 通过将 Sprague-Dawley 幼鼠 (N = 46) 从出生日开始至出生后 (P) 14 天暴露于 24 小时的 50±1% 和 10±1% 氧气交替期来诱导视网膜病。除了保持持续黑暗的测试日期外，将光周期控制在 12 小时 10-30 勒克司及 12 小时黑暗。控制光到暗转变的时间以与每

次氧气交替一致。

[0462] 从 P7 开始两周,在此转变期间,向第一窝和第四窝幼鼠口服施用 6mg/kg 的临床开发候选物;第二窝和第三窝只施用等体积的载体物 (20% 二甲亚砜, DMSO)。施用时间表被设计成在年龄范围内持续,该范围起始于视网膜的视紫红质含量的迅速增加,并持续至视紫红质含量超过成鼠量的 50% (Fulton 和 Baker, Invest Ophthalmol Vis Sci (1984) 25:647)。

[0463] 从 P7-14,在每次氧交替之间将治疗的大鼠保持在室内空气 (20.8% 氧) 中约 20 分钟。按照纵向设计评估大鼠,在 P20-22、P30-32 及 P60-62 进行测试。选择这些日期是因为其分别捕获血管异常的高点、明显恢复期及成鼠年龄。在各测试年龄下,使用非侵入性技术来评估神经视网膜的功能和视网膜脉管系统的形态。

[0464] 最后一次给药后不久 (0-2 天),通过视网膜电流扫描计术 (ERG) 评估化合物对神经视网膜的效果。刺激 (其被设计用来评估视杆光感受器及由视杆介导的感受器后神经功能) 的时机和强度在计算机控制下。进行了两组实验。第一组中,评估在经暗适应的视网膜中的视杆和由视杆介导的神经功能。第二组中,评估视杆光感受器从亮光、视紫红质光漂白刺激中的恢复。每一组实验都对约半数的来自每一窝的患者进行。

[0465] 为了评估 VCM 治疗是否影响视网膜脉管系统,获得显示每个 ERG 期后的视网膜主要血管的眼底宽视野图像。如图 19 所示,将图像复合以显示后极 (在由涡静脉约束并与视神经头同心的圆内的区域) 及视网膜区 (其在人类患者中对于高危 ROP 的诊断是最重要的) 的完整视图。用 RISA 定制图像分析软件分析小动脉 (Gelman, Invest Ophthalmol Vis Sci (2005) 46:4734)。

[0466] 实施例 6:由激光诱导的脉络膜新血管形成及黄斑变性的动物模型

[0467] 脉络膜新血管形成的鼠模型

[0468] 本文所述 VCM 化合物的效果可在脉络膜新血管形成的鼠模型中评估。

[0469] 简单地说,用盐酸氯胺酮:甲苯噻嗪 (100mg/kg :10mg/kg) 麻醉 4 至 5 周龄的 C57BL/6 小鼠并用 1% 托吡卡胺 (Alcon Laboratories, Inc Fort Worth, TX) 散瞳。使用光凝器的裂隙灯递送系统 (OcuLight ;Iridex, Mountain View, CA) 及手持式盖片作为接触镜片向每个视网膜递送三次 532nm 二极管激光光凝的烧灼 (75-μm 的斑点大小, 0.1- 秒的持续期间, 120mW)。在视网膜后极的 9、12 及 3 点钟位置处进行烧灼。激光处理时气泡的产生 (其指示布鲁赫膜的破裂) 是取得 CNV 中的重要因素;因此,此研究中仅包括其中产生气泡的烧灼。

[0470] 进行四个独立的实验以研究在布鲁赫膜破裂后第 0 天口服施用时临床开发候选物的效果。第 1-4 组中的小鼠每日分别口服施用剂量为 0.3、1、3 和 10mg/kg 的临床开发候选物。第 4 组仅接受载体物。

[0471] 14 天后,将小鼠麻醉并灌注荧光素标记的葡聚糖 ( $2 \times 10^6$  平均分子量, Sigma-Aldrich), 制备脉络膜平面封固 (flat mount)。简单地说,移出眼睛,在 10% 磷酸盐缓冲的福尔马林中固定 1 小时,并移除角膜和晶状体。将整个视网膜仔细地从视杯切开,从视杯边缘向所有四个象限的平分线做径向切口,将视网膜在水性固定介质 (Aquamount ;BDH, Poole, UK) 中平面封固。通过荧光显微镜术 (Axioskop ;Carl Zeiss Meditec, Thornwood, NY) 检查平面封固,并用三电荷耦合器件 (CCD) 彩色摄像机

(1K-TU40A, Toshiba, Tokyo, Japan) 对图像进行数字化。使用帧接受器图像分析软件来测量每个 CNV 病变的面积。采用 ANOVA 进行统计比较, 在多重比较时采用 Dunnett 校正。

[0472] 脉络膜新血管形成的抑制的鼠模型

[0473] 虽然动物本身不发展年龄相关性黄斑变性 (AMD), 但可以通过使用激光在布鲁赫膜及覆盖的视网膜色素上皮 (RPE) 中产生局灶破坏来产生类似于在 AMD 中所见的脉络膜新血管形成。这种伤害刺激下方的脉络膜毛细管异常生长进入 RPE 层和视网膜下腔。布鲁赫膜的破坏常见于所有形式的脉络膜新血管形成 (CNV), 包括表征湿型 AMD 的那些。

[0474] 在经激光诱导的脉络膜新血管形成模型中, 具有 9 或 10 只小鼠的组通过口服 (1) 临床开发候选物, 或 (2) 在激光损伤前一天及激光后第 2、5、8 和 11 天的无效治疗进行处理。在激光损伤后 14 天时, 向小鼠静脉内注射荧光素标记的葡聚糖 (50mg), 使其安乐死, 并将眼睛迅速切出以制成脉络膜平面封固, 或在包埋化合物的最佳切削温度下冷冻并切片以评估病变。

[0475] 通过荧光素血管造影术使 CNV 病变可视化并根据标准程序分级。

[0476] 实施例 7 : 在慢性的光诱导的脉络膜新血管形成中的疗效研究

[0477] 目的 : 本研究的目的是使用 Wistar 大鼠, 每天一次口服 0.3mg 和 3mg/kg/ 天的临床开发候选物治疗 3 个月 (90 天), 测试其在体内防护 3000 勒克司的光损伤的有效性。在大鼠中的长期光损伤 (3 个月) 已被表明会导致光感受器变性及脉络膜新血管形成 (CNV)。评估示例性临床开发候选物在防护光诱导的 ONL 丧失及 CNV 中的疗效。

[0478] 材料与方法 : 在开始给药前一天及每周一次, 共 13 周, 将临床开发候选物称重放入新的空玻璃闪烁小瓶中。将临床开发候选物溶解在去离子水中直至在所需的剂量体积 (0.5mL/ 动物) 下达到所需剂量需要的浓度。将给药溶液贮存于 4°C 并用于每天给药一次, 共 1 周。用于对照组给药的载体物为去离子水。本研究中使用 16 只雌性 Wistar 大鼠 (Charles River Laboratories)。开始给药时动物约为 12 周龄并且平均体重为 220 克。

[0479] 测定 : 每天一次在早上 (在光开启后 1 小时内), 使用配有 20 号口饲针的 1mL 注射器通过口服、管饲向动物施用分配的载体物对照物或测试制品。将动物置于循环光下, 以使笼子的中心有 12 小时的 3000 勒克司白光, 及 12 小时的黑暗交替。完成研究后, 用二氧化碳使动物安乐死, 接着创造气胸。在颈椎脱位后立即移出动物的两只眼睛以进行分析。此分析由切片染色和平面封固分析组成。在室温下, 将视杯在 4% PFA 中固定 1 小时。将一个视杯进行处理以供石蜡包埋, 切片并用 H&E 或异凝集素 B4 染色。将另一只眼睛固定以用于平面封固。将平面封固的眼睛切开至视网膜和脉络膜 / 巩膜综合体。用异凝集素 B4 对视网膜、脉络膜 / 巩膜综合体进行染色。

[0480] 研究设计

[0481]

治疗 指定 及动物分配 组	治疗	剂量 (mg/kg)	3000	每组的动物数	总计
			勒克司 暴露		
NC (1,2)	载体物	NA	否	2	4
3,2	载体物	NA	是	2	4
5,6	ACU-4429	3	是	2	4
7,8	ACU-4429	0.3	是	2	4

[0482] NC = 正常光对照

[0483] 数据分析：用 H&E 染色后，通过显微镜检查眼睛切片，并将靠近视神经的 ONL 区在  $40 \times 10$  的放大倍数下拍摄照片以供外核细胞计数。显微镜照片打印在  $8" \times 11"$  纸张上。计数被两条垂直线贯穿的、均匀地分散在照片上的 ONL 细胞核的数目，平均细胞数代表该眼睛的 ONL 厚度。用异凝集素 B4 将石蜡切片染色以确定是否存在脉络膜新血管形成。异凝集素 B4 可将血管染色。（参见图 16）。为了量化脉络膜新血管形成，对每一切片计数从脉络膜跨越并通过视网膜的血管数目，并在 Excel 中进行分析。在 10-33 个切片中计数血管并以每只动物的平均值报告数据。由于平面封固数据尚无定论，故在该报告中将其排除。

[0484] 结果

[0485] ONL 原始计数

[0486]

条件	DC				LC				4429, 3 mg/kg				4429, 0 3 mg/kg			
动物 #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

[0487]

计数 1	8	7	11	10	1	2	0	1	2	1	1	1	0	0	1	1
计数 2	8	6	9	12	1	1	0	1	3	2	1	1	1	1	1	2
计数 3	10	9	10	9	1	0	1	1	1	2	2	1	0	1	1	2
计数 4	10	9	10	9	1	0	1	2	3	2	2	1	0	1	1	1
平均值	9.0	7.8	10.0	10.0	1.0	0.8	0.5	1.3	2.3	1.8	1.5	1.0	0.3	0.8	1.0	1.5
组平均值	9.2				0.9				1.6				0.9			

[0488] 单因素 ANOVA

[0489]

Tukey 多重比较检验	平均差	q	P 值
LC (载体物) 相对于 DC	-8.325	25.25	P < 0.01
LC(载体物)相对于 4429, 3 mg/kg	-0.750	2.275	P > 0.05
LC (载体物) 相对于 4429, 0.3 mg/kg	0.00	0	P > 0.05
4429, 0.3 mg/kg 相对于 3 mg/kg	-0.750	2.275	P > 0.05

[0490] 图 22 说明在来自用环境光及 3000 勒克司 / 载体物或临床开发候选物处理的动物的 H&E 切片中, 外核层中的细胞核行数。数据为平均值 ± SEM。

[0491] 原始血管计数

[0492]

条件	DC				LC				3 mg/kg				0.3 mg/kg			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
血管计数	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	1	2	1	0	0	0	0	0	0	0	0	0

[0493]

条件	DC				LC				3 mg/kg				0.3 mg/kg				
	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	1
	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	0	0	0	0	0	2	2	2	0	0	1	0	1	0	0	2	
	0	0	0	0	0	1	0	2	0	0	1	0	0	0	0	0	1
	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0
	0	0	0	1	1	0	3	1	2	0	0	0	0	0	0	0	0
	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0
	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	
	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0		

[0494]

条件	DC				LC				3 mg/kg				0.3 mg/kg			
	0	0	1		0		0		0	0	0	1	0	1		
	0	0				0		0		0	0					
	0					0		0		0	0					
	0						0		0		1					
	0							0		0	0					
	0								0		0					
	0								0		0					
	0									0						
平均值	0.03	0	0	0	0.30	0.38	0.65	0.41	0.19	0.16	0.16	0.14	0.03	0.15	0	0.24
组平均值	<b>0.008</b>				<b>0.437</b>				<b>0.162</b>				<b>0.104</b>			

[0495] 单因素 ANOVA

[0496]

Tukey 多重比较检验	平均差	q	P 值
LC(载体物) 相对于 DC	0.4296	9.046	P<0.01
LC(载体物) 相对于 4429, 3mg/kg	0.2755	5.801	P<0.01
LC(载体物) 相对于 4429, 0.3mg/kg	0.3328	7.008	P<0.01
4429, 0.3mg/kg 相对于 3mg/kg	-0.0573	1.207	P>0.05

[0497] 图 23 说明穿越各层 / 切片的血管数。

[0498] 结论: 临床开发候选物保护视网膜免于由光诱导的 ONL 变薄。用临床开发候选物进行的治疗提供对抗脉络膜新血管形成的重要保护。

[0499] 实施例 8: ACU-4429 (一种新颖的视觉周期调节剂) 在健康志愿者中的 I 期剂量范围确定研究

[0500] 视觉周期调节 (VCM) 是指光子在视网膜中生物转化成电信号。(参见, 例如, 图 4A 和 4B)

[0501] 视网膜含有被称为“视杆”(负责夜间视觉)和“视锥”(负责白昼视觉)的光接受器细胞。视杆细胞比视锥细胞数目多得多且更活跃。视杆的过度活跃造成毒素在眼内的积聚, 而视锥提供我们绝大多数的视觉信息—包括颜色。VCM 基本上“减缓”视杆的活动并减少视锥上的代谢负荷。

[0502] 异构酶 /RPE65 代表一个抑制目标,因为它对视觉周期是特异性的。视杆细胞为 A2E 的主要来源 (光感受器细胞中的 90% )。

[0503] A2E 毒性 :

[0504] • 在光暴露时的自由基产生 ;

[0505] • 去污剂样性质可损伤 RPE 细胞膜 ;

[0506] • 抑制 RPE 溶酶体 (导致玻璃疣形成) ; 及

[0507] • 补体因子的活化。

[0508] ACU-4429 被设计成防止或抑制视觉周期的毒性副产物的产生,这可导致退化性眼疾病。它以口服的每日丸剂形式施用于患者,而非注射到眼内。临床前数据指出,ACU-4429 减缓视杆的视觉周期。

[0509] 1 期数据 :

[0510] 在 55-80 岁的健康志愿者中观察安全性和耐受性。通过视网膜电流扫描计术 (ERG) 观察视觉周期的剂量依赖性调节。

[0511] 临床安全性和耐受性

[0512] 向 125 名健康受试者施用 ACU-4429。ACU-4429 在健康受试者中耐受良好,没有与 DMC 有关的不良事件。在一些受试者中出现头痛,但是为短暂的且可能与药物无关。观察到轻度及短暂的视力不良事件。ACU-4429 产生非常良好的药理学反应,即使在较低的剂量下。视锥 ERG 中未观察到任何变化。

[0513] 总体而言,ACU-4429 具有口服生物利用度。剂量与 AUC 和  $C_{max}$  之间具有线性相关性,并且在首次剂量后达到稳定状态。观察到 ERG b 波振幅的剂量依赖性下降。

[0514] AUC 大致随剂量成比例增加,因此,在诊所可通过增加或减少 ACU-4429 的口服剂量很容易地调整全身性暴露。最大血浆浓度 ( $C_{max}$ ) 也随剂量呈线性增加。ACU-4429 很容易地从胃肠道吸收。(参见图 7)。ACU-4429 的 1a 期视杆 ERG 抑制 (24 h) 示于图 6 中。

[0515]

剂量	抑制
20mg	29% $\pm$ 35%
40mg	86% $\pm$ 10%
60mg	93% $\pm$ 4%
75mg	98% $\pm$ 1%

[0516] 1b 期研究设计

[0517]

<b>研究设计</b>	单中心、随机、双盲、安慰剂对照的、多剂量递增研究
<b>目的</b>	评估安全性、耐受性及药代动力学 (PK)
<b>剂量</b>	5 个小组, 以 6:2 随机化 5、10、20、30、40 mg 14 天/小组
<b>终点</b>	安全性、耐受性及 PK
<b>主要纳入标准</b>	两种性别的健康志愿者, 年龄 55-80, 体重 $\geq 50$ 且 $\leq 110$ kg
<b>主要排除标准</b>	<ul style="list-style-type: none"> <li>眼睛病况 (白内障、青光眼、葡萄膜炎、糖尿病性视网膜病及活动性结膜炎)</li> <li>在 28 天内更改慢性处方用药</li> <li>过去一年用类维生素 A 化合物治疗</li> </ul>

[0518]

	<ul style="list-style-type: none"> <li>上周内用 Viagra<sup>®</sup>、Cialis<sup>®</sup>、Levitra<sup>®</sup> 治疗</li> <li>伴随用以下药物治疗: 安眠药、抗抑郁药和精神活性物质; 洋地黄糖苷类(地高辛、哇巴因、洋地黄毒苷); L-DOPA; 氯喹或羟氯喹; 全身性皮质类固醇; 局部抗青光眼药物; 用于治疗“湿型”AMD 的药物</li> </ul>
--	---

[0519] 1b 期—人口统计学

[0520]

	ACU-4429 N=30	安慰剂 N=10
年龄, 平均值 (SD)	39.8 (8.48)	37.7 (8.55)
男性, n (%)	22 (73.3%)	8 (80%)
种族, n (%)		
白人	25 (83.3%)	5 (50.0%)
黑人或非裔美国人	5 (6.7%)	3 (30%)
亚洲人	0	1 (10%)
其他	0	1 (10%)

[0521] 1b 期—不良事件总结

[0522]

小组	具有视觉不良事件的受试者数目	视觉不良事件数目
5 mg	0	0
10 mg	2	21*

[0523]

20 mg	6	29
30 mg	6	26
40 mg	6	33

[0524] \*1 位受试者具有 19 项视觉不良事件 ;所有视觉不良事件均为轻度。

[0525] 1b 期 PK 数据

[0526]  $C_{max}$  为第一个及最后一个剂量后约 4 小时 ;PK 参数类似于 1a 期研究 ;第一个剂量后水平达到稳定状态。(参见图 7)。

[0527] 实施例 9 :用于测试 ACU-4935 是否降低由低氧条件造成的 VEGF 上调的实验

[0528] 图 8 描述用于测试 ACU-4935 是否降低由低氧条件造成的 VEGF 上调的方案。简单地说,使动物暗适应 16 小时,然后给予 ACU-4935。给药后 2 小时,以 50000 勒克司对动物进行光漂白 10 分钟,随后在黑暗中恢复 2 小时。以 6%  $O_2$  诱导低氧 6 小时。使一部分动物安乐死并在时间 0 时采集样品。将另一部分动物送回黑暗中超过 2 小时,然后施以安乐死并采集样品。

[0529] 检测样品的 VEGF 蛋白质 (图 9) 和 mRNA 表达 (图 10)。用 ACU-4935 治疗后观察到 VEGF 蛋白质表达有轻微差异。与载体物对照相比,用 ACU-4935 治疗后 VEGF mRNA 水平在时间 0 时降低,在低氧后 2 小时稍微增加。

[0530] 实施例 10 :[14C]-ACU-4429 在比格犬中的眼睛分布

[0531] ACU-4429 ( $C_{16}H_{25}NO_2 \cdot HC1$ ) 是一种口服视觉周期调节剂,已表明其降低视杆视觉系统的活性,从而减轻视网膜上的代谢负荷。

[0532] 进行下列实验来检查在 0.3mg/kg [<sup>14</sup>C]-ACU-4429 (40  $\mu$  Ci/kg) 单一和重复口服剂量后,ACU-4429 及其代谢产物在雄性比格犬中的药代动力学谱、眼睛分布和排泄。

[0533] 以单一口服剂量或重复剂量 (每日一次,连续 7 天) 的形式向未禁食的总共 36 只雄性比格犬施用作为胶囊中的粉末的 [<sup>14</sup>C]-ACU-4429 (0.3mg/kg, 40  $\mu$  Ci/kg)。在单一剂量后的 168 小时内,或在第一个每日剂量后的 336 小时内,评估质量平衡;分析尿液和粪便中的放射性和代谢特征谱。在最后一个剂量后的 0.25、1、2、4、8、12、48、72、96、168 和 192 小时收集血液;分析血液和血浆的放射性及血浆的代谢特征谱。在最后一个剂量后 4、8、12、24、48、72 和 168 小时 (3 只动物 / 时间点) 收集眼组织 (脉络膜、虹膜-睫状体 (iris-capillary body) 和 RPE) 并分析放射性 (右眼) 或代谢特征谱 (左眼)。

[0534] 在比格犬中,口服施用的 [<sup>14</sup>C]-ACU-4429 很容易被吸收 ( $T_{max} = 4$  小时) 并从血浆中排除;大部分放射性并不优先与 RBC 结合。放射性通过尿液和粪便 (分别为 46% 和 44%) 迅速排除,而从血浆中的清除到剂量后 48 小时基本上完成。其他数据指出,ACU-4429 母体分子优先分布至含有黑色素的眼组织,包括 VCM 作用的建议位点, RPE, 虽然有快速系统性清除 (见图 11 和 12)。连续 7 天口服给药后,眼组织中的 ACU-4429- $C_{max}$  比血浆中高出 278 倍 (930 相对于 3.34ng-eq/g) (图 11)。

[0535] 参考文献

[0536] <sup>1</sup>Kubota 等人, Retina, 2012, 32(1):183-188.

[0537] <sup>2</sup>Sparrow 等人, Vision Res., 2003, 43(28):2983-2990;Travis 等人, Ann. Rev. Pharmacol. Toxicol., 2007:47:469-512.

[0538] 实施例 11 :VCM 作为视网膜新血管形成的抑制剂

[0539] 在黑暗条件下,视网膜中的离子通道开启,从而允许多余的离子流入视网膜细胞。视网膜需要能量及氧泵出多余的离子流。在正常的健康条件下,对视网膜的血液供应刚刚足以支持此过程,这比其他细胞的任何功能产生更多的热量及消耗更多的氧。若血液供应

受损（如在糖尿病患者中经常发生的），会在视网膜中发生低氧。视网膜产生新的、小的渗漏血管来进行弥补，从而导致增生性糖尿病性视网膜病。

[0540] 视觉周期调节剂 (VCM)（如 ACU-4420 和 ACU-4935）抑制视觉周期异构酶<sup>2</sup>，从而模拟组成性光转导的状态并减少暗电流（见图 14）。不受理论所限制，据认为减少暗电流将会降低视网膜中的代谢压力和相关的氧需求，这应可减少低氧、低氧诱导的因子 1 (HIF-1  $\alpha$ ) 及血管内皮生长因子 (VEGF) 的产生，并导致对新血管生长的抑制。

[0541] 此研究评估 VCM ACU-4429 和 ACU-4935 在由氧诱导的视网膜病 (OIR) 的小鼠模型中对视网膜新血管形成的影响。<sup>3-5</sup>

[0542] 如图 15 中所示处理 129SvE 小鼠幼崽 (P0)。腹膜内施用 ACU-4429 (0.03 至 10mg/kg)、ACU-4935 (0.3mg/kg/ 天)、阳性对照物 (10mg/kg/ 天芦布妥林) 或载体物 2 次，共 4 天。

[0543] ACU-4429 及 ACU-4935 的参数

[0544]

VCM	化学式	IC <sub>50</sub> (体外异构酶活性)	ED <sub>50</sub> (体内异构酶分析单剂量, 小鼠)
ACU-4429	C <sub>16</sub> H <sub>25</sub> NO <sub>2</sub> ·HCl	4.4 nM	0.18 mg/kg
ACU-4935	C <sub>17</sub> H <sub>29</sub> NO <sub>2</sub>	5.2 nM	0.0004 mg/kg

[0545] 在 P17 使幼鼠安乐死，此时新血管形成达到最多，并移出眼睛以供分析。欲提取类维生素 A 时，在 P16 将小鼠移至暗室并在红灯下进行安乐死。

[0546] 利用平面封固制品的异凝集素染色对视网膜新血管形成区域进行可视化，并以 Adobe Photoshop 中的套索工具对其定量；新血管形成的总面积表示横跨视网膜的各个区域的总和，并且新血管形成 % 是相对于视网膜的总面积<sup>4</sup>。在红光下从右眼提取出类维生素 A 并分析 11- 顺式 -ROL- 脂含量以指示 11- 顺式 -ROL 浓度并作为周期异构酶活性的指示。

[0547] 使用 GraphPad Prism 软件进行统计分析。

[0548] 在具有 OIR 的小鼠中，与载体物处理相比，用 ACU-4420 或 ACU-4935 治疗显著降低了视网膜新生血管的面积。ACU-4429 (3mg/kg/ 天) 使视网膜新生血管面积减少 32%，ACU-4935 (0.3mg/kg/ 天) 使之减少 23%，芦布妥林 (10mg/kg/ 天，阳性对照) 使之减少 29%；这两种 VCM 的平均减少量显著 ( $p < 0.05$ ) 高于载体物，但与芦布妥林并无显著差异 ( $p < 0.05$ )。

[0549] ACU-4429 以剂量依赖的方式抑制新血管形成及 11- 顺式 -RAL 的产生，ED<sub>50</sub> 值分别为 0.46mg/kg 和 0.88mg/kg。

[0550] 参考文献

- [0551] 1. Arden 等人, Br. J. Ophthalmol., 2005;89(6):764-769.
- [0552] 2. Kubota 等人, Retina, 2012;32(1):183-188.
- [0553] 3. Chan 等人, Lab. Invest., 2005;85(6):721-733.
- [0554] 4. Connor 等人, Nat. Protoc., 2009;4(100):1565-1573.
- [0555] 5. Yoshida 等人, FASEB J., 2010;24(6):1759-1767.

[0556] 实施例 12 : 视网膜电流扫描计术材料与方法

[0557] 闪光的校准

[0558] 使用带有 ColorDome Ganzfeld 刺激器的 Espion e<sup>2</sup> (Diagnosys LLC, Lowell, MA) 递送 ERG 刺激。通过测量投射在位于大鼠角膜位置处的积分放射计 (IL1700 ; International Light, Newburyport, MA) 上的通量密度，并按照由 Lyubarsky 和 Pugh (1996) 所详述的程序来计算对于绿色 LED 闪光的每视杆的光异构化速率 (R\*)。该 LED 被视为单色光，而 1 等于 530nm。闪光的强度由以下公式得到

[0559]

$$i(\lambda) = Q(\lambda) \cdot T(\lambda) \cdot \frac{a_{瞳孔}}{a_{视网膜}} \cdot a_{视杆}(\lambda) \quad (1)$$

[0560] 其中  $i(\lambda)$  为  $R^*$ ,  $Q(\lambda)$  为计算出的角膜处的光子密度,  $T(\lambda)$  为眼介质及接受器前视网膜 (pre-receptor retina) 的透射率 (约 80% 在 530nm 处 ;Alpern 等人, 1987),  $a_{瞳孔}$ 、 $a_{视网膜}$  和  $a_{视杆}(\lambda)$  分别为放大的瞳孔的面积 (约  $\text{mm}^2$  ;Dodd 和 Echte, 1961)、视网膜表面的面积 (约  $50\text{mm}^2$  ;Hughes, 1979) 及视杆光感受器的端点光收集面积 (约  $1.5\text{mm}^2$  在 530nm 处) 的估计值。 $a_{视杆细胞}(\lambda)$  考虑外节段的长度、视杆的吸收谱和感光色素的光密度, 以及光感受器的半径 (Baylor 等人, 1979)。由于受 OIR 影响的大鼠视杆的这些参数值中有几个数值未知, 刺激被表示为成年对照大鼠的预期值。 $Q(\lambda)$  通过下式得出

$$[0561] \quad Q(\lambda) = \lambda \cdot \frac{P_\lambda}{h \cdot c} \quad (2)$$

[0562] 其中  $P_\lambda$  为辐射通量 (W),  $h$  为普朗克 (Plank) 常数, 而  $c$  为光速 (Wyszecki 和 Stiles, 1982)。为了评估“白”氙弧闪光的强度, 记录散开的绿色和白色闪光的强度系列, 而根据暗视 b 波的刺激 / 响应曲线的移动来估计该等同的光。

[0563] 漂白光的校准

[0564] 使用具有 EXR 300W 卤素灯 (色温  $3350^\circ$  K) 的 Ektographic III B 幻灯机 (Eastman Kodak, Rochester, NY) 产生漂白。为了将光漫射, 将对切的乒乓球放置在眼睛上。将投影机放置在一个平台上从而使其镜头距离球的表面约 6cm。使用布置在大鼠头部位置的乒乓球下方的放射计 (其集成功能关闭) 测量光的功率。假设  $\lambda = 500\text{nm}$ , 使用公式 (2) 计算投射在光检测器上的光子数 (量子  $\text{cm}^{-2}\text{s}^{-1}$ )。通过下式估计漂白的强度

$$[0565] \quad R_0(t) = \exp\left(-\frac{Q(\lambda) \cdot t}{Q_e}\right) \quad (3)$$

[0566] 其中  $1-R_0$  为光暴露结束时漂白的视紫红质的分安徽,  $t$  为暴露的持续时间 (60s), 而  $Q_e$  (量子  $\text{cm}^{-2}$ ), 光敏度的倒数, 为使  $1/e$  的视紫红质未漂白所需的能量 (Perlman, 1978)。早期的测量指示, 在 Sprague Dawley 大鼠中的  $Q_e$  值为约  $15.8 \log$  量子  $\text{cm}^{-2}$  (Fulton 和 Baker, 1984)。因此, 产生约  $15.9 \log$  量子  $\text{cm}^{-2}$  的光将视网膜中的约 60% 视紫红质漂白。

[0567] 准备

[0568] 用腹膜内注射的约  $75\text{mg kg}^{-1}$  氯胺酮和  $8\text{mg kg}^{-1}$  甲苯噻嗪的加载剂量麻醉经暗适应的受试者。随后肌肉内施用加强剂量 (加载剂量的 50 %)。用 1 % 盐酸去氧肾上腺素和 0.2 % 盐酸环戊通 (Cyclomydril ;Alcon, Fort Worth, TX) 的组合将瞳孔

放大。用一滴 0.5% 盐酸丙美卡因将角膜麻醉。将 Burian-Allen 双极电极 (Hansen Laboratories, Coralville, IA) 放置在角膜上并将接地电极放置在尾部。熄灭红灯, 将动物保持在完全黑暗中另外 10 分钟以使其在实验开始前回复至完全暗适应的状态。

[0569] 光转导的活化

[0570] 在第一次测试日期, 将动物分配为一半危险 (half-hazard), 从而使每窝的一半 (如果是单数则向上舍入) 参与光转导的活化和去活化以及感受器后视网膜功能的研究; 其余的参与漂白实验。通过将涉及光转导活化的 Lamb 和 Pugh (1992; Pugh 和 Lamb, 1993) 生化过程模型的 Hood 和 Birch (1992) 公式的参数拟合至由五次最亮闪光所引起的 a 波, 从 ERG 估计视杆光响应的特性:

[0571]

$$P_3(i, t) = Rm_{p3} \cdot (1 - \exp(-1/2 \cdot i \cdot S \cdot (t - t_d)^2))$$

对于  $t_d < t < 20 \text{ ms}$ . (4)

[0572] 在此模型中,  $i$  为闪光强度 ( $R^*$ ), 且  $t$  为经过的时间 (s)。模型中的自由参数  $Rm_{p3}$ 、 $S$  和  $t_d$  的数值使用将平方偏差总和最小化的常规程序 (fmins; MATLAB R11, The Math-works, Natick, MA) 进行优化。 $Rm_{p3}$  为饱和视杆响应的振幅 ( $\mu \text{V}$ ); 其与暗电流的幅度成比例且取决于 ROS 中可通过光关闭的通道数 (Lamb 和 Pugh, 1992; Pugh 和 Lamb, 1993), 其在正常条件下转而直接取决于 ROS 的长度 (Reiser 等人, 1996)。 $S$  为敏感性 ( $R^{*-1}S^{-2}$ ) 参数, 如果正确地具体指明刺激强度, 则其与放大常数  $A$  (其总结由视紫红质光异构化所引发的一系列过程的动力学) 相关, 并造成光感受器质膜中的通道的关闭。 $t_d$  为短暂延迟 (s)。模型的拟合局限于 a 波的前缘。

[0573] 光转导的去活化

[0574] 在相同的大鼠中, 使用双闪光范式得出视杆对产生约  $150R^*$  的‘绿色’ ( $\lambda_{\max} \approx 530\text{nm}$ ) 条件化闪光 (CF) 的响应的时间过程。此绿色闪光虽然引发小于饱和视杆响应的一半的 a- 波, 但仍然足以完全抑制暗电流。首先, 单独记录对 CF 的响应。然后, 测定对强烈的、视杆饱和 (约  $10,000R^*$ ) 的‘白’氙弧探针闪光的响应的振幅。在出现后 8 毫秒 (就在 a 波谷底之前) 测量的 PF 响应的振幅  $a_{\max}$  ( $\mu \text{V}$ ) 被认为与最大视杆暗电流成比例。接着, CF 和 PF 一起出现, 相隔 10 个预定的刺激间隔 (10ms、20ms、50ms、0.1s、0.15s、0.2s、0.4s、0.7s、1 s 及 1.4s)。在双闪光条件下, 单独记录的对 CF 的响应作为用于测量在每个刺激间时间  $t$  处对 PF 的响应的振幅  $a_{\text{sat}, t}$  的基线。因此, 在经过时间  $t$  时被 CF 抑制的暗电流的比例  $SF_t$  由下式得出

[0575]  $SF_t = 1 - \frac{a_{\text{sat}, t}}{a_{\max}}$  (5)

[0576] 为了得到去活化的时间过程的数值, 确定视杆响应的波谷并拟合通过恢复期的线。记录达到 50% 恢复前的等待时间 (ms)。

[0577] 感受器后功能

[0578] 在相同的动物中, 从 ERG 的 b 波评估由视杆细胞介导的感受器后功能。使用一系列 13 个产生约  $0.075$  至  $300R^*$  的‘绿色’闪光来引发 b 波响应。针对这类响应的振幅 ( $\mu \text{V}$ ), 优化 Naka-Rushton 函数的参数,

$$[0579] \quad \frac{V(i)}{V_m} = \frac{i}{i + \sigma} \quad (6)$$

[0580] 在此公式中,  $V(i)$  为对  $i$  强度的闪光 ( $R^*$ ) 的响应的振幅,  $V_m$  为  $b$  波的饱和振幅,  $a$  为引起具有  $V_m$  一半振幅的  $b$  波的强度。该函数仅适配于首先观察到  $a$  波侵入的那些强度。若  $i$  被正确地具体指明, 则  $\log \sigma$  为感受器后敏感性的测量值。

[0581] 从光漂白恢复

[0582] 在第二组在小组中进行的实验中评估从漂白中恢复的暗电流。使用呈现给经暗适应的眼睛的视杆饱和  $PF(10,000R^*)$  来测定暗电流的幅度。在漂白暴露后, 以 2min 的间隔监测对  $PF$  的响应约 40min。每一次, 计算恢复的暗电流的分数 ( $1-SF_t$ )。通过将函数 (7) 的参数最优化来找出到饱和视杆光响应恢复 50% 前的时间  $t_{50}$ ,

$$[0583] \quad t(P) = -t_0 \cdot \ln \left( \frac{P - P_0}{B} \right) \quad (7)$$

[0584] 然后求解  $P = 50\%$  的公式。在此公式中,  $t(P)$  为  $a$  波达到其暗适应值的  $P\%$  所需的时间,  $t_0$  为再生的时间常数,  $P_0$  为暗适应  $a$  波的归一化振幅 (100%), 而  $B$  为一个标量。通常,  $t_{50}$  长于记录期间, 因此通过外推取得。

[0585] 刺激递送

[0586] ERG 刺激的时机和强度受计算机控制。用于评估感受器和感受器后响应敏感性及振幅的、针对强度系列求平均值的刺激间间隔及扫描数目详述于下文中。对于去活化实验, 对条件化闪光的响应为 8 次的平均值, 对探针闪光的响应为 4 次的平均值, 而在双闪光条件下, 所有痕迹均为两次扫描的平均值, 记录间隔为 1 分钟。在漂白实验中, 每 2min 一次递送探针闪光。

[0587] ERG 强度系列

[0588]

光源	强度 <sup>a</sup> ( $R^*$ )	扫描 (最少)	I. S. I. (s)
‘绿色’ LED	0.075	32	0.35
	0.15	24	0.40
	0.30	24	0.45
	0.60	18	0.50
	1.0	18	0.60
	2.5	14	0.75
	5.0	14	1.0
	9.5	11	1.5

	20	11	2.0
	40	8	2.5
	75	8	4.0
	150	6	5.5
	300	6	8.0
氩弧	1000	5	18
	2500	4	27
	5000	4	40
	10,000	3	60
	20,000	3	90

[0589]

[0590] <sup>a</sup>‘绿色’LED 及氩弧闪光的效率 ( $R^*cd^{-1}m^2$ ) 分别在约 150 和约 75 计算。

[0591] 视网膜血管的分析

[0592] 使用非侵入性技术对受试者的两只眼睛评估血管弯曲性,这是此纵向研究中所必需的。本研究中采用的OIR模型的特征在于NV发生率为100%;其特征也在于弯曲的视网膜血管。在患者中,后极为对于高危ROP的诊断而言最重要的区域。相应地,在每一ERG期间后,取得并复合显示出视网膜的主要血管的眼底宽视野图像以显示后极的完整视图,后极在此处被定义为在由涡静脉约束并与视神经头同心的圆内的区域;该涡静脉界定平分线。如前所述识别小动脉,并使用RISA软件测量其弯曲度(Akula等人,2007;Akula等人,2008;Gelman等人,2005;Hansen等人,2008;Martinez-Perez等人,2002,2007)。简单地说,从主图像获取各血管并单独分割。若需要时,手工编辑分割后的图像,以去除无关的特征,如背景脉络膜脉管系统。RISA构建骨架并标记终端和分叉点。然后,使用者选择用于分析的血管节段,而RISA自动计算各血管的选定节段的整合曲率,IC。IC捕获任何离开线性过程的偏离,并且是沿着血管的角度的总和,用血管长度对其进行归一化(弧度像素<sup>-1</sup>)。因此,理论上的直血管具有IC=0。高IC值充分捕获医生很可能会指明为弯曲的血管。对于每位受试者,将小动脉弯曲度TA(弧度像素<sup>-1</sup>)计算为两只眼睛中所有可测量的小动脉的平均整合曲率(中位数10)。

[0593] 实施例13:早产儿视网膜病的人体临床试验

[0594] 目的:这项研究的主要目的是评估临床试验候选物在口服施用于具有ROP的新生儿时的安全性。本研究的进一步的目的是根据ROP的严重程度,通过在不同的时间间隔计划的系列眼科检查,评估与接受常规治疗(由ETROP合作组采用的治疗)的对照组中所观察到的相比,临床试验候选物对于减少ROP的进展的疗效。

[0595] 方法:进行介入性先导性随机对照试验,以评估相对于仅使用传统方法(由ETROP

合作组采用的治疗)治疗具有第2期ROP(第II-III区无阳性(plus))的早产儿(胎龄小于32周),除传统方法外还使用临床试验候选物的安全性和疗效。

[0596] 在加入该研究时若符合以下任何排除标准则将该患者排除:(1)早产儿心动过缓事件发作超过10次/天( $HR < 90\text{bpm}$ );(2)心房-心室(A-V)阻滞(第二或第三级);(3)明显的先天性心脏异常(不包括动脉导管未闭、卵圆孔未闭或小室间隔缺损);(4)心力衰竭;(5)低血压(平均血压 $< 45\text{mmHg}$ );(6)低血糖( $< 50\text{mg/dL}$ );及(7)血小板计数 $< 100000/\text{mm}^3$ 。

[0597] 为了比较治疗组和对照组中进展到更严重的ROP的新生儿的比例,考虑正态分布、0.05的 $\alpha$ 误差及80%的指数(power),计算估计的样本大小。每一组的样本大小为22名参与者。从第2期ROP进展到更高期的发病率随着胎龄的减少而增加。为了确保两个组(治疗和对照组)中的胎龄的均匀分布,招募的新生儿将根据胎龄被随机化并分成三个不同的组:第1组(23-25周)、第2组(26-28周)及第3组(29-32周)。

[0598] 在研究开始时,将每个妊娠组中的患者进一步分为两组,一组口服接受剂量为0.5mg/kg/6小时的悬浮液形式的临床开发候选物,另一组则接受悬浮液形式的安慰剂。在治疗和安慰剂两组中,继续进行由ETROP合作组采用的传统治疗。治疗和安慰剂两组均在40周胎龄时接受眼科检查。眼科医生不知道哪些患者接受临床开发候选物,哪些患者接受安慰剂。

[0599] 评估:为了评估临床开发候选物的安全性,持续监测心脏和呼吸系统参数(心跳频率、血压、氧饱和度、呼吸支持)。一旦被诊断为第2期ROP就立即进行血液采样,以检查肾、肝和代谢平衡。使用Kruskal-Wallis检验来评估接受临床开发候选物的新生儿与接受安慰剂的新生儿之间可能的差异。也通过相对风险(RR)来评估安全性。RR被计算为治疗组的副作用几率相对于对照组的比例。RR也可计算为治疗组中ROP进展至更严重的ROP的几率相对于对照组的比例。在此情况下,小于1的RR值与治疗效力相关联。如果有必要,获得每一胎龄组的RR。

[0600] 对于效力,使用最新公布的被设计用来评估视觉功能各方面的一系列行为试验(Ricci等人,Early Hun Dev. 2008年2月;84(2):107-13)在40周胎龄时评估所有新生儿(治疗和对照组),这些行为试验包括以下项目:评估眼球运动(自发行为及响应于目标的行为)、固定并追踪黑/白目标(水平地、垂直地及以弧形)的能力、对彩色目标的反应、区分空间频率逐渐增加的黑色和白色条纹的能力,以及对慢慢远离婴儿移动的目标保持注意力的能力。在1、41/2、12、18和24个月的校正年龄,具体针对视敏度(双眼和单眼)再次评估视觉功能(Ricci等人,J Pediatr. 2010年4月;156(4):550-5),基于优先强迫选择(Teller视敏度卡)、立体视觉和眼球运动力,通过众所周知的工具进行测量。

[0601] 实施例14:脉络膜新血管形成的人体临床试验

[0602] 目的:这项研究的主要目的是评估临床开发候选物在口服施用于患有继发于年龄相关性黄斑变性(AMD)的脉络膜新血管形成(CNV)的患者时的安全性。本研究的进一步的目的是评估与接受安慰剂处理的对照组中所观察到的相比较,该临床开发候选物在治疗继发于年龄相关性黄斑变性(AMD)的脉络膜新血管形成(CNV)中的效力。

[0603] 方法:进行介入性先导性随机对照试验,以比较临床开发候选物相对于安慰剂对患有继发于年龄相关性黄斑变性(AMD)的脉络膜新血管形成(CNV)的患者的安全性和效力。如果符合以下条件,则患者是适格的:(1)其为50岁或以上的男性或女性;(2)其被诊

断为患有原发或复发的继发于 AMD 的中心凹下 CNV, 包括具有主要为典型性、最低典型性或活跃的隐匿性病变但无典型组分的那些病症 ;(3) 在研究的眼睛中, 其 BCVA 得分介于 73 和 24 个字母之间 ( 包括在内 ) ( 斯内伦视力等值为约 20/40 至 20/320) ;(4) 包含在病变内的 CNV 的总面积 ( 包括典型性和隐匿性组分 ) 至少为病变总面积的 50% , 及 (5) 病变总面积不超过 12 个视盘区域。

[0604] 若符合下列条件之一则该患者是不适宜的 : (1) 对侧眼的斯内伦视力等值低于 20/200 的患者 ;(2) 在研究的眼中存在血管样条纹、假定的眼组织胞浆菌病综合征、近视 ( 超过 -8 屈光度 ) 或继发于 AMD 以外的原因的 CNV ;(3) 研究的眼睛中有中心凹下纤维化或萎缩 ;(4) 在研究的眼睛中有玻璃体出血、视网膜撕裂或孔源性视网膜脱离或黄斑裂孔的病史 ( 第 3 或 4 期 ) ;(5) 在筛选前 30 天之内, 在研究的眼睛中有活跃的, 或眼部炎症或感染病史 ;(6) 在研究的眼睛中有不受控的青光眼 ;(7) 在筛选前 30 天之内用维替泊芬、外线束放射疗法、中心凹下焦点激光光凝术、玻璃体切割术、黄斑下手术或经瞳孔热疗法治疗所研究的眼睛 ;(8) 先前用抗血管生成药物 ( 培加尼布、雷珠单抗、贝伐珠单抗、醋酸阿奈可他、皮质类固醇、蛋白激酶 C 抑制剂、角鲨胺、 siRNA 、 VEGF-Trap 等 ) 治疗所研究的眼睛中的新生血管性 AMD ;(9) 所研究的眼睛中的眼内手术史, 包括睫状环玻璃体切割术, 除了超过筛选前 60 天的不复杂的白内障手术外 ; 筛选前 30 日内在研究的眼睛中的 YAG 激光晶状体后囊切开术史。

[0605] 在研究开始时, 将患者分成六组。将剂量为 2 、 5 、 7 、 10 和 20mg/ 天的片剂形式的临床开发候选物通过口服分别施用给前 5 组患者共 3 个月。在同一期间将片剂形式的安慰剂通过口服施用给第六组患者。治疗组和安慰剂组均在每个月底进行眼科检查。眼科医生不知道哪些患者接受临床开发候选物, 哪些患者接受安慰剂。

[0606] 评估 : 为了评估临床开发候选物的安全性, 在口服施用临床开发候选物后监测心脏和呼吸系统参数 ( 心跳频率、血压、氧饱和度、呼吸支持 ) 。也进行血液采样以检查肾、肝脏和代谢平衡。借助于相对风险 (RR) 进一步评估临床开发候选物的安全性。 RR 被计算为治疗组的副作用几率相对于对照组的比例。 RR 也可计算为治疗组中 DR 进展至更严重的 DR 的几率相对于对照组的比例。在此情况下, 低于 1 的 RR 值将与治疗效力相关联。

[0607] 为了评估临床开发候选物的效力, 结果测量包括眼睛和非眼睛不良事件的发生率、从基线开始 3 个月时获得  $\geq 15$  个字母的视敏度 (VA) 的患者的百分比、从基线开始 3 个月时丧失  $\geq 15$  个字母的 VA 的患者的百分比, 以及从基线开始 3 个月时 VA 和中心视网膜厚度 (CRT) 的平均变化。

[0608] 实施例 15 : 与葡萄膜炎相关的视网膜新血管形成的人临床试验

[0609] 目的 : 这项研究的主要目的是评估临床开发候选物当口服施用于患有与葡萄膜炎相关的视网膜新血管形成 (RNV) 的患者时的安全性。本研究的进一步的目的是评估与接受安慰剂处理的对照组所观察到的相比较, 该临床开发候选物对于治疗与葡萄膜炎相关的视网膜新血管形成 (RNV) 的效力。

[0610] 方法 : 进行介入性先导性随机对照试验, 以比较临床开发候选物相对于安慰剂, 对患有与葡萄膜炎相关的视网膜新血管形成 (RNV) 的患者的安全性和效力。如果符合以下条件, 则患者是适宜的 : (1) 其为至少有一只眼睛具有非感染性中间或后葡萄膜炎或全葡萄膜炎的 18 至 70 岁 ( 包括在内 ) 男性或女性患者, 他们在其他方面的健康状况良好 ;(2) 黄

斑水肿,平均中心视网膜厚度 $\geq 250 \mu\text{m}$ ; (3) 玻璃体混浊得分 $\geq 1$ ,但 $\leq 3$ (根据国立眼科研究所(National Eye Institute)分级系统); (4) 最佳矫正视敏度不差于20/400且不优于20/40; 及 (5) 每日泼尼松剂量 $<1\text{mg/kg}$ 。

[0611] 若符合下列条件之一则患者是不格的:(1) 具有脉络膜新血管形成的患者;(2) 具有匐行性脉络膜病、急性多病灶鱼鳞板状色素上皮病或白点视网膜脉络膜病的患者(例如多发一过性白点综合征(MEWDS)或多病灶性脉络膜炎);(3) 与其他眼部疾病(例如糖尿病性视网膜病)相关的黄斑水肿;(4) 先前曾进行玻璃体切割术的患者;(5) 可能影响视敏度及视网膜厚度评估的任何眼睛病况;(6) 某些免疫抑制剂的同时使用(不同试剂的特定清除期在方案中定义);(7) 目前或过去6个月内使用已知对晶状体、视网膜或视神经有毒的全身性药物(例如去铁胺、氯喹及乙胺丁醇);(8) 其他方案定义的纳入/排除标准可适用。

[0612] 在研究开始时,将患者分为六组。将剂量为2、5、7、10和20mg/天的片剂形式的临床开发候选物分别口服施用于前5组患者共3个月。在同一期间将片剂形式的安慰剂口服施用于第六组患者。治疗组和安慰剂组均在每个月底进行眼科检查。眼科医生不知道哪些患者接受临床开发候选物,哪些患者接受安慰剂。

[0613] 评估:为了评估临床开发候选物的安全性,在口服施用临床开发候选物后监测心脏和呼吸系统参数(心跳频率、血压、氧饱和度、呼吸支持)。也进行血液采样以检查肾、肝脏和代谢平衡。借助于相对风险(RR)进一步评估临床开发候选物的安全性。RR被计算为治疗组的副作用几率相对于对照组的比例。RR也可计算为治疗组中DR进展至更严重的DR的几率相对于对照组的比例。在此情况下,低于1的RR值将与治疗效力相关联。

[0614] 为了评估临床开发候选物的效力,在预定的每月眼科检查时由经认证的检查者评估最佳矫正视敏度(BCVA)及中心视网膜厚度(CRT)。结果测量包括眼睛和非眼睛不良事件的发生率、从基线开始3个月时获得 $\geq 15$ 个字母的视敏度(VA)的患者的百分比、从基线开始3个月时丧失 $\geq 15$ 个字母的VA的患者的百分比,以及从基线开始3个月时VA和中心视网膜厚度(CRT)的平均变化。

[0615] 尽管在本文中已经显示并描述了本发明的某些实施方式,但对本领域技术人员显而易见的是,这些实施方式仅作为示例来提供。可以进行多种变化、改变和替代而不偏离实施方式的范围。应当理解,可以使用本文描述的实施方式的多种备选方案。意图以下列权利要求限定本发明的范围并由此涵盖这些权利要求的范围中的方法和结构及其同物。

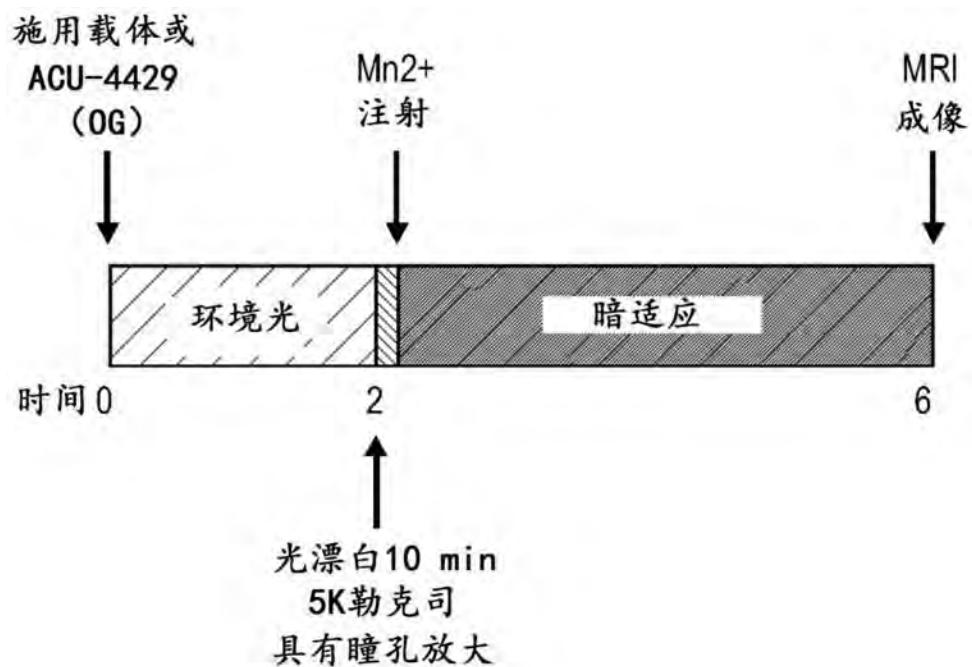


图 1

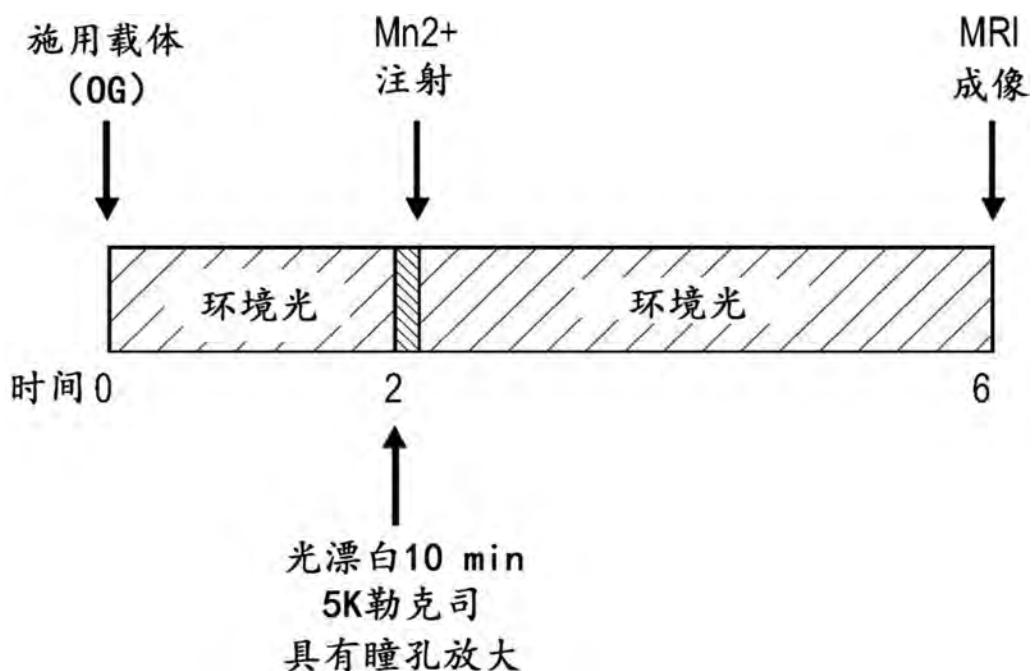


图 2

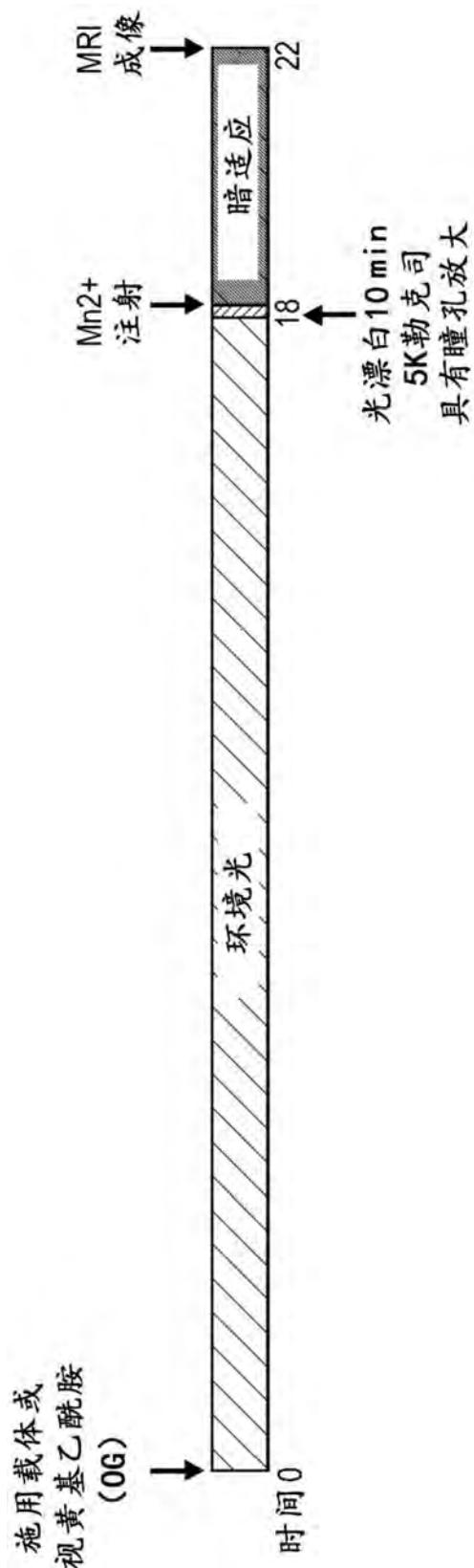


图 3

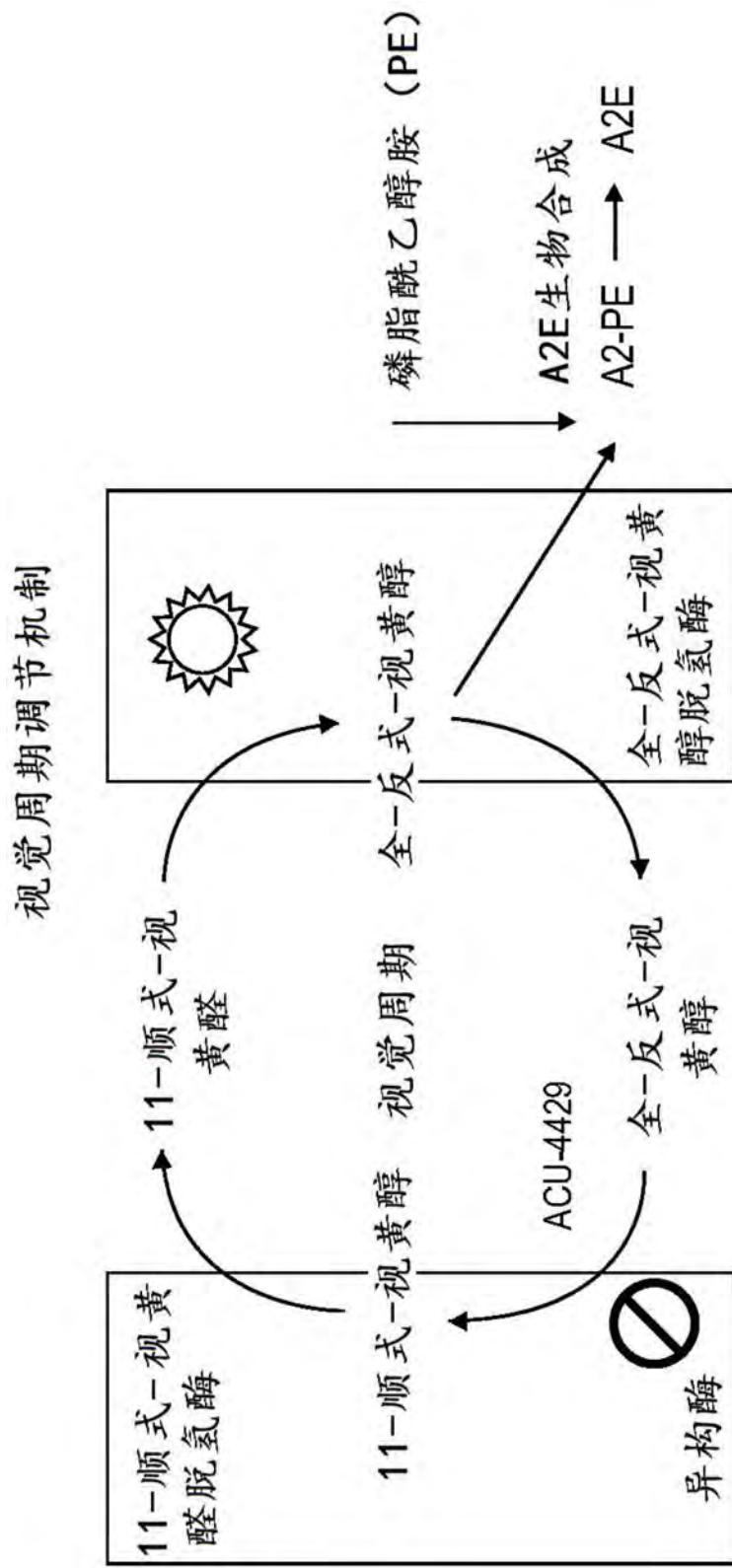


图 4A

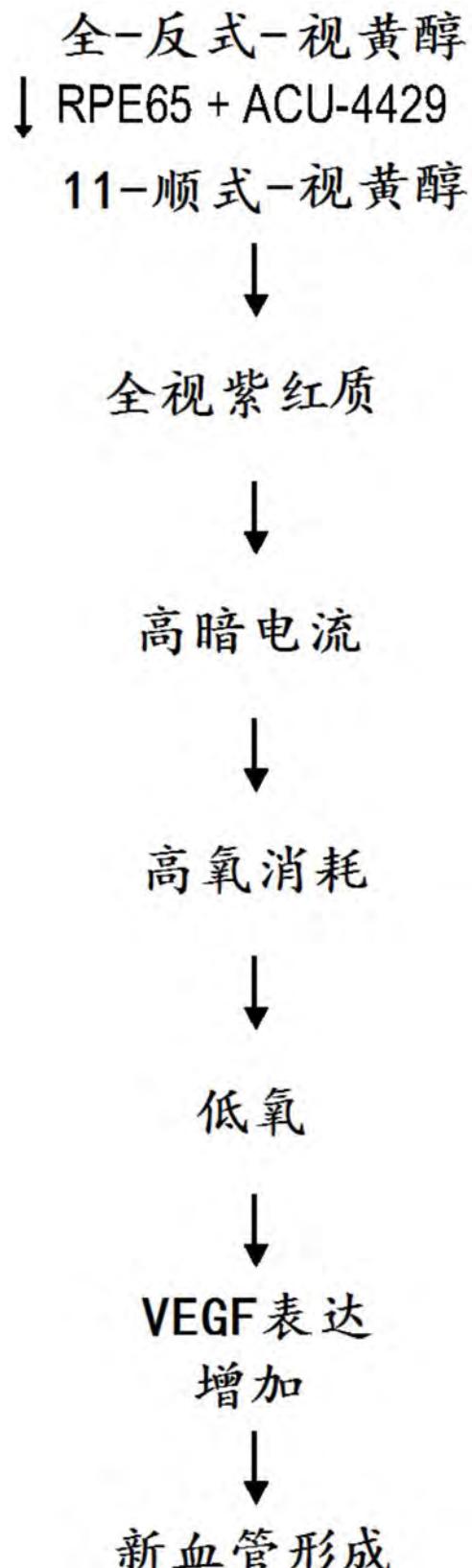


图 4B

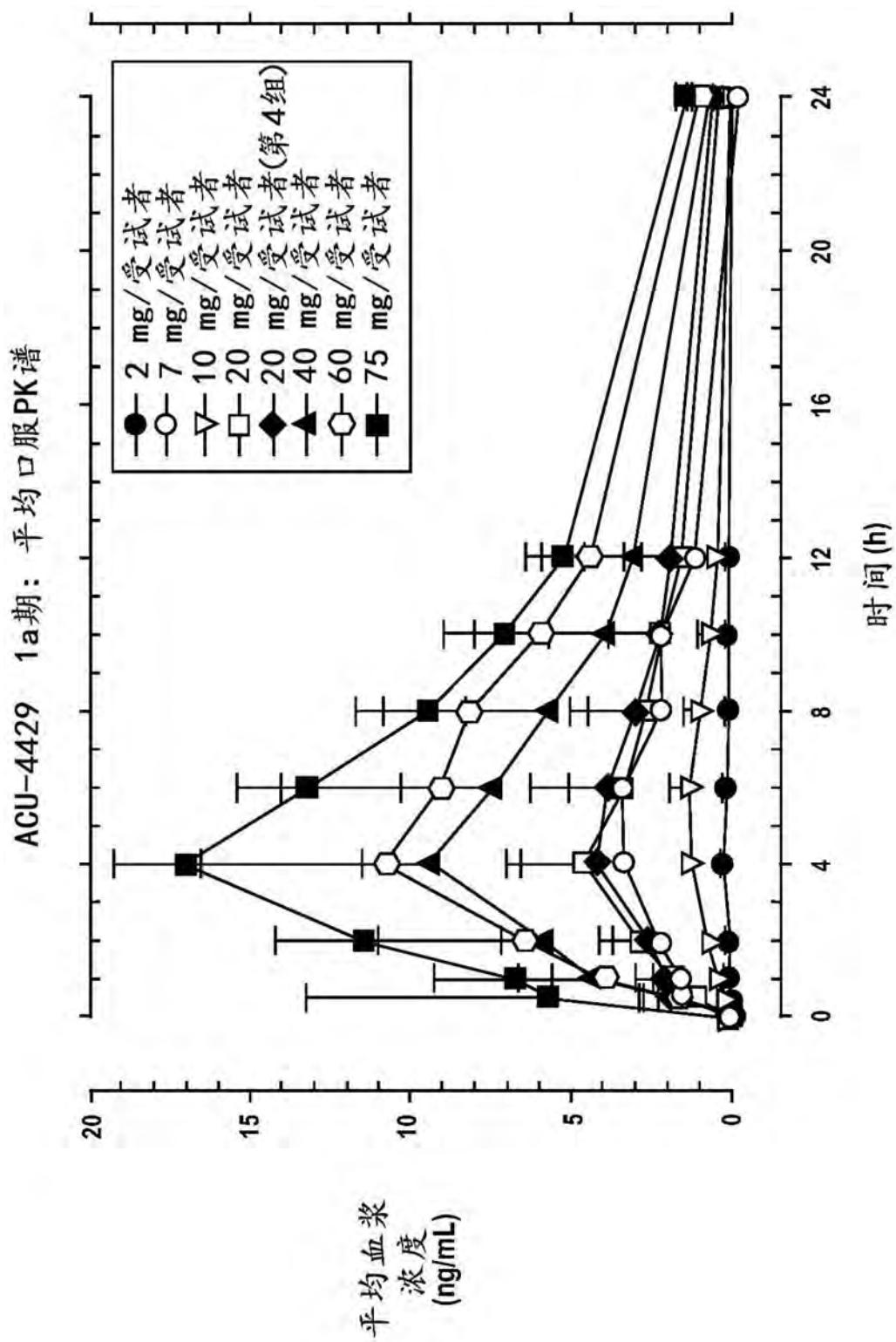


图 5

ACU-4429 1a期：视杆ERG抑制(24 h)

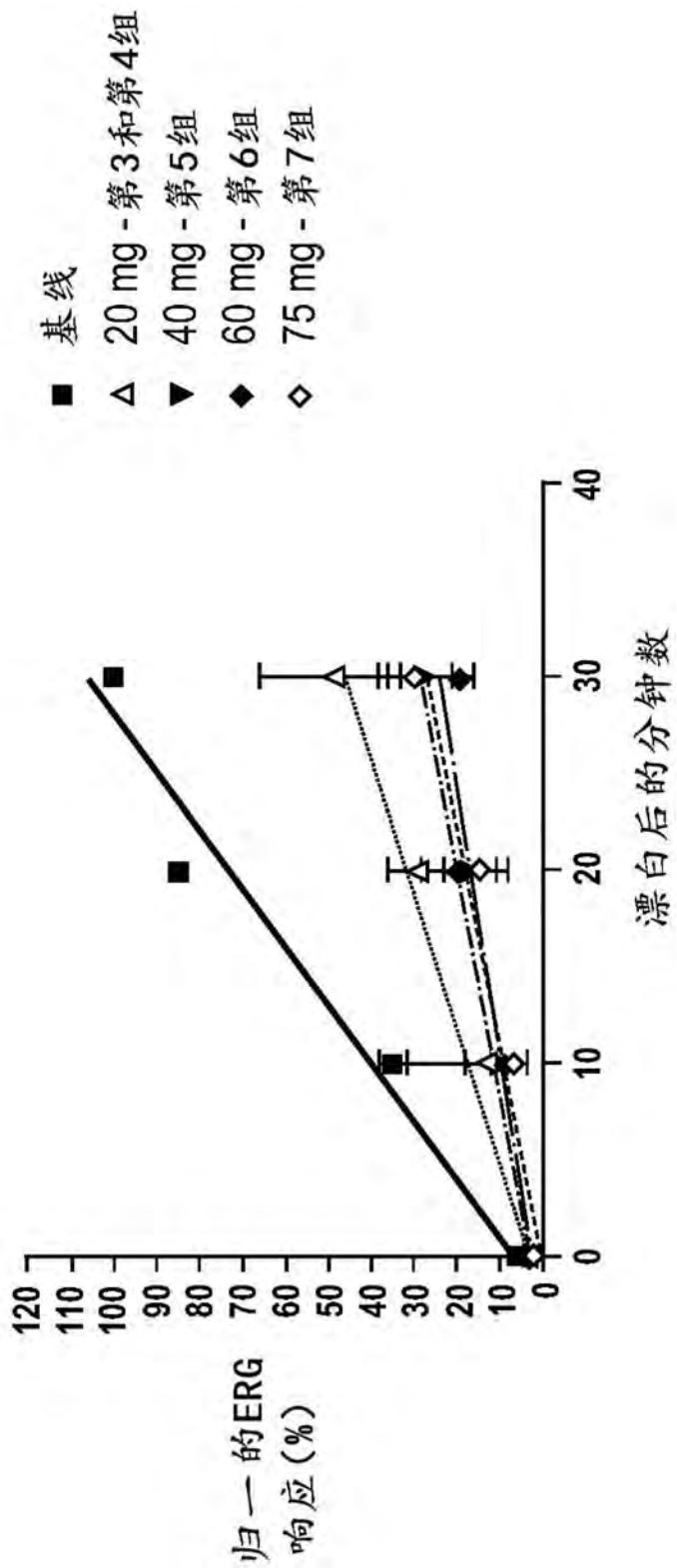


图 6

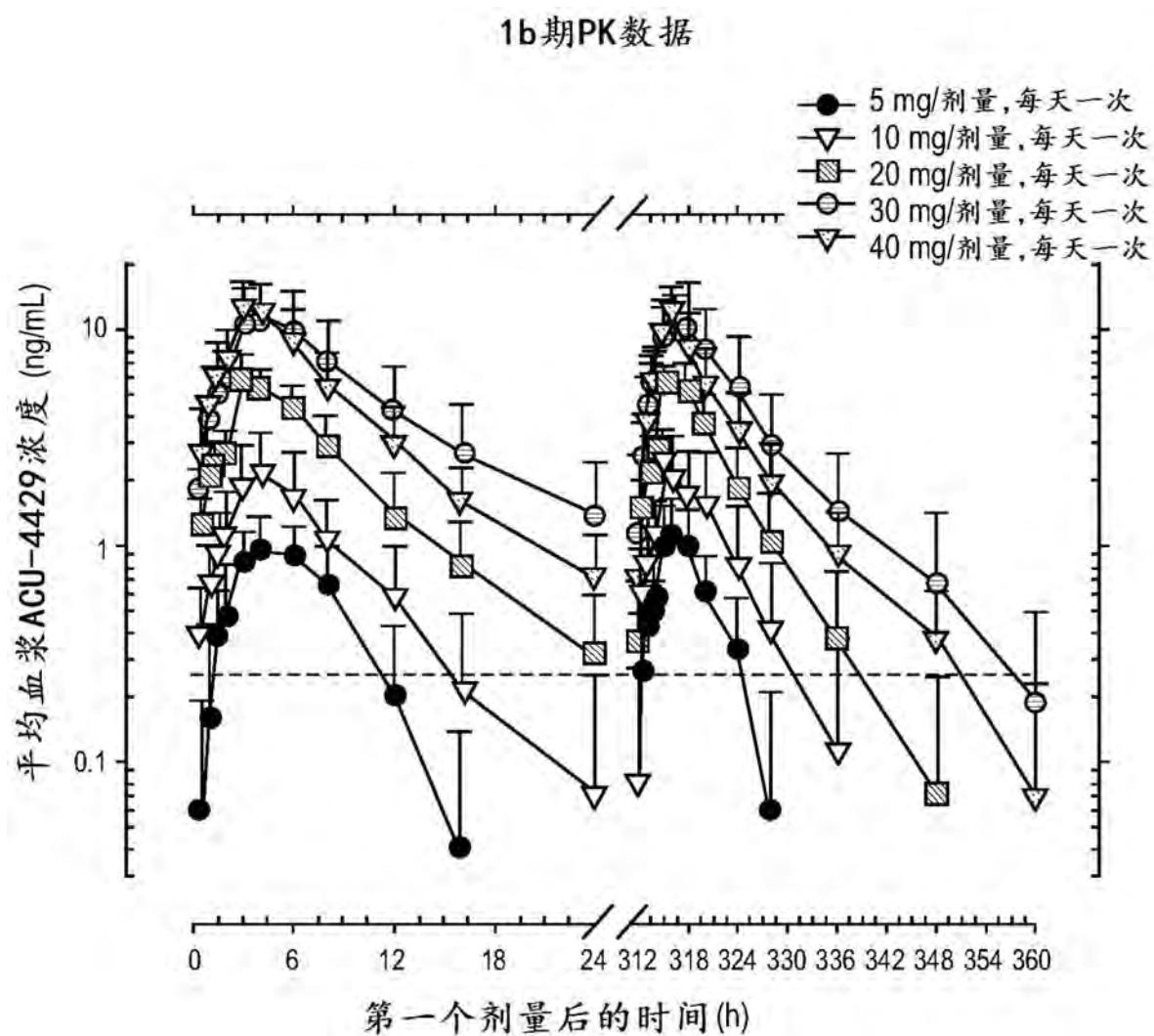


图 7

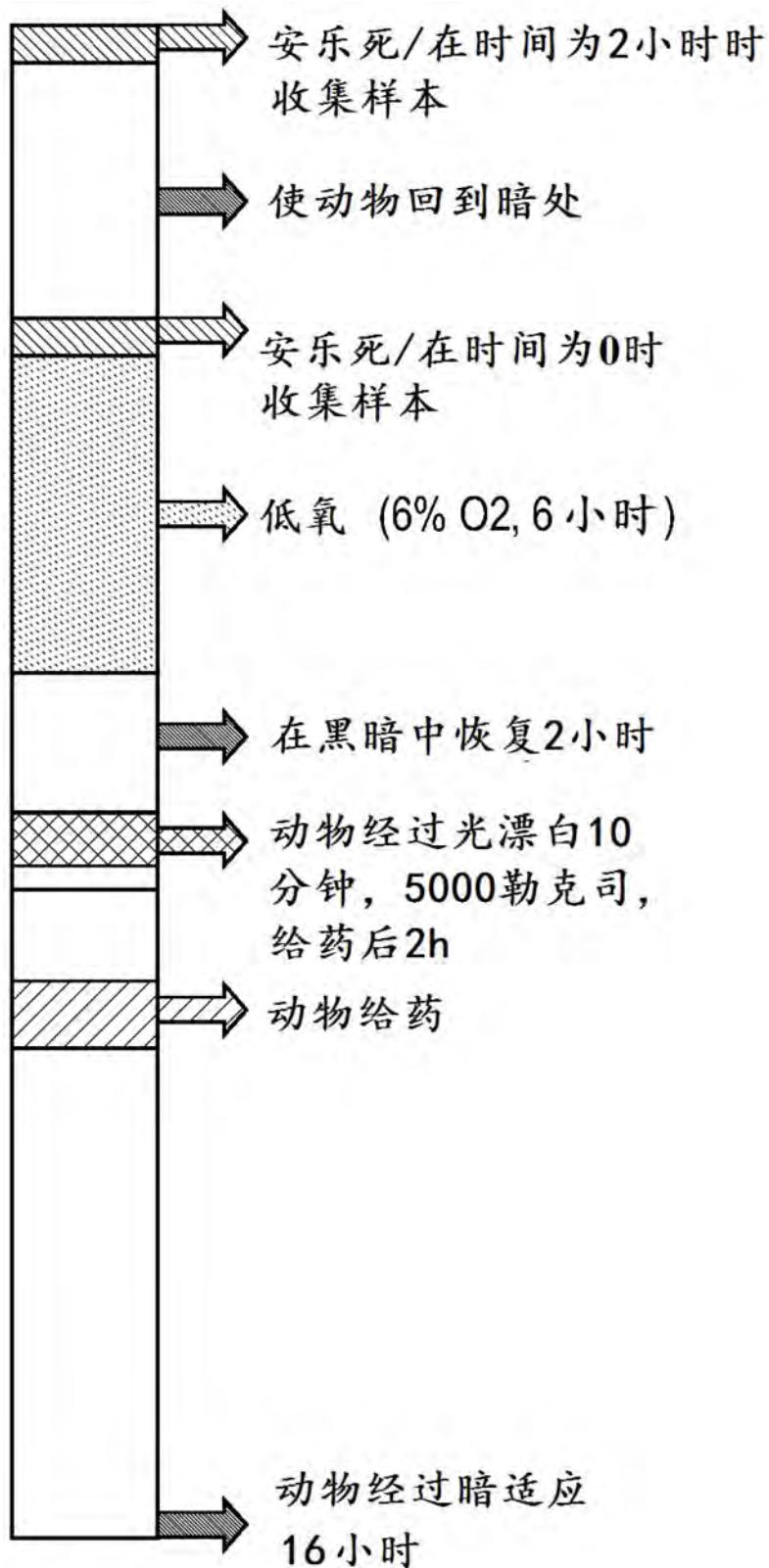


图 8

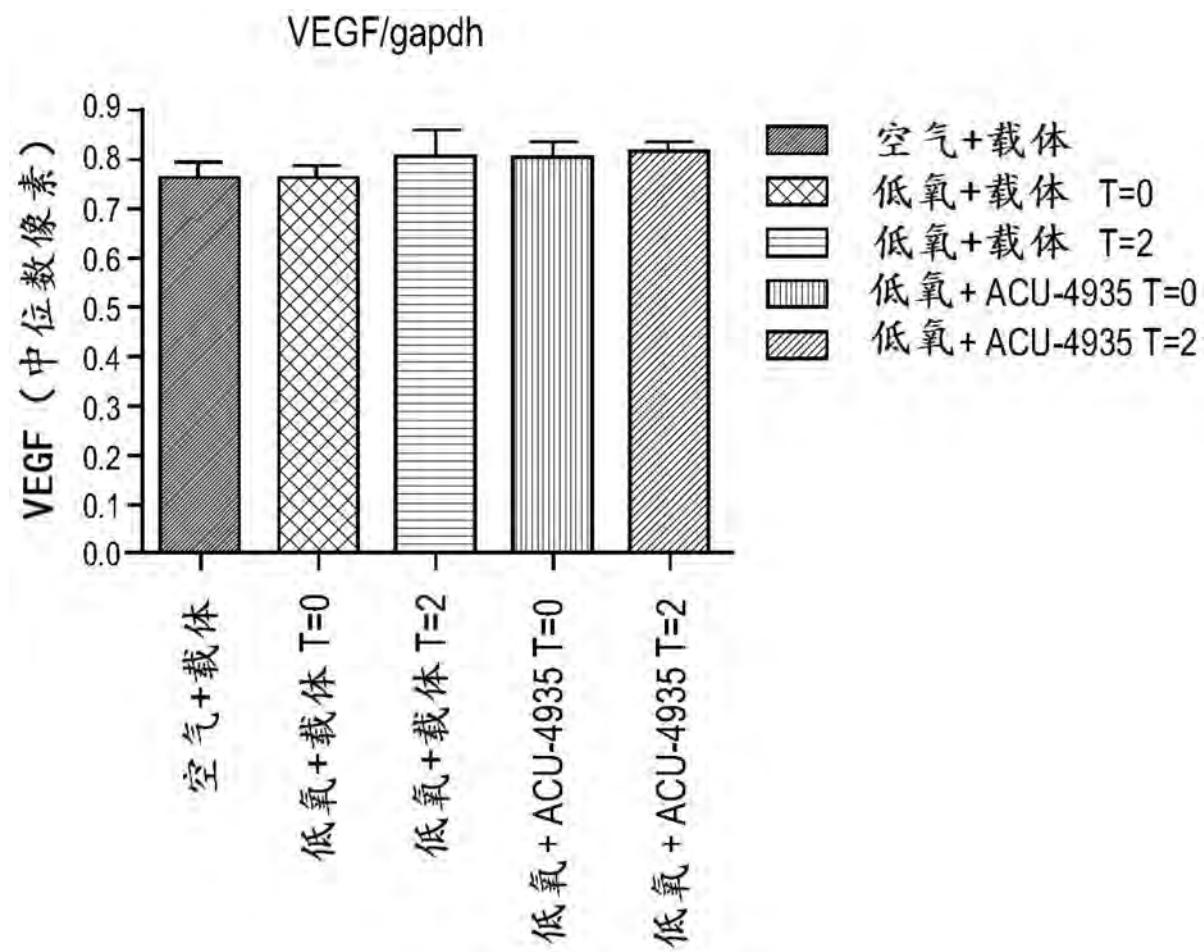


图 9

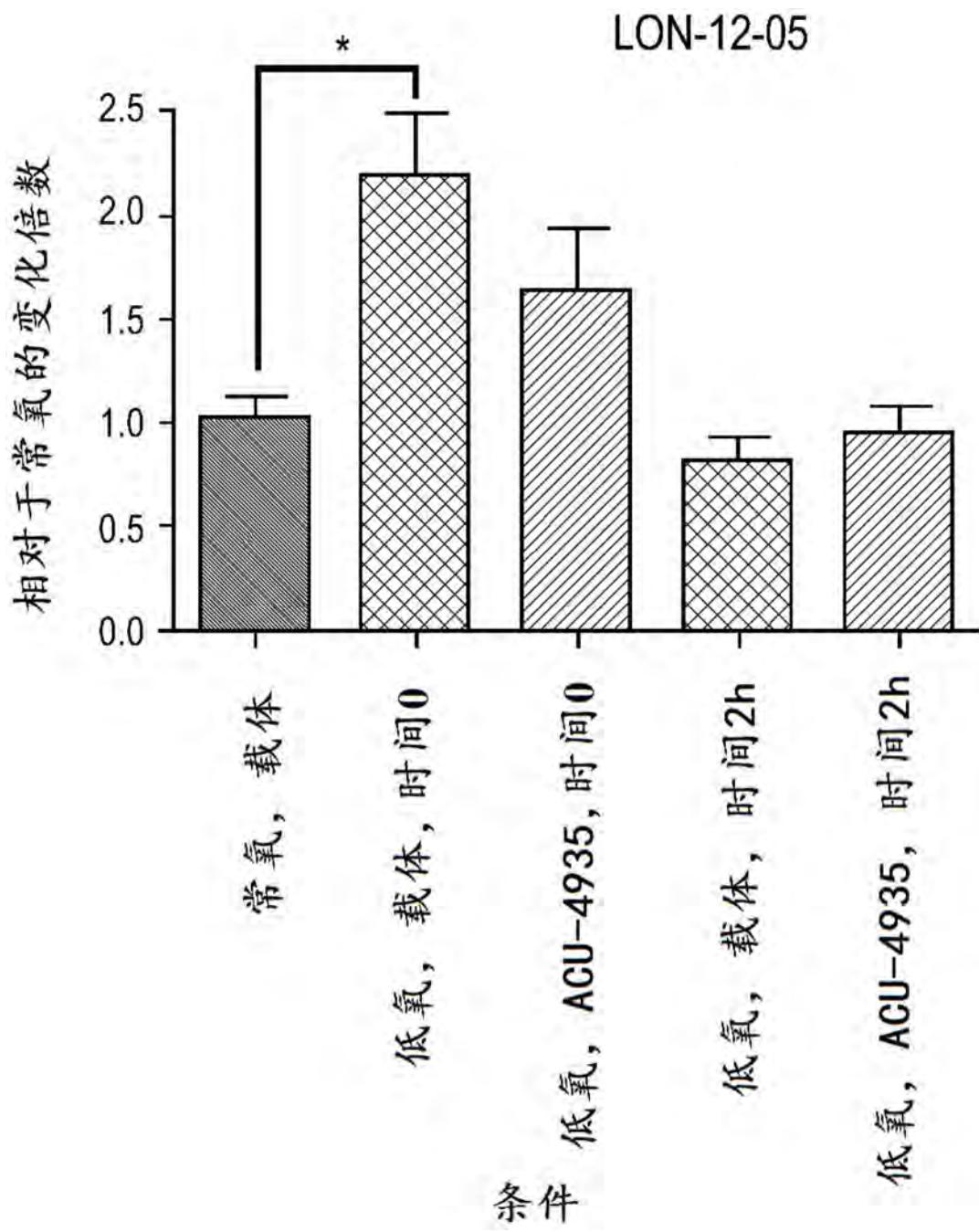


图 10

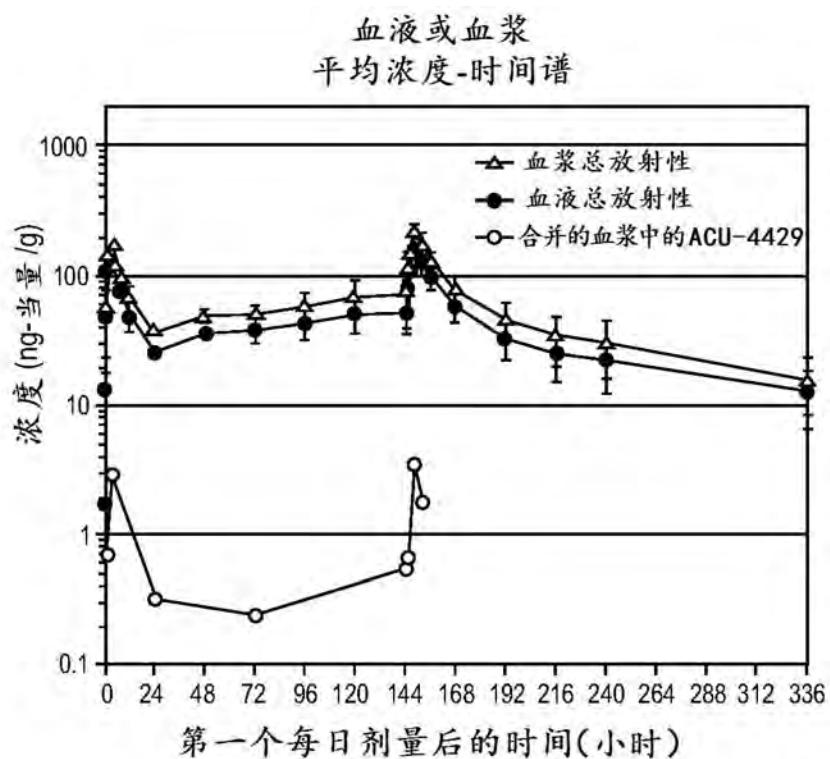


图 11A

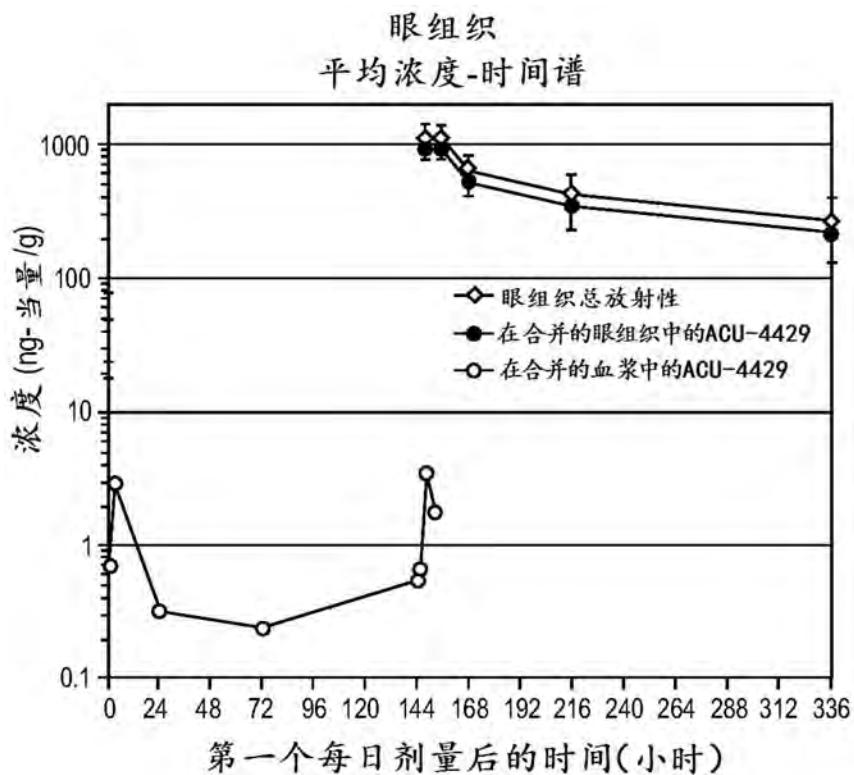


图 11B

## 第7天给药后4小时的代谢物放射谱

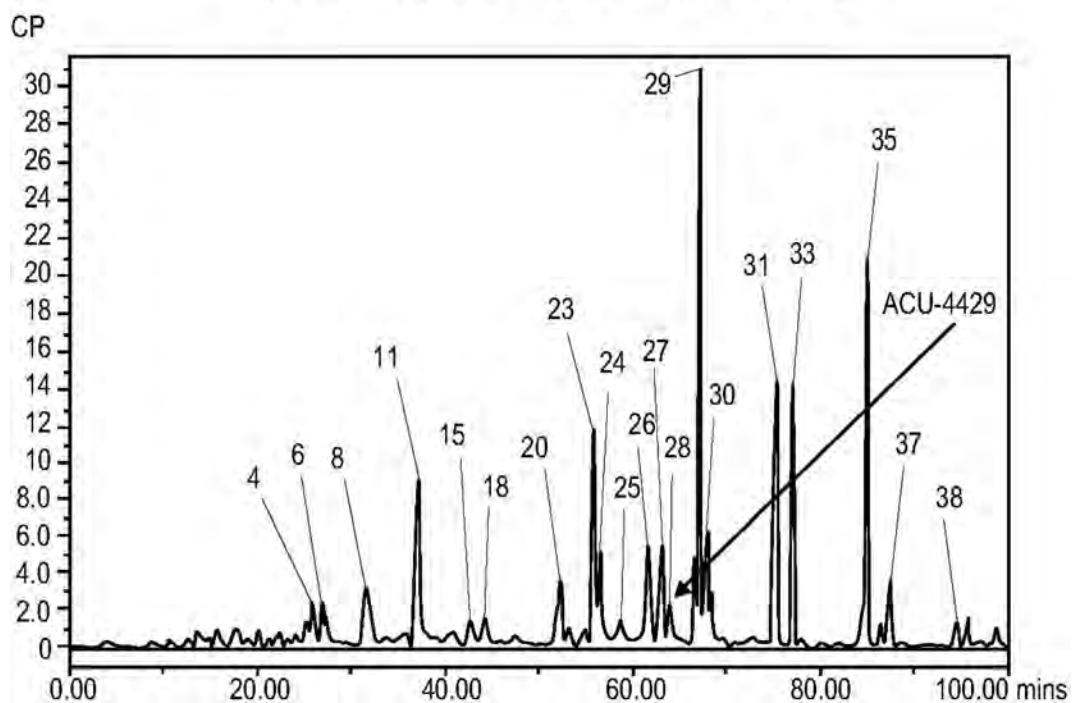


图 12A

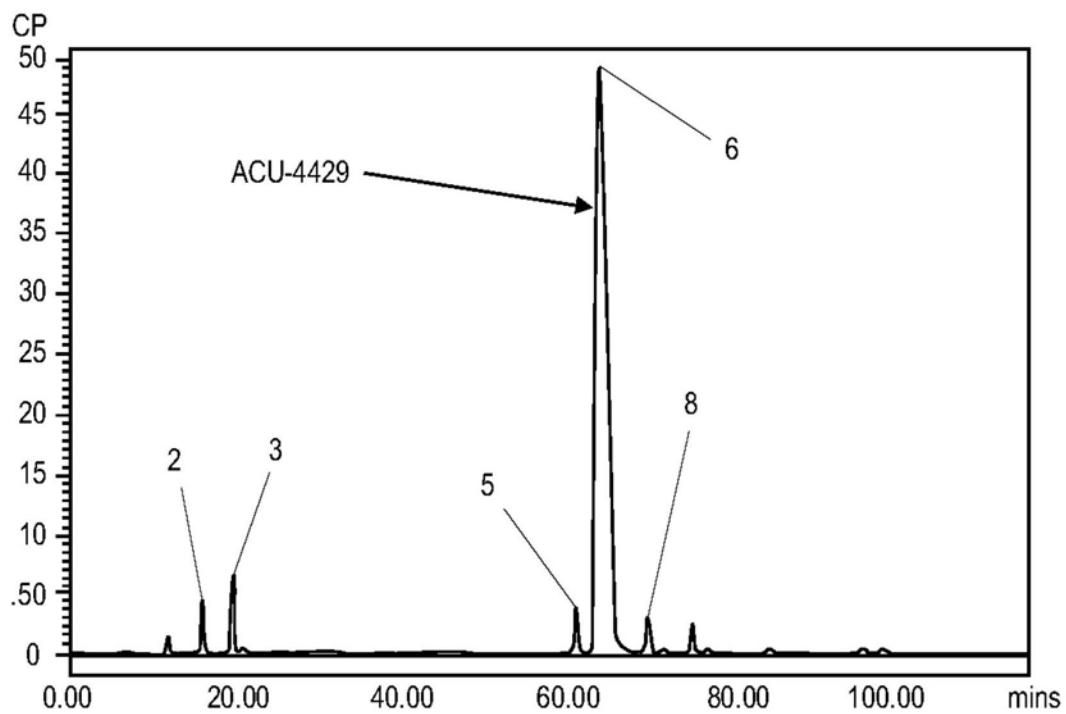


图 12B

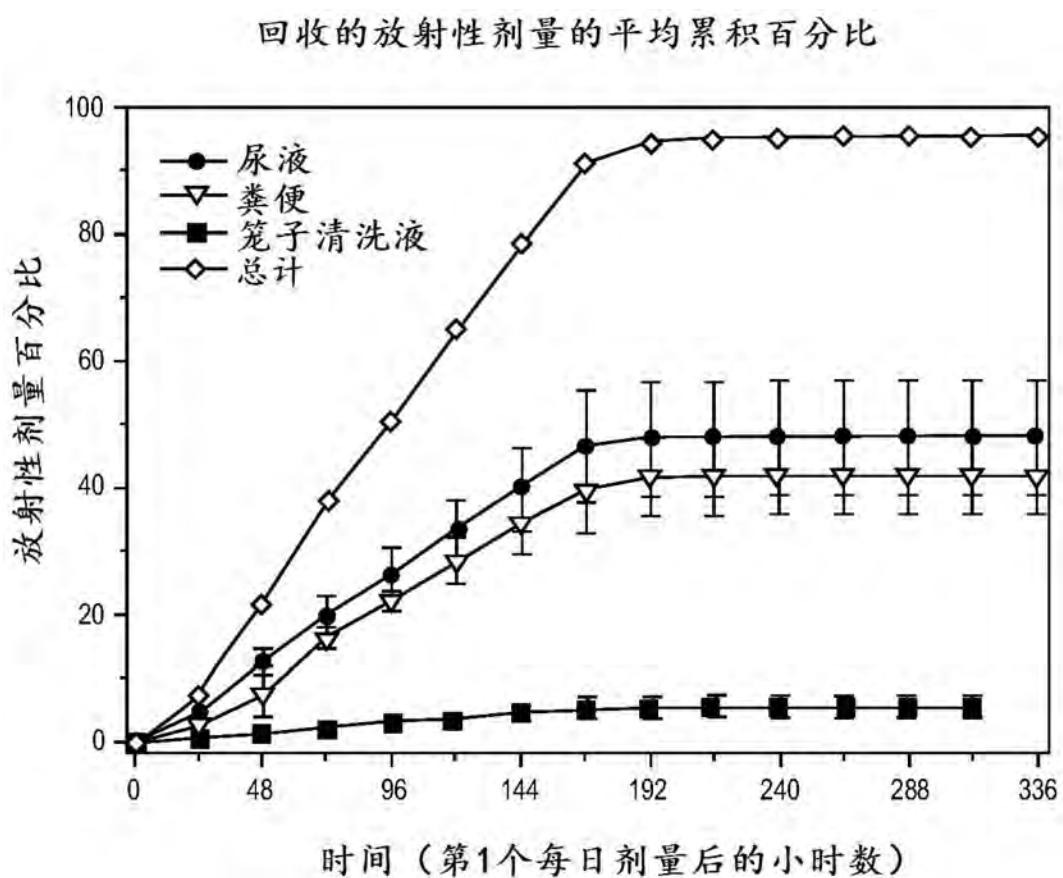


图 13

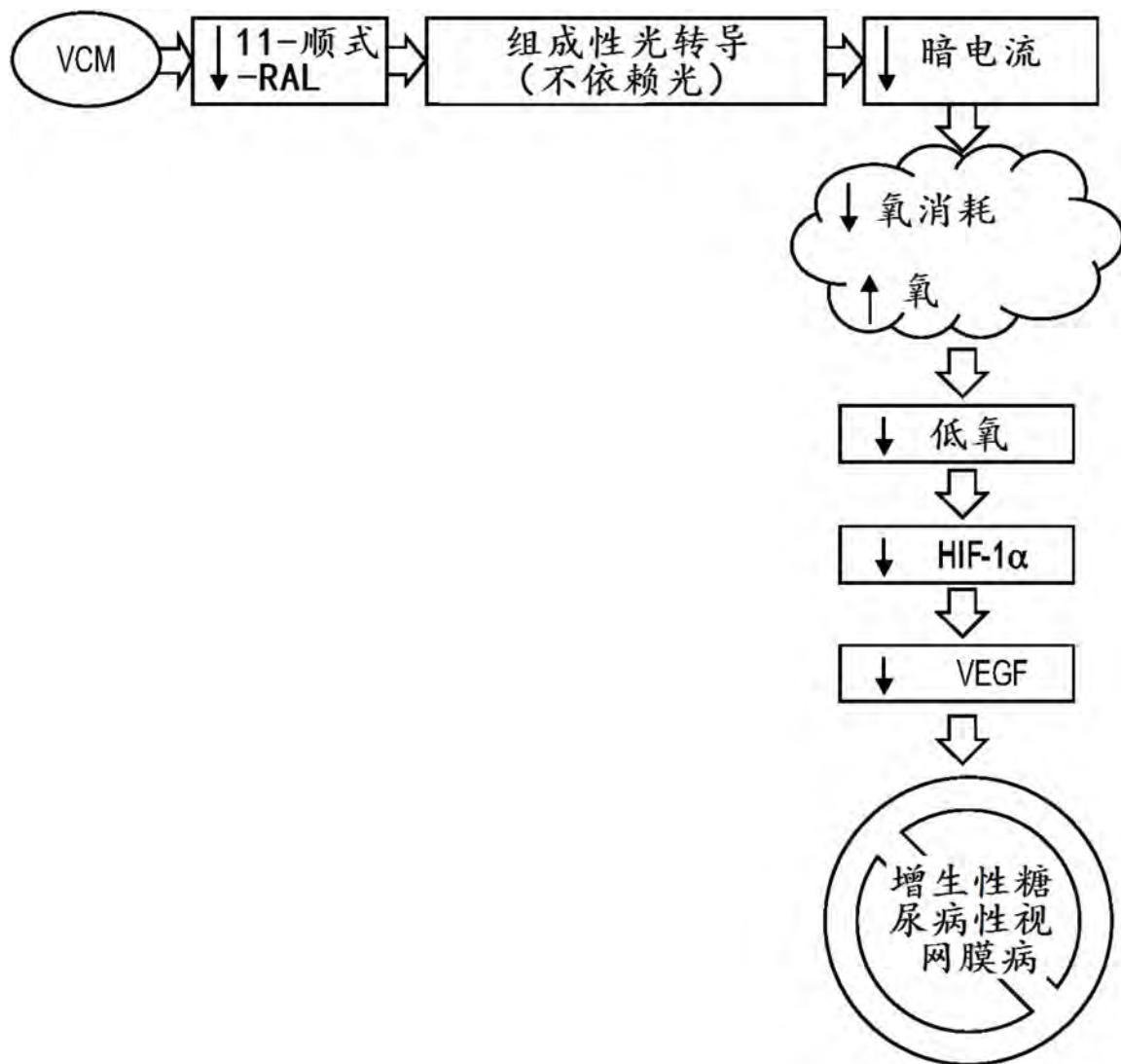


图 14

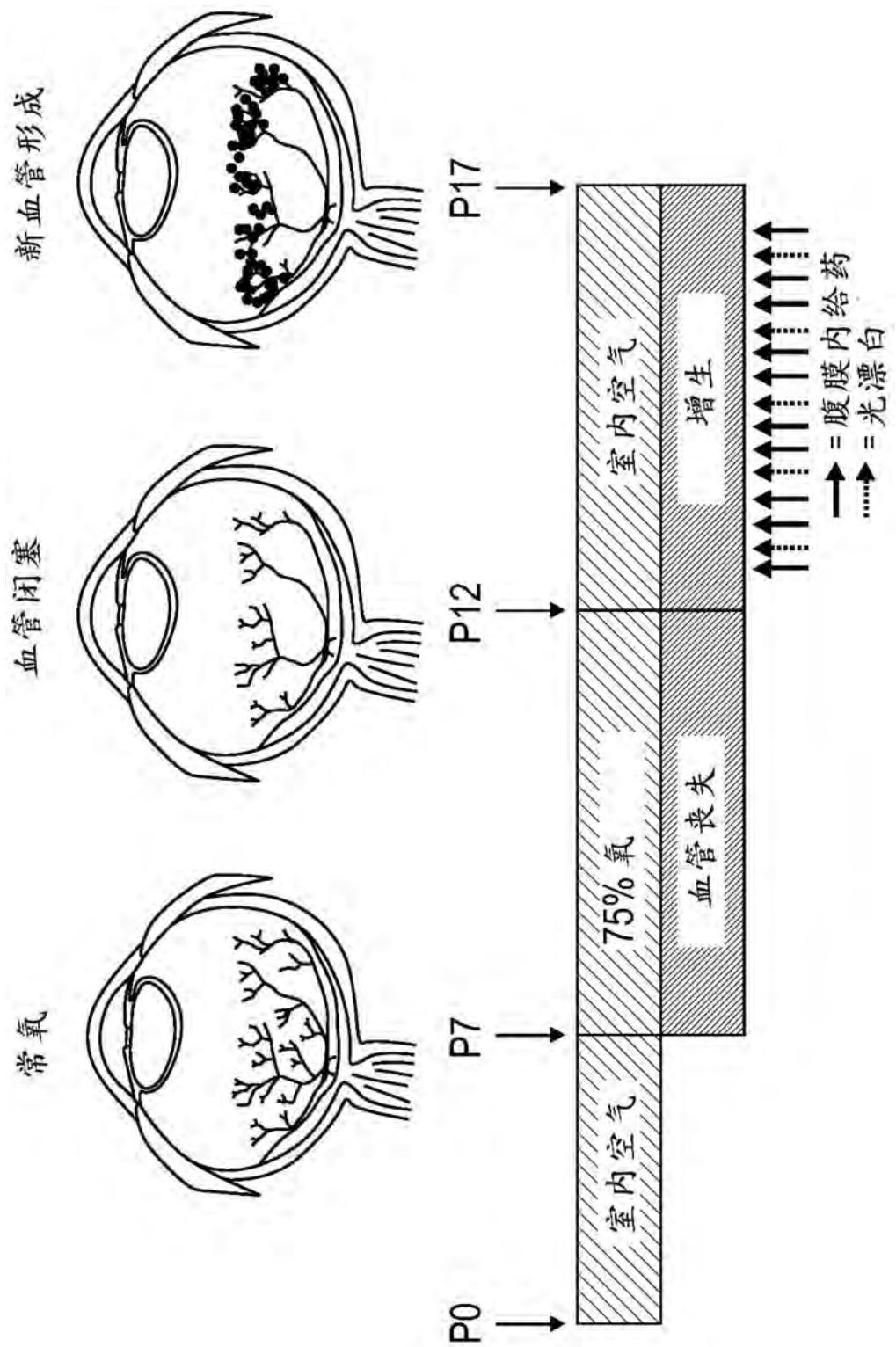


图 15

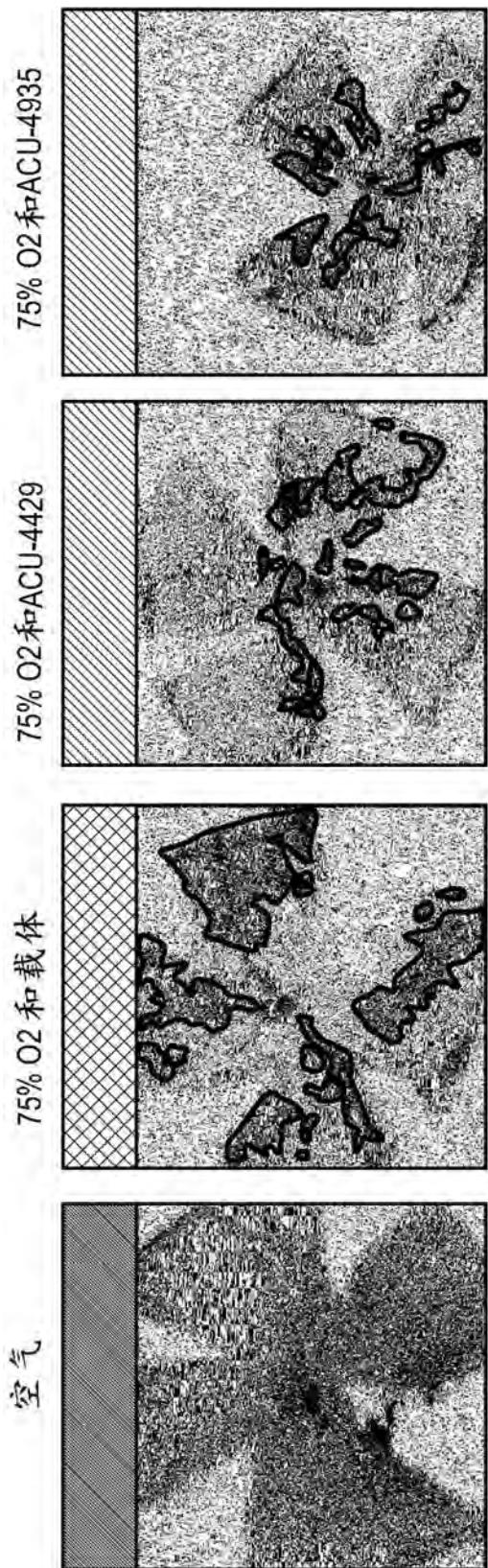


图 16A

相对于载体归一的  
%新血管形成

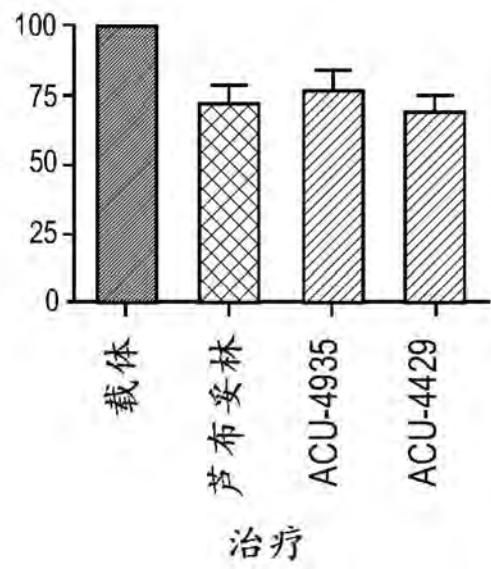


图 16B

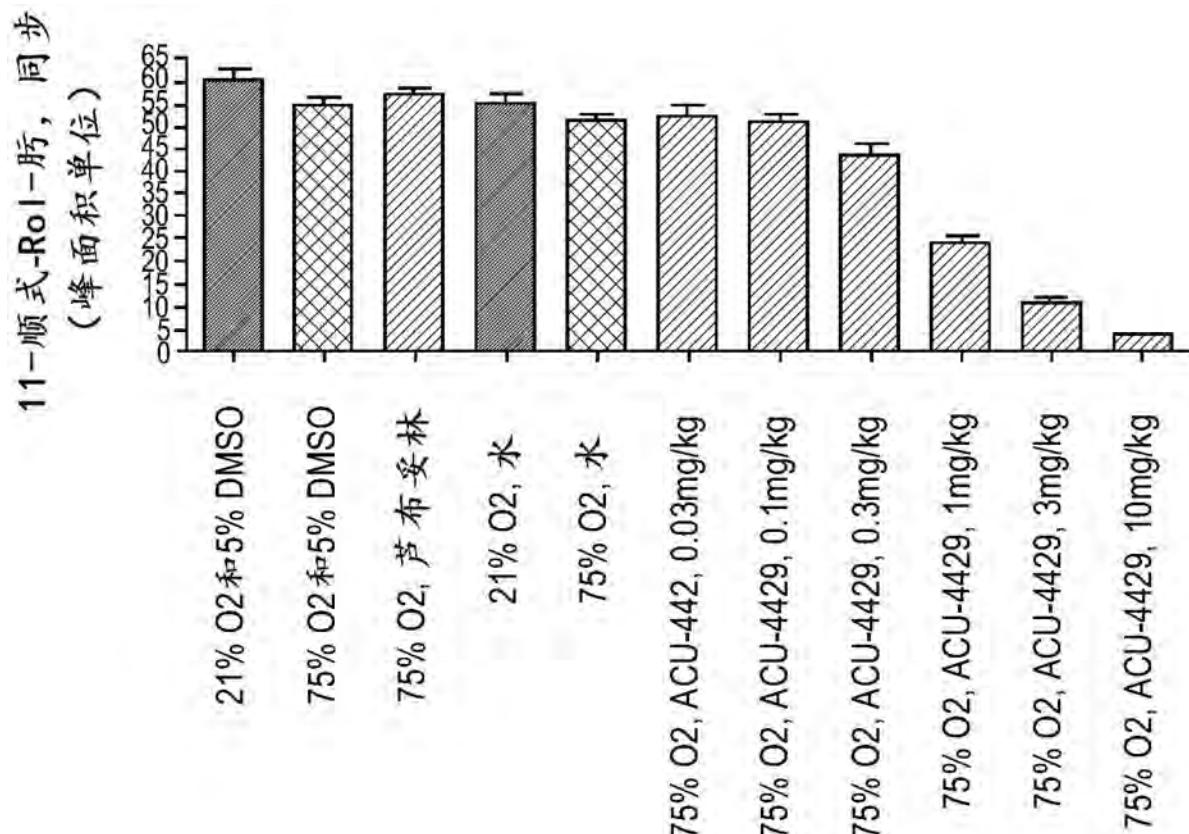


图 16C

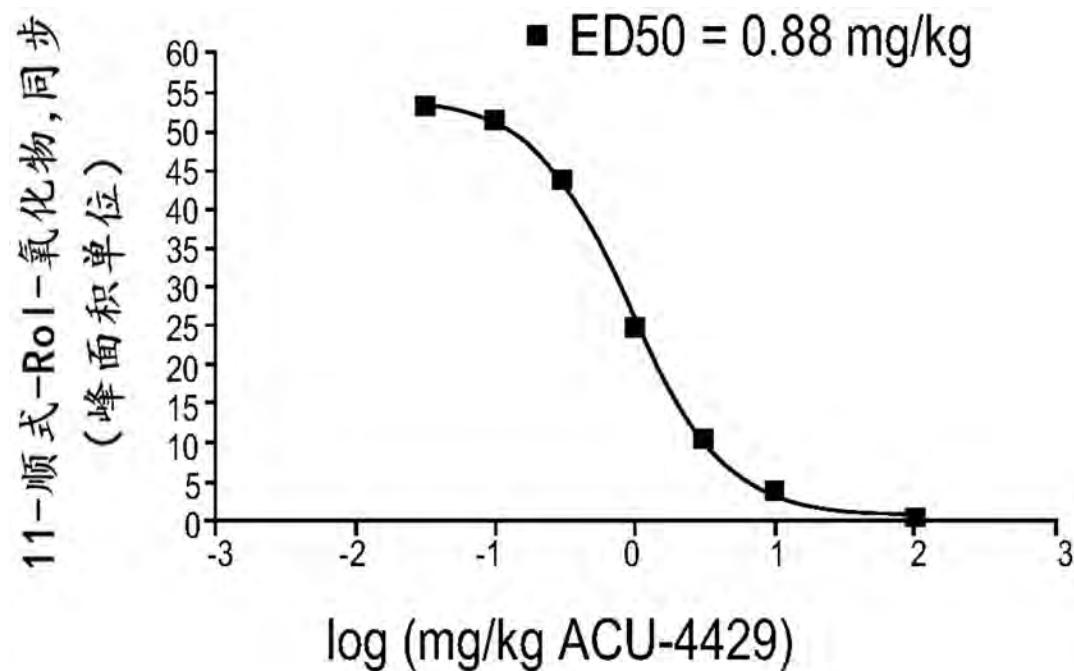


图 16D

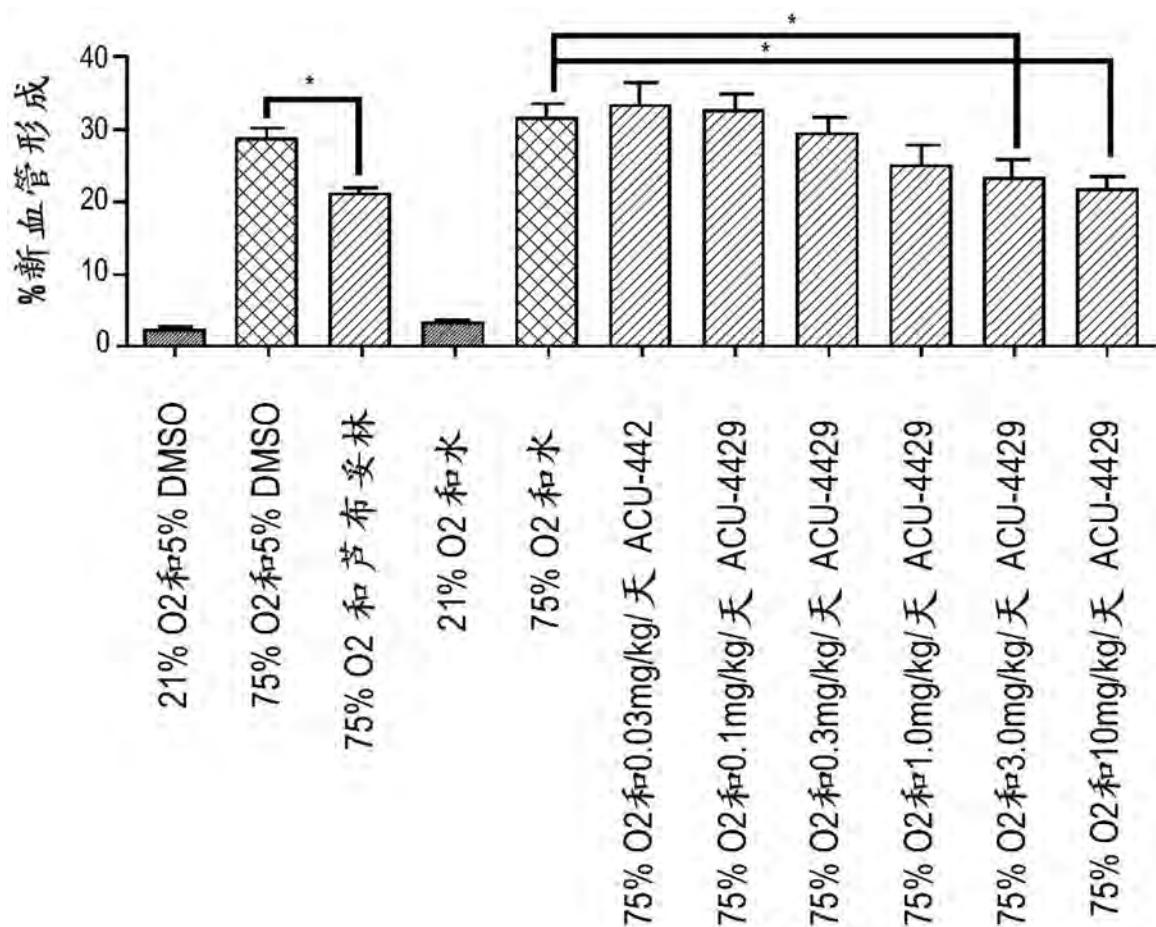


图 16E

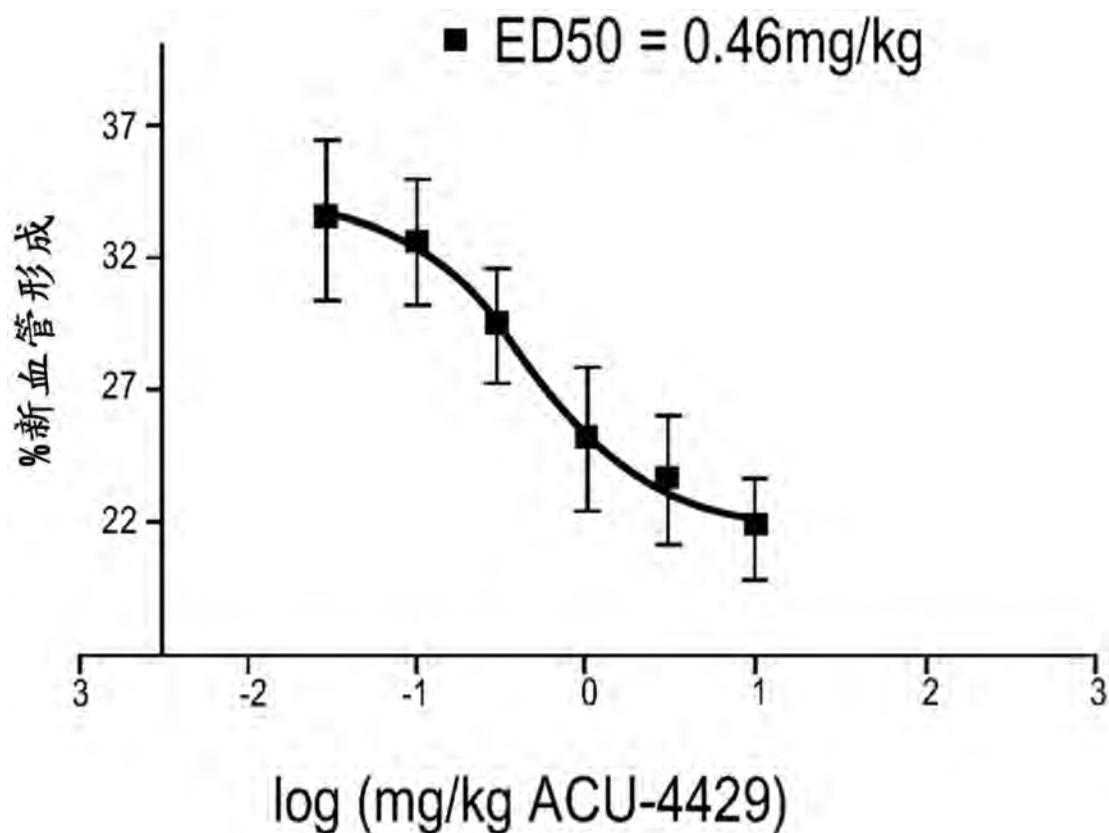


图 16F

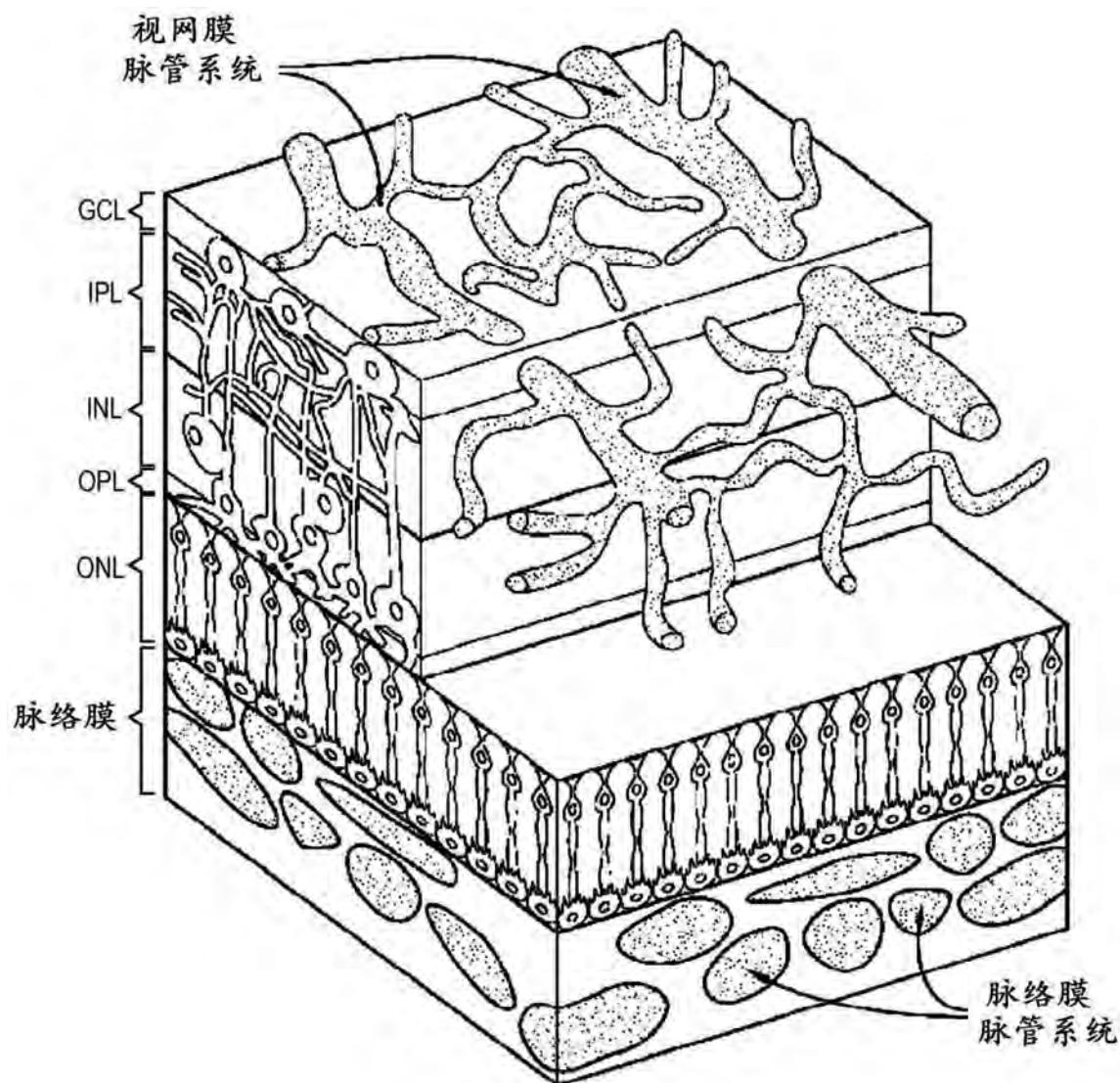


图 17

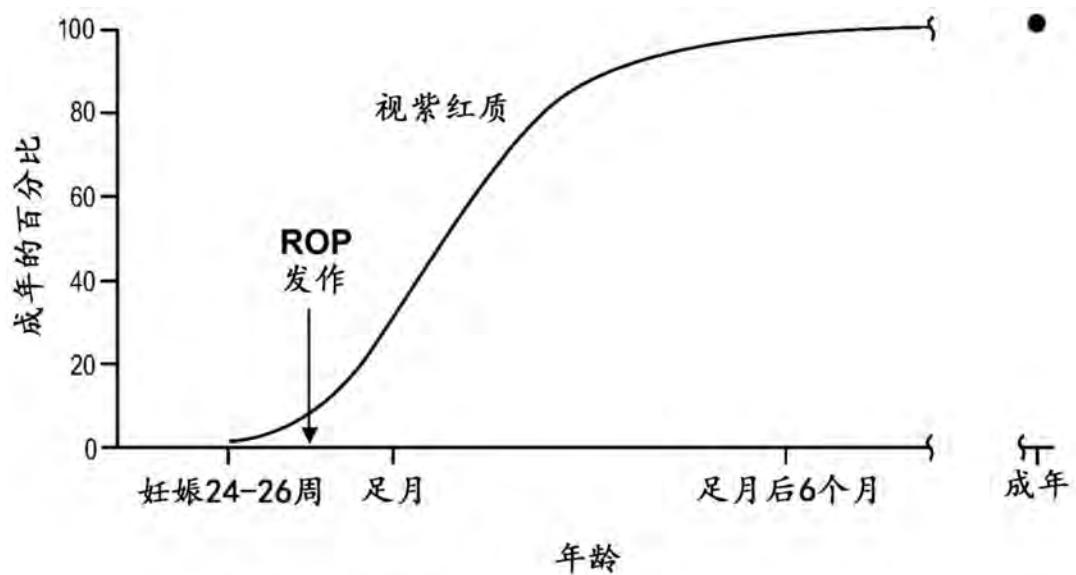


图 18

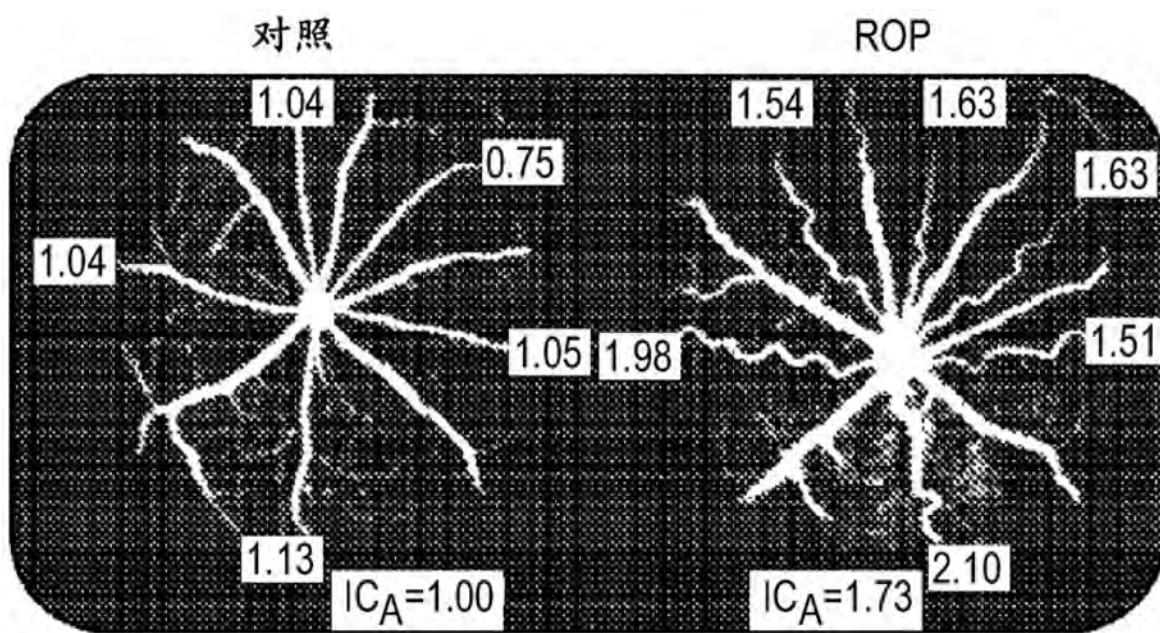


图 19

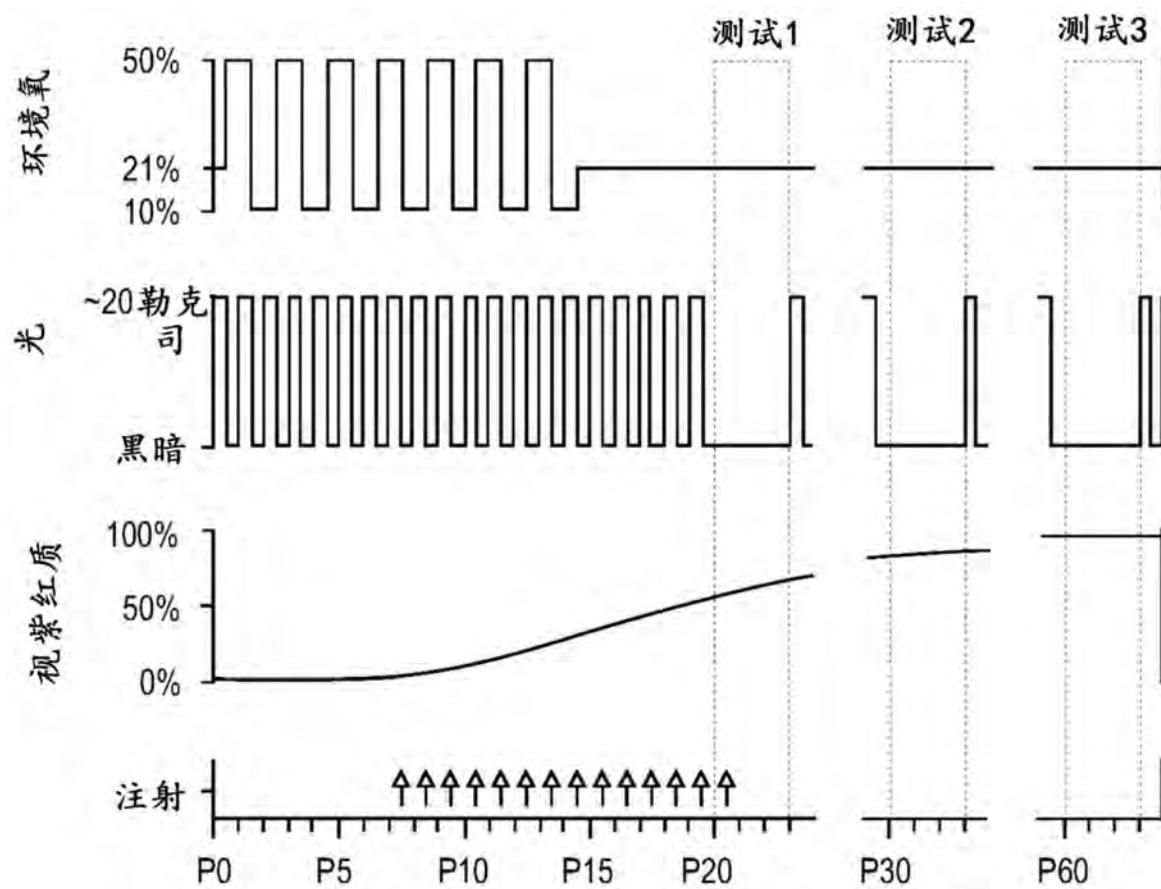


图 20

## ONL的比较

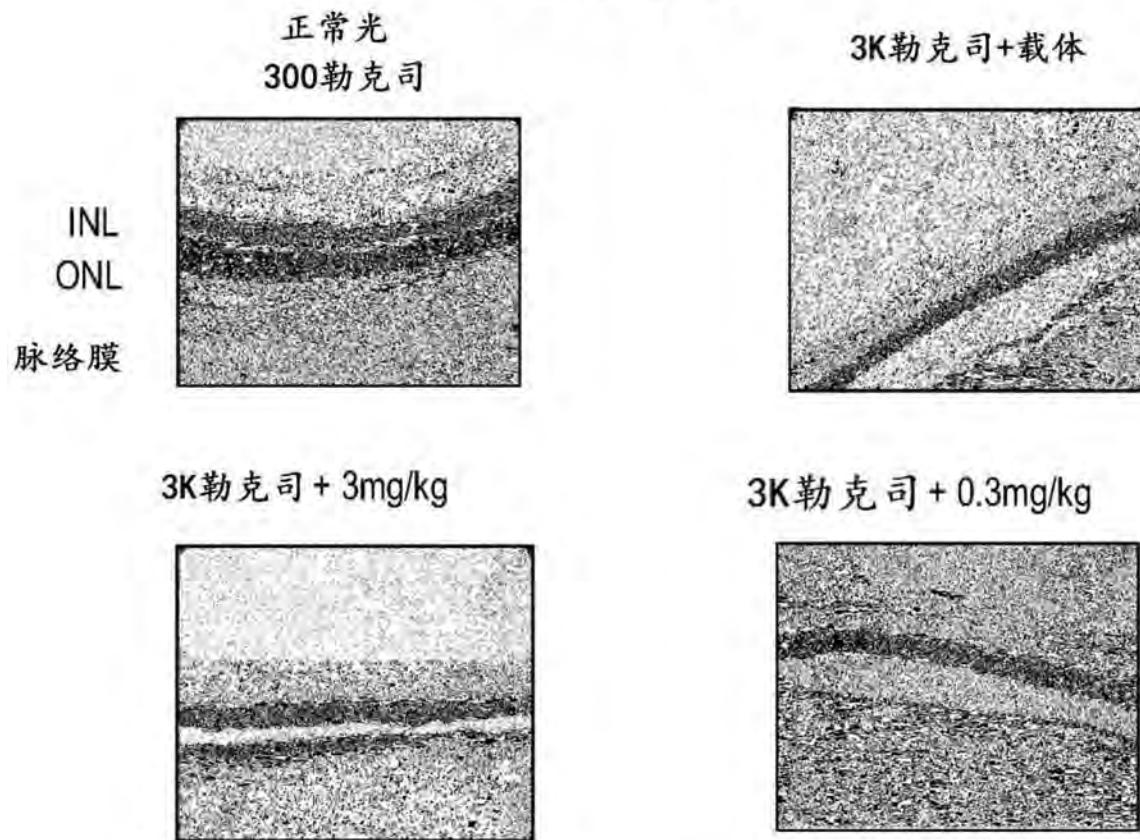


图 21

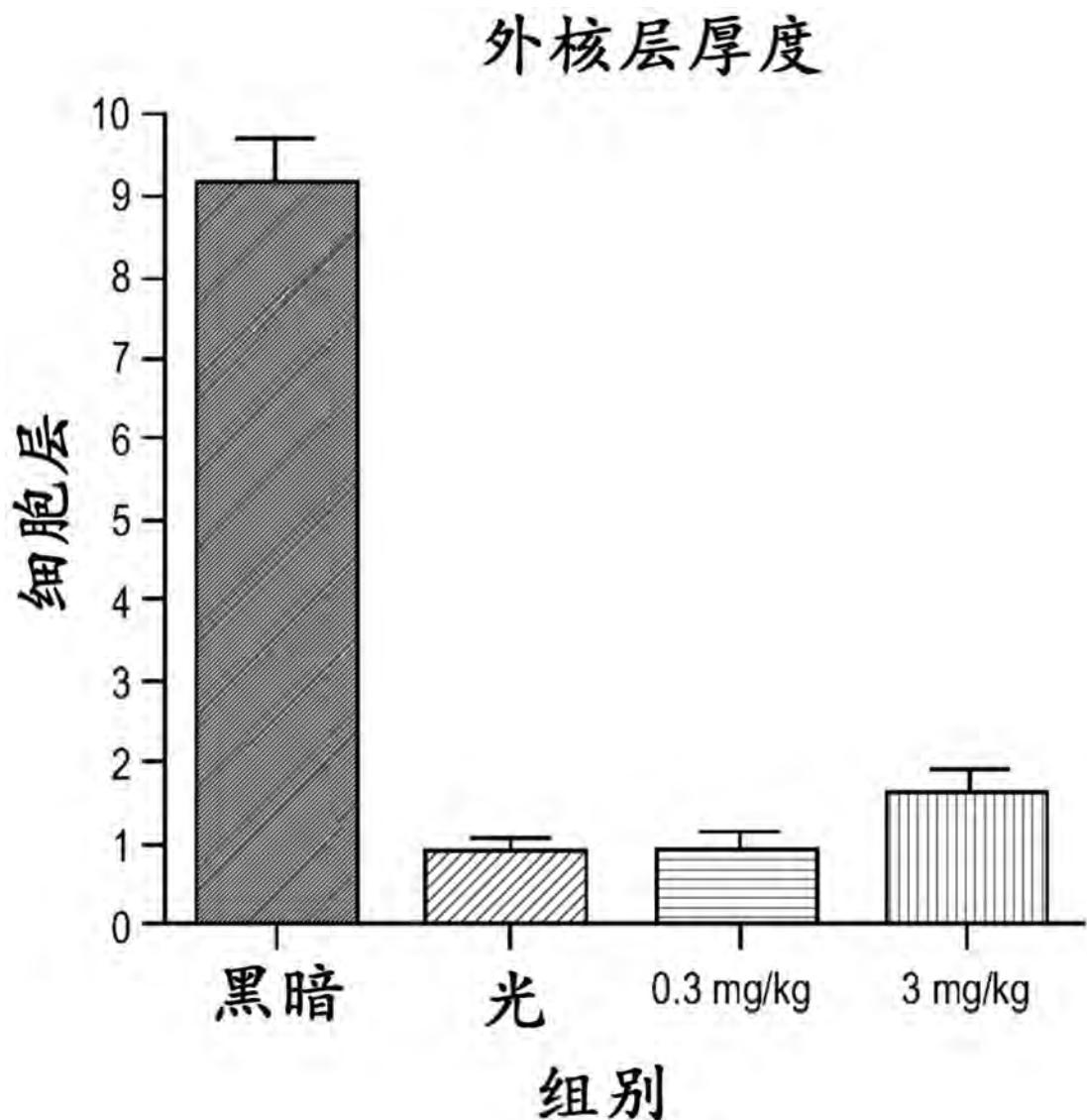


图 22

## WIS-11-04

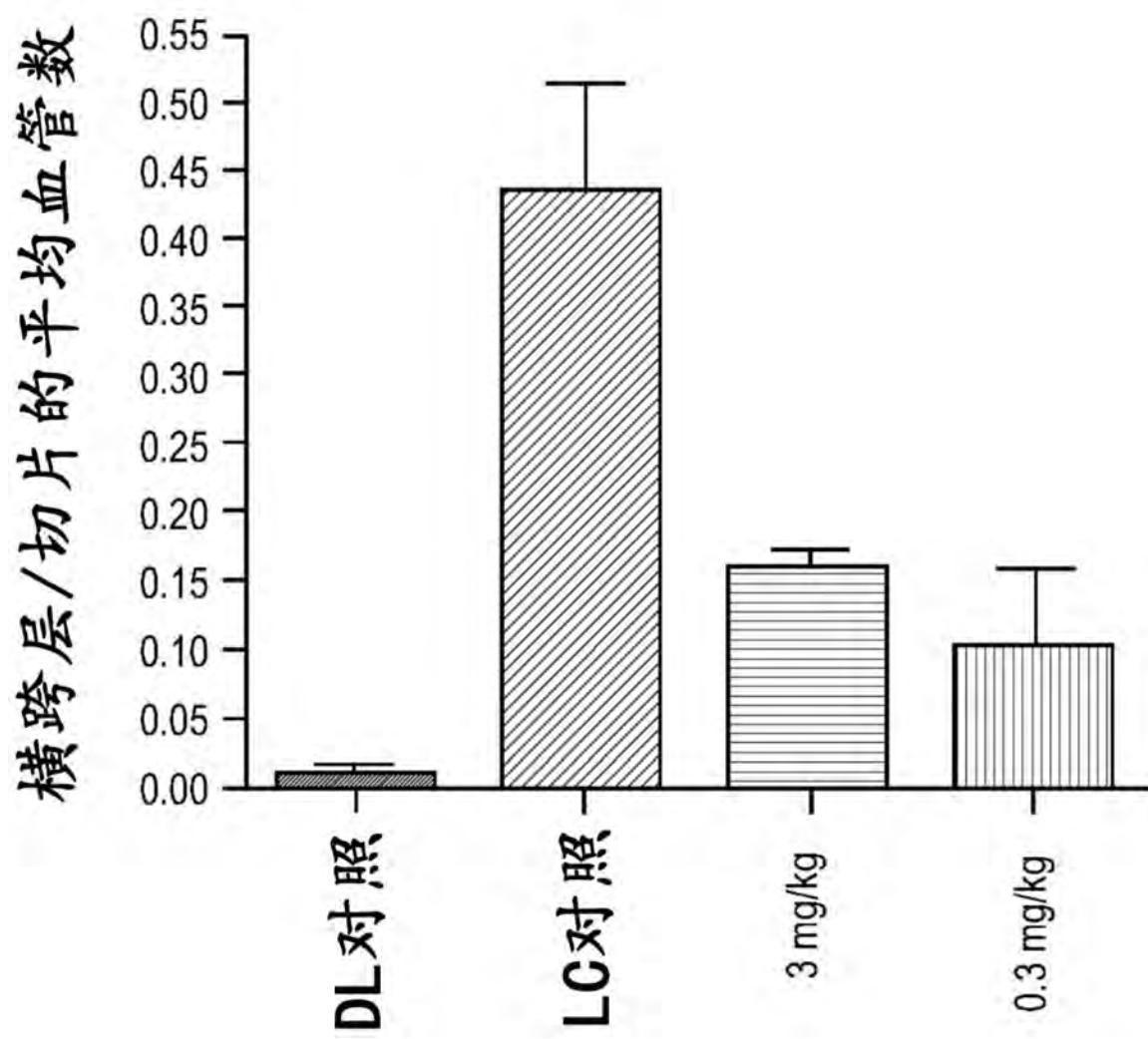


图 23

### **Abstract**

Methods are provided herein for the treatment of ophthalmic diseases or conditions such as an ophthalmic disease or disorder associated with diabetes in a patient. Also provided herein are methods of treating retinopathy of prematurity in a patient. Further, provided herein are methods for treating wet age-related macular degeneration in a patient. The methods comprise administration of compounds disclosed herein to a patient in need thereof that inhibit or slow one or more signs or symptoms of such conditions.