(54) Title: Ophthalmic Compositions Comprising PPAR-Alpha Agonists and Methods of Production and Use Thereof

(57) Abstract: Ophthalmic compositions comprising at least one PPAR-alpha agonist. The PPAR-alpha agonist is fenofibrate, and is in a range of from about 0.001% to 95%. The ophthalmic composition is formulated as an eye drop. Methods of treating retinal conditions by administering to the eye of a patient an ophthalmic composition comprising at least one PPAR-alpha agonist.
Declarations under Rule 4.17:

- as to applicant’s entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant’s entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- with international search report (Art. 21(3))
Ophthalmic Compositions Comprising PPAR-Alpha Agonists and Methods of Production and Use Thereof

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 61/540,621, filed on September 29, 2011, which is incorporated by reference in its entirety herein.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with Government support under Grant Nos. EY019309, EY012231, and EY018659 awarded by the National Institutes of Health; and Grant No. P20RR024215 awarded by the National Center for Research Resources. The Government has certain rights in the invention.

TECHNICAL FIELD

The present disclosure is related to ophthalmic compositions, and more particularly, but not by way of limitation, to ophthalmic compositions comprising PPAR-alpha agonists, as well as methods of production and use thereof.

BACKGROUND

Abnormal or aberrant neovascularization is associated with a number of diseases and disorders, including but not limited to, cancer, inflammatory disease, macular degeneration and diabetic retinopathy (DR).

The Wnt signaling pathway plays a crucial role in neovascularization and many other associated biological processes, including retinal vessel development and the inflammation process. Mutation of Wnt signaling pathway genes Frizzled-4 (Fz4), LRP5, or LRP6 leads to inhibition of retinal angiogenesis in familial exudative vitreoretinopathy (FEVR) patients, while Fz4 knockout mice exhibit incomplete retinal vascularization. Moreover, VEGF is upregulated as a result of mutational activation of Wnt signaling in colon cancer and human endothelial cells. VEGF is a potent mediator of vascular permeability and angiogenesis, and is an established therapeutic target for a number of angiogenesis associated diseases, including cancer and age related macular
degeneration. A number of other angiogenic regulators are also Wnt target genes including, but not limited to, FGF18, endothelin-1, Cx43, uPAR, MMP7, and MMP3.

Among the aberrant neovascularization associated diseases, diabetic retinopathy (DR) is a very common complication of diabetes mellitus and one of the four common sight-threatening conditions in developed countries. Almost 100% of patients with type I diabetes and 60% of type II diabetic patients will develop some degrees of retinopathy in their lifetime. Approximately 10% of diabetic patients develop a severe visual handicap after 15 years of diabetes. DR is a chronic and progressive disorder, primarily affecting retinal capillaries. Breakdown of the blood-retinal barrier is a common pathological change in patients with diabetes and in streptozotocin (STZ)-induced diabetic animal models. In the early stages of DR, the retinal vascular permeability is increased without the appearance of clinical retinopathy. Retinal vascular leakage and thickening of the retina lead to diabetic macular edema (DME). In the late stages of DR, over-proliferation of capillary endothelial cells results in retinal neovascularization (NV), the abnormal formation of new vessels from preexisting capillaries in the retina and vitreous. This, in turn, leads to proliferative diabetic retinopathy (PDR). The abnormal angiogenesis can ultimately cause severe vitreous cavity bleeding and/or retinal detachment, resulting in severe vision loss.

It has been shown that multiple growth factors in the eye, such as but not limited to, VEGF, bFGF, IGF-1, and PEDF, are implicated in the pathogenesis of DR. Alterations of these growth factors and their receptors in diabetes have been identified in both experimental and clinical studies. Increased VEGF levels are at least partly responsible for retinal vascular leakage, retinal vascular hyper-permeability and retinal NV in patients with DR. VEGF therefore plays an important role in the development and pathogenesis of DR. The up-regulated expression of retinal VEGF and its receptors correlates with retinal NV in OIR. Inhibition of VEGF and VEGF receptors has been shown to prevent retinal NV in diabetic and OIR animal models.

Accumulating evidence indicates that the Wnt signaling pathway not only mediates inflammation, i.e. TNF-alpha, NF-κB translocation and VEGF, but also regulates angiogenesis in the eye. Studies demonstrated that both Frizzled-4 (Fz4) and
Lrp5/6 are expressed in adult murine retinal vasculature. Mutations in the Fz4 or LRP5 or LRP6 gene in the human lead to inhibition of normal retinal angiogenesis in familial exudative vitreoretinopathy (FEVR) patients, and Fz4 knockout (fz4-/-) mice exhibited an incomplete retinal vascularization. Meanwhile, it has been shown that seven β-catenin/TCF binding sites occur in the gene promoter for VEGF-A. Under hypoxia conditions, HIF-1α competes with TCF-4 to form a new complex with β-catenin instead of β-catenin/TCF in the HIF-1α gene promoter region. Moreover, VEGF is upregulated as a result of mutational activation of the Wnt/β-catenin signaling in colon cancer cells and in human endothelial cells. A variety of other angiogenic regulators have previously been reported as Wnt target genes including but not limited to, FGF18, endothelin-1, Cx43, uPAR, MMP7, and MMP3. Thus Wnts may regulate angiogenesis through induction of multiple angiogenic genes.

The canonical pathway is initiated when a Wnt ligand binds to a member of the Frizzled serpentine receptor family and its co-receptor LRP6 or a close relative such as LRP5. When the Wnt-induced Fz-LRP6 or Fz-LRP5 complex forms, LRP6/LRP5 will be phosphorylated at its PPPSP motif and is then capable of binding Axin in a phosphorylation-dependent manner to the plasma membrane, thereby resulting in the inhibition of β-catenin phosphorylation and degradation. LRP5 and LRP6 are of critical importance in human diseases. The LRP6 cytoplasmic domain is essential for Axin binding, and its deletion in LRP6 ΔC results in a dominant negative receptor that binds Wnt but is unable to bind Axin. The LRP6 extracellular domain has auto-inhibitory activity, because its deletion in LRP6ΔN results in a constitutively activated receptor that binds Axin in the absence of Wnt ligand.

As stated herein above, retinal NV is a major pathological feature leading to vision loss in DR. VEGF is a well-known key factor in stimulating the retinal NV formation in the DR.

Current treatments for DR are still quite limited. Besides systemic therapy such as glycemic control, blood pressure control, lipid-lowering therapy and ocular therapy (including but not limited to, laser photoacoagulation, surgical intervention, intravitreal injection of anti-VEGF agents, etc.). Recently, a FIELD study in Australia demonstrated
that oral administration of 200 mg/d fenofibrate to diabetic patients slowed down the progress of diabetic retinopathy and decreased the ratio of need for laser treatment (Keech et al., 2007). Fenofibrate is a traditional small-molecule agonist of peroxisome proliferator-activated receptor alpha ("PPAR-alpha" or "PPARα"), which mainly reduces blood triglycerides, VLDL and LDL levels and increases HDL levels (Rosenson, 2008). Fenofibrate also suppresses VEGF expression on endothelium in Type 2 diabetes and tumors (Skrha et al., 2004; and Panigrahy et al., 2008). There are also reports showing that fenofibrate attenuates endothelial monocyte adhesion, possibly through the prevention of upregulation of cell adhesion molecules such as ICAM-1 by endothelial cells in response to inflammation in chronic heart failure, metabolic syndrome, and diabetic nephropathy (Huang et al., 2009; Rosenson, 2009; and Chen et al., 2008). However, fenofibrate exhibits pleiotropic effects (Tsimihodimos et al., 2009); thus, in certain instances, it is desirable to limit systemic exposure to fenofibrate to limit the number of distinct and unrelated effects caused by the drug as well as to potentially isolate a particular, desired pathway/effect.

Further, oral administration is not efficient to deliver fenofibrate to the retina because of the blood-brain barrier. As a result, high doses are required to reach effective levels in the retina. However, high doses of fenofibrate are associated with renal toxicity. Thus, new compositions that reach effective concentrations of fenofibrate with little systemic exposure – and thus less chance of systemic toxicity – are desired.

Therefore, there exists a need for new and improved compositions containing PPARα agonists, such as, but not limited to, fenofibrate, as well as methods of production and use thereof. Such compositions and methods would be useful in the treatment and prevention of neovascularization-associated and/or Wnt signaling pathway associated diseases, including but not limited to, inflammation, fibrosis, angiogenesis and/or tumorigenesis. The present disclosure is directed to compositions and methods, which overcome the disadvantages and defects of the prior art.
SUMMARY

Provided herein is an ophthalmic composition comprising at least one PPAR-alpha agonist. In some embodiments, the at least one PPAR-alpha agonist is fenofibrate. In some embodiments, the concentration of PPAR-alpha agonist in the ophthalmic composition is in a range selected from the group consisting of a range of from about 0.0001% to about 95%, a range of from about 0.001% to about 50%; a range of from about 0.005% to about 40%; a range of from about 0.01% to about 35%; a range of from about 0.05% to about 30%; a range of from about 0.1% to about 25%; a range of from about 0.1% to about 20%; a range of from about 0.1% to about 15%; and a range of from about 1% to about 10%.

The ophthalmic composition can be formulated in any reasonable manner to allow administration to the eye. For example, the ophthalmic composition can be formulated as an eye drop. In some embodiments, the ophthalmic composition comprises an emulsion. In some embodiments, the ophthalmic composition comprises at least one lipid.

In some embodiments, the composition further comprises at least one delivery agent that assists in the penetration of the surface of the eye. For example, the composition can further comprise a delivery agent that assists in delivery of the composition to the cornea of the eye and/or the retina of the eye.

Non-limiting examples of components of the ophthalmic composition include: glycerin, castor oil, soybean lecithin, polyoxyethylene-polyoxypropylene block copolymer, alpha tocopherol, and carboxymethyl cellulose. In some embodiments, the ophthalmic composition includes at least one of the above; at least two of the above; at least three of the above; at least four of the above; or at least five of the above. In some embodiments, the ophthalmic composition comprises glycerin, castor oil, soybean lecithin, polyoxyethylene-polyoxypropylene block copolymer, alpha tocopherol, and carboxymethyl cellulose.

Also provided herein is a kit comprising a ophthalmic composition as provided herein.

This disclosure also provides a pharmaceutical composition comprising an ophthalmic composition as provided herein and a pharmaceutically acceptable carrier. In
some embodiments, the pharmaceutical composition further comprises a second therapeutic agent. For example, the pharmaceutical composition can include at least one of an anti-angiogenic agent or an anti-VEGF reagent. In some embodiments, the pharmaceutical composition includes a second therapeutic agent selected from the group consisting of: AVASTIN® (bevacizumab), LUCENTIS® (ranibizumab), KENALOG® (triamicinolone acetonide), ganciclovir, foscarinet, vancomycin, ceftazidime, amikacin, amphotericin B, dexamethasone, ACULAR® (ketorolac tromethamine), ACUVAIL® (ketorolac tromethamine), AK-CON-A® (naphazoline), AKTEN® (lidocaine hydrochloride), ALAMAST® (pemirolast potassium), ALPHAGEN® (brimonidine), ALREX® (loteprednol etabonate), ASTEPRO® (azelastine hydrochloride), AZASITE® (azithromycin), BEPREVE® (bepotastine besilate), BESIVANCE® (besifloxacin), BETAXON® (levobetaxolol hydrochloride), COSOPT® (dorzolamide hydrochloride – timolol maleate), DUREZOL® (difluprednate), EYLEA® (aflibercept), LOTEMAX® (loteprednol etabonate), LUMIGAN® (bimatoprost), MACUGEN® (pegaptanib), OCUFLOX® (ofloxacin), OCUIHIST® (naphazoline with pheniramine), OZURDEX® (dexamethazone), QUIXIN® (levofloxacin), RESCULA® (unoprostone isopropyl), RESTASIS® (cyclosporine), SALAGEN® (pilocarpine hydrochloride), TRAVATAN® (travoprost), VALCYTE® (valganciclovir HCl), VIROPTIC® (trifluridine), VISTIDE® (cidofovir), VISUDYNE® (verteporfin), VITRAVENE® (fomivirsen), ZADITOR® (ketotifen), ZIOPTAN® (tafluprost), ZIRGAN® (ganciclovir), ZYMAXID® (gatifloxacin), and combinations thereof.

Further provided herein is a method of inhibiting enzyme activity and/or enzyme production of at least one angiogenic, inflammatory, or fibrogenic factor of diabetic retinopathy (DR). The method comprises administering to at least one eye of a patient suffering from or predisposed to DR at least one of the ophthalmic compositions or pharmaceutical compositions as provided herein. In some embodiments, the factor of DR is selected from the group consisting of VEGF, ICAM-1, TNF-α, and CTGF.

This disclosure also provides a method of treating at least one retinal condition selected from the group consisting of retinal leukostasis, inflammation, vascular leakage, fibrosis, abnormal neovascularization and carcinogenesis in the retina. The method
comprising administering to at least one eye of a patient suffering from or predisposed to the at least one retinal condition at least one of the ophthalmic compositions or pharmaceutical compositions as provided herein.

Provided herein is a method of inhibiting and/or decreasing the occurrence and/or severity of at least one condition/disorder selected from the group consisting of ocular inflammation, corneal inflammation, diabetic retinopathy, diabetic macular edema, macular degeneration, uveitis, retinal inflammation, retinal vascular leakage, and retinal neovascularization. The method comprises administering to at least one eye of a patient suffering from or predisposed to the at least one condition at least one of the ophthalmic compositions or pharmaceutical compositions as provided herein.

This disclosure also provides a method of inhibiting activation of Wnt signaling pathway in an eye of a patient, comprising administering to at least one eye of a patient at least one of the ophthalmic compositions or pharmaceutical compositions as provided herein.

Further provided herein is a method of inhibiting phosphorylation of low-density lipoprotein receptor-related protein 6 (LRP6) or LRP6, comprising administering to at least one eye of a patient at least one of the ophthalmic compositions or pharmaceutical compositions as provided herein.

In some embodiments, of the methods described above, the method can further include administration of a second therapeutic agent to the patient. For example, the second therapeutic agent can be at least one of an anti-angiogenic agent or an anti-VEGF reagent. In some embodiments, the second therapeutic agent is selected from the group consisting of: AVASTIN® (bevacizumab), LUENTIS® (ranibizumab), KENALOG® (triamcinolone acetonide), ganciclovir, foscarnet, vancomycin, ceftazidime, amikacin, amphotericin B, dexamethasone, ACULAR® (ketorolac tromethamine), ACUVAIL® (ketorolac tromethamine), AK-CON-A® (naphazoline), AKTEN® (lidocaine hydrochloride), ALAMAST® (pemirolast potassium), ALPHAGEN® (brimonidine), ALREX® (loteprednol etabonate), ASTEPRO® (azelastine hydrochloride), AZASITE® (azithromycin), BEPREVE® (bepotastine besilate), BESIVANCE® (besifloxacin), BETAXON® (levobetaxolol hydrochloride), COSOPT® (dorzolamide hydrochloride –
timolol maleate), DUREZOL® (difuaprednate), EYLEA® (afibercept), LOTEMAX®
(loteprednol etabonate), LUMIGAN® (bimatoprost), MACUGEN® (pegaptanib),
OCUFLOX® (ofloxacin), OCUHIST® (naphazoline with pheniramine), OZURDEX®
(dexamethazone), QUIXIN® (levofloxacin), RESCULA® (unoprostone isopropyl),
RESTASIS® (cyclosporine), SALAGEN® (pilocarpine hydrochloride), TRAVATAN®
(travoprost), VALCYTE® (valganciclovir HCl), VIROPTIC® (trifluridine), VISTIDE®
(cidofovir), VISUDYNE® (verteporfin), VITRAVENE® (fomivirsen), ZADITOR®
(ketotifen), ZIOPTAN® (tafluprost), ZIRGAN® (ganciclovir), ZYMAXID®
(gatifloxacin), and combinations thereof.

Administration as described herein can include injecting the composition into the
vitreous of the eye of the patient. In some embodiments, administration includes
topically applying the composition to a surface of the eye of the patient.

The details of one or more embodiments of the invention are set forth in the
accompanying drawings and the description below. Other features, objects, and
advantages of the invention will be apparent from the description and drawings, and from
the claims.

DESCRIPTION OF DRAWINGS

FIG. 1 is a bar graph showing the tissue distribution of fenofibrate in animals
following fenofibrate treatments. Normal adult mice, rats or rabbits were treated with
fenofibrate eye drops (3%, 4 times/day, 5 days) or oral fenofibrate (120 mg/kg/d, 5 days).
Fenofibrate or fenofibrlic acid levels in tissue extracts were determined by HPLC/MS
(mean±SD, n=5).

FIG. 2 is a bar graph illustrating that topical application of fenofibrate reduces
retinal vascular leakage in OIR rats. OIR rats were topically administrated 15 μl of 3%
fenofibrate at P12 (immediately after they were removed from a 75% oxygen
environment), and the same volume of vehicle served as a control. At P16, retinal
vascular leakage was quantified by the vascular permeability assay (mean±SD, n=5).

**P<0.01.
FIG. 3 illustrates that topical application of fenofibrate ameliorates ischemia-induced retinal NV in OIR rats. Rats were exposed to 75% oxygen from P7 to P12. Right after returned to room air, rats were topically administrated 15 μl of 3% fenofibrate (5 times/day x 5 days), while the same amount of vehicle served as a control. FIG. 3A and 3B: at P18, retinal vasculature was visualized by fluorescein angiography. FIG. 3C: Non-vascular perfusion area of each subject group was calculated and compared (mean±SD, n=6) **P<0.01.

FIG. 4 shows topical application of fenofibrate eye drops down-regulates retinal VEGF in the OIR rats. At P12, OIR rats were topically administrated with fenofibrate ((3%, 15 μl/eye, 5 times/day), and the same amount of vehicle served as control. FIG. 4A: At P16, equal amounts (50 μg) of retinal proteins were blotted with an antibody against VEGF with β-actin as a loading control. Each lane represents an individual rat. FIG. 4B: Relative to the vehicle, fenofibrate eye drops significantly reduced retinal VEGF levels induced by hypoxia (mean ± SD, n = 3). **P<0.01.

FIG. 5 illustrates that topical application of fenofibrate eye drops reduced retinal vascular leakage in STZ-induced diabetic rats. 6 weeks following diabetes onset, STZ-induced diabetic rats were topically administrated fenofibrate (3% fenofibrate, 15 μl/eye, 3 times/day for 4 weeks) and the same volume of vehicle served as control. Four weeks after treatment, retinal vascular leakage was quantified by the vascular permeability assay (mean±SD, n=5).

FIG. 6 shows that fenofibrate eye drops decrease retinal vascular leukostasis in type 1 diabetic animals. The retinal vascular endothelium and adherent leukocytes were stained with FITC-conjugated Con-A after removal of circulating leukocytes. The retinae were then flat-mounted, and adherent leukocytes visualized by fluorescence microscopy. Representative images of retinal flat mounts from non-diabetic rats (FIG. 6A), STZ-induced diabetic rats treated with vehicle (FIG. 6B), and diabetic rats treated with fenofibrate eye drops (FIG. 6C) (3% fenofibrate, 30 μl/eye, 3 times/day, 4 weeks) are shown. Scale bar, 50 μm. FIG. 6D: Quantification of leukocytes showed that fenofibrate eye drops significantly reduced adherent leukocytes in the diabetic rats (mean±SD, n=7).
FIG. 7 illustrates that fenofibrate eye drops suppress the diabetes-induced over-expression of pathogenic factors. Soluble ICAM-1 (sICAM-1) levels were measured using ELISA in retinas from STZ-induced diabetic rats treated with fenofibrate eye drops or vehicle (3% fenofibrate, 30 µl, 3 times/day, 4 weeks) and expressed as ng/mg of total retinal protein (mean±SD, n=3). *P<0.05.

FIG. 8 shows that topical fenofibrate eye drops do not cause abnormalities to the anterior segment or retina in treated rats. FIG. 8A: Slit lamp examination of fenofibrate-treated rats (non-diabetic and diabetic) revealed no abnormalities in the anterior segment structures (iris, pupil, and cornea). FIG. 8B: ERG showed no significantly changes of the A wave and B wave in the eyes treated with fenofibrate eye drops and vehicles in rats.

FIG. 9 illustrates that fenofibrate blocks REC migration and tube formation and prevents HG induced oxidative stress. FIGs. 9A&B: Primary RECs treated with 50 µM fenofibrate or the same volume of vehicle were cultured on Matrigel for six hours. In the absence of fenofibrate, REC formed a capillary pattern (FIG. 9A), which was blocked by fenofibrate (FIG. 9B). FIGs. 9C&D: RECs were cultured in the presence or absence of 50 µM fenofibrate on a gelatin-coated plate and an acellular area was generated by a scratch. As visualized 24 hours after scratch wounding, the scratch width in the control cells was smaller than in the wounded cells treated with fenofibrate. FIG. 9E: Relative to the control cells, fenofibrate significantly reduced REC migration. (mean ± SD, n = 3). **P<0.01. FIGs. 9F-H: The undersurfaces of a 96-well Transwell motility chamber inserts were coated with 10 mg/ml mouse cellular fibronectin. RECs were added to the upper chambers of the Transwell and cultured in the presence (FIG. 9G) or absence (FIG. 9F) of various concentrations of fenofibrate for 6 hours. The cells migrated to the undersurface were stained with DiI and quantified, after removal of cells from the upper surface. Scale bar, 100 µm. FIG. 9H: Relative to the control cells, fenofibrate significantly reduced REC migration to the undersurface of the Transwell motility chamber (mean ± SD, n = 3). **P<0.01

FIG. 10 illustrates inhibition of expressions of inflammatory and vasculogenesis factors in HUVEC cells by fenofibrate. FIGs. 10A & B: HUVEC cell treated with 30 mM D-glucose 48 hours or 400 µM CoCl₂ 8 hours together with different doses of
fenofibrate; ICAM-1, CTGF and VEGF expression levels were dose-dependently decreased by fenofibrate in all studies.

**FIG. 11** shows that treatment with a 3% fenofibrate eye drop formulation decreased retina leakage in STZ (**FIG. 11A**) and OIR (**FIG. 11B**) rats. The permeability assay shows fenofibrate significantly decreased the Evans Blue leakage in retinas in both STZ (n=4) and OIR (n=4) models following administration for 8 weeks and 5 days, respectively.

**FIG. 12** illustrates the results of a leukostasis assay in normal and STZ rats. **FIGS. 12A, B & C** show the retina vessels of rats without treatment, STZ+ vehicle eye drop and STZ + 3% fenofibrate eye drop, respectively. **FIG. 12D**: Compared with normal rats (A), STZ induced T1DM rats administered a control eye drop formulation (B) showed significantly increased leukocytes in the total retina vessels, which dramatically decreased in the induced rats treated with topical administration of 3% fenofibrate eye drops. (P<0.01)

**FIG. 13** shows that administration of an eye drop formulation of fenofibrate inhibits an inflammation reaction in both STZ induced T1DM DR and OIR models. **FIGS. 13A & B**: Western blot results shows retina ICAM-1 expressions were dramatically induced by oxygen in control P16 rats, but this expression significantly decreased by 5-day of topical 3% fenofibrate eye drop treatment. **FIG. 13C**, Elisa results show retina ICAM-1 expression of T1DM rat models were also significantly increased on control subjects, but expression was inhibited following 8-week topical 3% fenofibrate eye drop treatment.

**FIG. 14** illustrates that administration of 3% fenofibrate eye drop formula ameliorates angiogenesis. **FIG. 14A**: angiography and HE staining of retinas in control subjects, while **FIG. 14B** shows staining in rats treated with the 3% fenofibrate formula. **FIG. 14C**: Fenofibrate eye drop treatment decreases central 1/3 non-vessel area of OIR retinas (Photoshop CS5, pixels, n=4). **FIG. 14D**: illustrates Western blot results of vascular endothelial growth factor (VEGF) expression following relative hypoxia after 5 days in a 70% oxygen chamber.
FIG. 15 illustrates that the fenofibrate eye drop formulation did not injure the cornea or visual function. FIGs. 15A & B provide the ERG data for STZ rats following treatment with the eye drop formulation for 8 weeks.

DETAILED DESCRIPTION

Before explaining at least one embodiment of the present disclosure in detail by way of exemplary drawings, experimentation, results, and laboratory procedures, it is to be understood that the present disclosure is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings, experimentation and/or results. The present disclosure is capable of other embodiments or of being practiced or carried out in various ways. As such, the language used herein is intended to be given the broadest possible scope and meaning; and the embodiments are meant to be exemplary - not exhaustive. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well-known and commonly used in the art. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well-known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al. Molecular Cloning: A Laboratory Manual (2nd ed., Cold Spring Harbor Laboratory Press, Cold
Spring Harbor, N.Y. (1989) and Coligan et al. Current Protocols in Immunology (Current Protocols, Wiley Interscience (1994)), which are incorporated herein by reference. The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which present disclosure pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of the present disclosure have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the present disclosure. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the present disclosure as defined by the appended claims.

As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.” The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.” Throughout this application, the term “about” is used to indicate that a value includes the inherent
variation of error for the device, the method being employed to determine the value, or
the variation that exists among the study subjects. The use of the term “at least one” will
be understood to include one as well as any quantity more than one, including but not
limited to, 2, 3, 4, 5, 10, 15, 20, 30, 40, 50, 100, etc. The term “at least one” may extend
up to 100 or 1000 or more, depending on the term to which it is attached; in addition, the
quantities of 100/1000 are not to be considered limiting, as higher limits may also
produce satisfactory results.

As used in this specification and claim(s), the words “comprising” (and any form
of comprising, such as “comprise” and “comprises”), “having” (and any form of having,
such as “have” and “has”), “including” (and any form of including, such as “includes”
and “include”) or “containing” (and any form of containing, such as “contains” and
“contain”) are inclusive or open-ended and do not exclude additional, unrecited elements
or method steps.

The term “or combinations thereof” as used herein refers to all permutations and
combinations of the listed items preceding the term. For example, “A, B, C, or
combinations thereof” is intended to include at least one of: A, B, C, AB, AC, BC, or
ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA,
ACB, BAC, or CAB. Continuing with this example, expressly included are combinations
that contain repeats of one or more item or term, such as BB, AAA, MB, BBC,
AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand
that typically there is no limit on the number of items or terms in any combination, unless
otherwise apparent from the context.

As used herein, “substantially pure” means an object species is the predominant
species present (i.e., on a weight basis it is more abundant than any other individual
species in the composition). Generally, a substantially pure composition will comprise
the object species as more than about 50% percent of all species present in the
composition, such as more than about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%,
95%, and 99%. In one embodiment, the object species is purified to essential
homogeneity (contaminant species cannot be detected in the composition by conventional
detection methods) wherein the composition consists essentially of a single macromolecular species.

The term “agent” refers to a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials.

The term “antagonist” refers to an agent that reduces an activity of a protein/enzyme. The term “agonist” refers to an agent that increases an activity of a protein/enzyme.

The term “patient” includes human and veterinary subjects. In certain embodiments, a patient is a mammal. In certain other embodiments, the patient is a human.

The terms “treating” and “treatment” mean causing a therapeutically beneficial effect, such as ameliorating existing symptoms, preventing additional symptoms, ameliorating or preventing the underlying metabolic causes of symptoms, postponing or preventing the further development of a disorder and/or reducing the severity of symptoms that will or are expected to develop. Those in need of treatment include, but are not limited to, individuals already having a particular condition. The term “treating” refers to administering an agent to a patient for therapeutic and/or prophylactic/preventative purposes.

A “therapeutic agent” refers to an agent that may be administered to bring about a therapeutic and/or prophylactic/preventative effect.

“Synergism” or “synergistic” refers to a combination of two or more active agents that create a result which is greater than the sum of the individual active agents.

A “disorder” is any condition that would benefit from treatment with the compounds and compositions provided herein. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders encompassed by the present disclosure include diabetic retinopathy, ocular inflammation, corneal inflammation, diabetic macular edema, macular degeneration, uveitis, retinal inflammation, retinal vascular leakage, retinal neovascularization, cancer, and the like.
The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, renal cancer, prostate cancer, vulvar cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer. In some embodiments, the cancer is a cancer that affects the eye or the ocular structures. Non-limiting examples of such cancers include: uveal melanoma (melanoma of the iris, choroid or ciliary body); retinoblastoma, basal cell carcinoma of eyelid; squamous cell carcinoma of eyelid; sebaceous carcinoma of eyelid; melanoma of eyelid; Merkel cell carcinoma of eyelid; adnexal carcinoma of eyelid; squamous cell carcinoma of conjunctiva; melanoma of conjunctiva; MALT lymphoma of conjunctiva; orbital lymphoma; orbital sarcoma (including rhabdomyosarcoma, myxoid sarcoma, alveolar soft part sarcoma); orbital and optic nerve menigioma; metastatic tumors of orbit; lymphoma of lacrimal gland; adenoid cystic carcinoma of lacrimal gland; pleomorphic adenoma of lacrimal gland; other epithelial tumors of lacrimal gland; squamous cell carcinomas of lacrimal sac; transitional cell carcinoma of lacrimal sac and duct; and lacrimal sac lymphoma.

“Mammal” for purposes of treatment refers to any animal classified as a mammal, including human, domestic and farm animals, nonhuman primates, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc.

The term “effective amount” refers to an amount of a biologically active molecule (e.g., a compound or composition provided herein) sufficient to exhibit a detectable therapeutic effect without undue adverse side effects (such as toxicity, irritation and allergic response) commensurate with a reasonable benefit/risk ratio when used in the manner of the present disclosure. The therapeutic effect may include, for example, but not by way of limitation, inhibiting and/or neutralizing at least one activity of LRP6, LRP5, or PARPα. The effective amount for a patient will depend upon the type of
patient, the patient's size and health, the nature and severity of the condition to be treated, the method of administration, the duration of treatment, the nature of concurrent therapy (if any), the specific formulations employed, and the like. Thus, it is not possible to specify an exact effective amount in advance. However, the effective amount for a given situation can be determined by one of ordinary skill in the art using routine experimentation based on the information provided herein.

As used herein, the term “concurrent therapy” is used interchangeably with the terms “combination therapy” and “adjunct therapy”, and will be understood to mean that the patient in need of treatment is treated or given another drug for the disease/disorder in conjunction with the compositions of the present disclosure. This concurrent therapy can be sequential therapy, where the patient is treated first with one drug and then one or more additional drugs, or the two or more drugs may be administered simultaneously.

The term “pharmaceutically acceptable” refers to compounds and compositions which are suitable for administration to humans and/or animals without undue adverse side effects such as toxicity, irritation and/or allergic response commensurate with a reasonable benefit/risk ratio.

By “biologically active” is meant the ability to modify the physiological system of an organism. A molecule can be biologically active through its own functionalities, or may be biologically active based on its ability to activate or inhibit molecules having their own biological activity.

The compositions of the present disclosure may be administered to a patient by any method known in the art, including but not limited to, oral, topical, transdermal, parenteral, subcutaneous, intranasal, intramuscular, intraperitoneal, intravitreal, intraocular, ophthalmic, and intravenous routes, including both local and systemic applications. In some embodiments, the compositions may be administered topically. In some embodiments, the compositions may be administered by intraocular injection. In some embodiments, the compositions may be administered by intravitreal injection.

The term “Wnt” or the plural “Wnts” as used herein will be understood to refer to a group of secreted, cysteine-rich glycoproteins which bind to a co-receptor complex of frizzled (Fz) receptors and low-density lipoprotein receptor-related proteins 5 or 6.
(LRP5/6) and regulate the expression of a number of target genes through an intracellular signaling pathway, namely the Wnt pathway. In humans, the Wnts include WNT1, WNT2, WNT2B, WNT3, WNT3A, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A and WNT9B. In the absence of Wnt ligands, β-catenin, a downstream effector of the canonical Wnt pathway, is phosphorylated by a protein complex containing glycogen synthase kinase-3β (GSK-3β). The phosphorylated β-catenin is constantly degraded, to prevent its accumulation. Upon binding of certain Wnts to the Fz-LRP5/6 co-receptors, phosphorylation of β-catenin is inhibited, which prevents the degradation of β-catenin and results in its accumulation. β-catenin is then translocated into the nucleus, where it associates with T cell factor for DNA binding and thus regulates expression of target genes including but not limited to VEGF.

The term “ophthalmic composition” as used herein will be understood to refer to any composition for direct or local administration to an eye of a patient. The composition may be administered topically to an eye surface or may be injected into the eye (e.g., intravitreal injection, subconjunctival injection, sub-tenon injection, retrobulbar injection, subretinal injection, suprachoroidal injection, and the like). The ophthalmic composition may be provided in any form that allows local or direct administration thereof to the eye, including but not limited to, a solution, drops, mist/spray, plasters and pressure sensitive adhesives, ointment, lotion, cream, gel, lyophilized/spray-dried forms, rods, beads, emulsions, lenses, patch, plug, elixir, and the like. The ophthalmic compositions provided herein typically vary according to the particular active agent (i.e., PPARα agonist) used, the preferred drug release profile, the condition being treated, and the medical history of the patient. In addition, the ophthalmic compositions of the present disclosure may be designed to provide delayed, controlled or sustained release using formulation techniques which are well known in the art.

The term “PPAR-alpha agonist” as used herein refers to any agonist of peroxisome proliferator-activated receptor alpha (“PPAR-alpha” or “PPARα”). For example, a PPARα agonist is a compound or composition which when combined with PPARα directly or indirectly (e.g., binding directly to PPARα) stimulates or increases an in vivo or in vitro reaction typical for the receptor, e.g., transcriptional regulation activity.
as measured by an assay known to one skilled in the art including, but not limited to, the “co-transfection” or “cistrans” assays described or disclosed in U.S. Patent Nos. 4,981,784; 5,071,773; 5,298,429; 5,506,102; WO89/05355; WO91/06677; WO92/05447; WO93/11235; WO93/23431; WO94/23068; and WO95/18380. PPARα agonists may also be identified according to an assay described in U.S. Patent No 6,008,239.

Non-limiting examples of PPARα agonists include fibrate drugs. Fibrates are a class of amphipathic carboxylic acids and esters, such as, but not limited to, clofibrate, aluminum clofibrate, simfibrate, ronifibrate, etofibrate, clofibric acid, clidrofibrate, gemfibrozil, ciprofibrate, bezafibrate, binifibrate, etofylline clofibrate, pirifibrate, fenofibrate, fenofibric acid, or a pharmaceutically acceptable salt thereof. In some embodiments, the fibrate drug is a derivative, for example, an acid or ester derivative. In some embodiments, the PPARα agonist is fenofibrate. PPARα compounds also include those disclosed in Tontonez et al., Cell 79:1147-1156 (1994), Lehmann et al., J. Biol. Chem. 270(22):1-4, 1995, Amri et al., J. Lipid Res. 32:1449-1456 (1991), Kliewer et al., Proc. Natl. Acad. Sci. USA 94:4318-4323 (1997), Amri et al., J. Lipid Res. 32:1457-1463, (1991) and Grimaldi et al., Proc. Natl. Acad. Sci. USA 89:10930-10934 (1992). PPARα agonist compounds also include those described in U.S. Pat. No. 6,008,239, WO97/27847, WO97/27857, WO97/28115, WO97/28137, WO97/28149, WO92/10468, and WO01/80852.

Turning now to certain particular embodiments of the present disclosure, an ophthalmic composition comprising at least one PPAR-alpha agonist, or a pharmaceutically acceptable salt thereof, is disclosed and claimed herein. In one embodiment, at least one of the PPAR-alpha agonists is fenofibrate, or a pharmaceutically acceptable salt thereof. In some embodiments, an ophthalmic composition as provided herein comprises fenofibrate, or a pharmaceutically acceptable salt thereof. The ophthalmic composition may be provided in any formulation that allows the ophthalmic composition to function in accordance with the present disclosure; for example, but not by way of limitation, the ophthalmic composition may be provided in the form of a solution, drops, mist/spray, plasters and pressure sensitive adhesives,
ointment, lotion, cream, gel, lyophilized/spray-dried forms, and the like. In one particular
embodiment, the ophthalmic composition is provided in the form of an eye drop.

The PPAR-alpha agonist may be present in the ophthalmic composition at any
concentration that allows the ophthalmic composition to function in accordance with the
present disclosure; for example, but not by way of limitation, the PPAR-alpha agonist
may be present in a range having a lower level selected from 0.0001%, 0.005%, 0.001%,
0.005%, 0.01%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%,
1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9% and 2.0%; and an upper level
selected from 3%, 3.5%, 4%, 4.5%, 5%, 5.5%, 6%, 6.5%, 7%, 7.5%, 8%, 8.5%, 9%,
9.5%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%,
40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% and 95% by weight of the
composition. Non-limiting examples of particular ranges include a range of from about
0.0001% to about 95% by weight of the composition; a range of from about 0.001% to
about 50% by weight of the composition; a range of from about 0.005% to about 40% by
weight of the composition; a range of from about 0.01% to about 35% by weight of the
composition; a range of from about 0.05% to about 30% by weight of the composition; a
range of from about 0.1% to about 25% by weight of the composition; a range of from
about 0.1% to about 20% by weight of the composition; a range of from about 0.1% to
about 15% by weight of the composition; a range of from about 1% to about 10% by
weight of the composition; a range of from about 1% to about 5% by weight of the
composition; and the like. In some embodiments, the PPAR-alpha agonist may be
present in an amount of about 3% by weight of the composition.

In some embodiments, the PPAR-alpha agonist is fenofibrate, or a
pharmaceutically acceptable salt thereof. In some instances of this embodiments,
fenofibrate, or a pharmaceutically acceptable salt thereof, may be present in the
ophthalmic composition at any concentration that allows the ophthalmic composition to
function in accordance with the present disclosure; for example, but not by way of
limitation, the fenofibrate may be present in a range having a lower level selected from
0.0001%, 0.005%, 0.001%, 0.005%, 0.01%, 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%,
0.6%, 0.7%, 0.8%, 0.9%, 1.0%, 1.1%, 1.2%, 1.3%, 1.4%, 1.5%, 1.6%, 1.7%, 1.8%, 1.9%
and 2.0%; and an upper level selected from 3%, 3.5%, 4%, 4.5%, 5%, 5.5%, 6%, 6.5%, 7%, 7.5%, 8%, 8.5%, 9%, 9.5%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% and 95% by weight of the composition. Non-limiting examples of particular ranges include a range of from about 0.0001% to about 95% by weight of the composition, a range of from about 0.001% to about 50% by weight of the composition; a range of from about 0.005% to about 40% by weight of the composition; a range of from about 0.01% to about 35% by weight of the composition; a range of from about 0.05% to about 30% by weight of the composition; a range of from about 0.1% to about 25% by weight of the composition; a range of from about 0.1% to about 20% by weight of the composition; a range of from about 0.1% to about 15% by weight of the composition; a range of from about 1% to about 10% by weight of the composition; a range of from about 1% to about 5% by weight of the composition; and the like. In some embodiments, the fenofibrate may be present in an amount of about 3% by weight of the composition.

The pharmaceutical composition may be administered at once, or may be divided into a number of smaller doses to be administered at intervals of time. It is understood that the precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by extrapolation from in vivo or in vitro test data. It is to be noted that concentrations and dosage values may also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular patient, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that the concentration ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed compositions.

Any of the ophthalmic compositions described and claimed herein may further comprise at least one delivery agent that assists in the penetration of a surface of an eye; in certain embodiments, the delivery agent may assist in delivery to the cornea and/or retina of the eye. For example, in order for a topical application to be effective, the composition may need to be able to penetrate the surface of the eye so that it can travel to
the desired tissue. This may include penetrating the conjunctiva and/or the cornea. Also, the penetration rate should be sufficient to impart an effective dose.

Pharmaceutically acceptable salts of the compounds described herein include the acid addition and base salts thereof.

Suitable acid addition salts are formed from acids which form non-toxic salts. Examples include the acetate, adipate, aspartate, benzoate, besylate, bicarbonate/carbonate, bisulphate/sulphate, borate, camsylate, citrate, cyclamate, edisylate, esylate, formate, fumarate, gluceptate, gluconate, glucuronate, hexafluorophosphate, hibenzate, hydrochloride/chloride, hydrobromide/bromide, hydroiodide/iodide, hydrogen phosphate, isethionate, D- and L-lactate, malate, maleate, malonate, mesylate, methylsulphate, 2-napsylate, nicotinate, nitrate, orotate, oxalate, palmitate, pamoate, phosphate/hydrogen, phosphate/phosphate dihydrogen, pyroglutamate, saccharate, stearate, succinate, tannate, D- and L-tartrate, 1-hydroxy-2-naphthoate tosylate and xinafoate salts.

Suitable base salts are formed from bases which form non-toxic salts. Examples include the aluminium, arginine, benzathine, calcium, choline, diethylamine, diolamine, glycine, lysine, magnesium, meglumine, olamine, potassium, sodium, tromethamine and zinc salts.

Hemisalts of acids and bases may also be formed, for example, hemisulphate and hemicalcium salts.

Generally, the pharmaceutical formulations provided herein include one or more pharmaceutically acceptable excipients. The term “excipient” is used herein to describe any ingredient other than the PPARα agonist provided herein. The choice of excipient will to a large extent depend on factors such as the particular mode of administration, the effect of the excipient on solubility and stability, and the nature of the dosage form.

Non-limiting examples of pharmaceutical excipients suitable for administration of the compounds provided herein include any such carriers known to those skilled in the art to be suitable for the particular mode of administration. Pharmaceutically acceptable excipients include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, self-emulsifying drug delivery systems (SEDDS) such as d-α-tocopherol
polyethylene glycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens or other similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, sterile water, saline, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium-chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethyl cellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, and wool fat. Cyclodextrins such as α-, β, and γ-cyclodextrin, or chemically modified derivatives such as hydroxyalkylcyclodextrins, including 2- and 3-hydroxypropyl-b-cyclodextrins, or other solubilized derivatives can also be advantageously used to enhance delivery of compounds of the formulae described herein. In some embodiments, the excipient is a physiologically acceptable saline solution.

The pharmaceutical compositions are provided for administration to humans and animals in unit dosage forms, such as powders, granules, sterile parenteral solutions or suspensions, and liquid solutions or suspensions, and oil-water emulsions containing suitable quantities of the PPARα agonist or pharmaceutically acceptable derivatives thereof. The pharmaceutical compositions are, in one embodiment, formulated and administered in unit-dosage forms or multiple-dosage forms. Unit-dose forms as used herein refers to physically discrete units suitable for human and animal patients and packaged individually as is known in the art. Each unit-dose contains a predetermined quantity of the therapeutically active compound sufficient to produce the desired therapeutic effect, in association with the required pharmaceutical carrier, vehicle or diluent. Examples of unit-dose forms include, for example, ampoules and syringes and individually packaged bottles or vials. Unit-dose forms may be administered in fractions or multiples thereof. A multiple-dose form is a plurality of identical unit-dosage forms packaged in a single container to be administered in segregated unit-dose form. Examples of multiple-dose forms include vials, bottles, or bottles of pints or gallons. Hence, multiple dose form is a multiple of unit-doses which are not segregated in packaging.
In some embodiments, the ophthalmic formulations described herein are conveniently packaged in forms suitable for metered application, such as in containers equipped with a dropper, to facilitate application to the eye. Containers suitable for dropwise application are usually made of suitable inert, non-toxic plastic material, and generally contain between about 0.5 and about 15 ml solution. One package may contain one or more unit doses.

Preservative-free solutions are often formulated in non-resealable containers containing up to about ten (e.g., up to about five) unit doses, where a typical unit dose is from one to about 8 drops, preferably one to about 3 drops. The volume of one drop usually is about 20-35 μl.

Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, or otherwise mixing an active compound as defined above and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, glycols, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting agents, emulsifying agents, solubilizing agents, pH buffering agents and the like, for example, acetate, sodium citrate, cyclodextrin derivatives, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and other such agents.

Pharmaceutical compositions suitable for the delivery of compounds described herein and methods for their preparation will be readily apparent to those skilled in the art. Such compositions and methods for their preparation may be found, for example, in Remington's Pharmaceutical Sciences, 19th Edition (Mack Publishing Company, 1995).

In some embodiments, the ophthalmic composition is a solution for topical administration (e.g., an eye drop formulation). For such applications, the solutions can be prepared using a physiological saline solution as a major vehicle. The pH of such ophthalmic solutions can be maintained between 4.5 and 8.0 with an appropriate buffer system. In some embodiments, the solution has a neutral pH (e.g., about 6.8 to about 7.8). The formulations may also contain conventional, pharmaceutically acceptable preservatives, stabilizers and surfactants.
Examples of preservatives that may be used in the ophthalmic compositions of include, but are not limited to, benzalkonium chloride, chlorobutanol, thimerosal, phenylmercuric acetate, phenylmercuric nitrate, and PURITE®. In some embodiments, a surfactant includes Tween 80. Non-limiting examples of vehicles that can be used in the ophthalmic preparations include, but are not limited to, polyvinyl alcohol, povidone, hydroxypropyl methyl cellulose, poloxamers, carboxymethyl cellulose, hydroxyethyl cellulose cyclodextrins, and purified water.

Tonicity adjustors may be added as needed or convenient. These include, but are not limited to, salts, particularly sodium chloride and potassium chloride, mannitol, and glycerin, or any other suitable ophthalmically acceptable tonicity adjustor.

Various buffers and means for adjusting pH may be used so long as the resulting preparation is ophthalmically acceptable. Accordingly, buffers can include acetate buffers, citrate buffers, phosphate buffers, and borate buffers. Acids or bases may be used to adjust the pH of these formulations as needed.

In a similar vein, an ophthalmically acceptable antioxidant for use in the present formulations includes, but is not limited to, sodium metabisulfite, sodium thiosulfate, acetylcysteine, butylated hydroxyanisole, and butylated hydroxytoluene.

Other excipient components which may be included in the ophthalmic preparations include chelating agents. For example, edentate disodium, although other chelating agents may also be used in place of or in conjunction with it.

In some embodiments, the ingredients can be used in the following amounts:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active ingredient</td>
<td>0.001 – 5</td>
</tr>
<tr>
<td>Preservative</td>
<td>0 – 0.10</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0 – 40</td>
</tr>
<tr>
<td>Tonicity adjustor</td>
<td>0 – 10</td>
</tr>
<tr>
<td>Buffer</td>
<td>0 – 10</td>
</tr>
<tr>
<td>pH adjustor</td>
<td>As needed to achieve a pH of 4.5 – 8.0</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>As needed</td>
</tr>
<tr>
<td>Surfactant</td>
<td>As needed</td>
</tr>
<tr>
<td>Purified water</td>
<td>As needed to bring formulation to 100%</td>
</tr>
</tbody>
</table>
In certain embodiments, the ophthalmic composition may comprise an emulsion. In other certain embodiments, the ophthalmic composition may further include at least one lipid.

In some embodiments, the ophthalmic composition includes at least one of a viscosity enhancing agent or an antioxidant.

The viscosity enhancing agent typically enhances the viscosity of the ocular solution to increase retention time of the solution on the eye, and in some instances, to provide a protective layer on the eye surface. Viscosity enhancing agents include, among others, carbopol gels, dextran 40 (molecular weight of 40,000 Daltons), dextran 70 (molecular weight of 70,000 Daltons), gelatin, glycerin, polyoxyethylene-polyoxypropylene block copolymer, carboxymethylcellulose (CMC), hydroxyethyl cellulose, hydroxypropyl methylcellulose, (HPMC) methylcellulose, ethylcellulose, polyethylene glycol, poloxamer 407, polysorbate 80, propylene glycol, polyvinyl alcohol, and polyvinylpyrrolodine (povidone), in various molecular weights and in various compatible combinations. Viscosity of a solution is given in poise units, with a viscosity between about 25 and 50 cps being suitable for ophthalmic solutions. The amount of agent for use in the ocular formulations can be determined by one of skill in the art, and can provide residence times in the eye of 15 min or more, 30 min or more, 1 hr or more, 2 hrs or more, 3 hrs or more, 4 hrs or more, 6 hrs or more, 8 hrs or more, 12 hr or more as would be suitable for the condition being treated and the desired retention time of the solution on the eye.

Suitable antioxidants, include, by way of example and not limitation, EDTA (e.g., disodium EDTA), sodium bisulphite, sodium metabisulphite, sodium thiosulfate, thiourea, and alphatocopherol.

In one embodiment, the ophthalmic composition may include at least one of glycerin, castor oil, soybean lecithin, polyoxyethylene-polyoxypropylene block copolymer, alpha tocopherol, and carboxymethyl cellulose. In other embodiments, the ophthalmic composition may include at least two of the above reagents, at least three of the above reagents, at least four of the above reagents, at least five of the above reagents, or all of the above reagents.
In some embodiments, the ophthalmic composition includes one or more additives.

In some embodiments, the additive is one or more wetting agents. Generally, wetting agents can hydrate and limit drying of the eye. Wetting agents generally are hydrophilic polymers, including, by way of example and not limitation, polysorbate 20 and 80, poloxamer 282, and tyloxapol. In some embodiments, wetting agents also include, among others, cellulose based polymers, such as HPMC and CMC; polyvinylpyrrolodine; and polyvinyl alcohol.

In some embodiments, the additive is one or more lubricating agents. Ocular lubricants can approximate the consistency of endogenous tears and aid in natural tear build-up. Lubricating agents can include non-phospholipid and phospholipid-based agents. Ocular lubricants that are non-phospholipid based include, but are not limited to, propylene glycol; ethylene glycol; polyethylene glycol; hydroxypropylmethylcellulose; carboxymethylcellulose; hydroxypropylcellulose; dextran, such as, dextran 70; water soluble proteins, such as gelatin; vinyl polymers, such as polyvinyl alcohol, polyvinylpyrrolidone, povidone; petrolatum; mineral oil; and carbomers, such as, carbomer 934P, carbomer 941, carbomer 940, and carbomer 974P. Non-phospholipid lubricants can also include compatible mixtures of any of the foregoing agents.

In some embodiments, the ocular lubricating agent is a phospholipid-based lubricant. As used herein, “phospholipid lubricant” refers to aqueous compositions which comprise one or more phospholipids. Tear film has been shown to comprise a lipid layer, which is secreted by tear glands and is composed of various types of phospholipids (see, e.g., McCulley and Shine, 2003, The Ocular Surface 1: 97-106). Examples of phospholipid lubricant formulations include those disclosed in U.S. Pat. Nos. 4,804,539; 4,883,658; 4,914,088; 5,075,104; 5,278,151; 5,294,607; 5,371,108; and 5,578,586; all of which are incorporated herein by reference. Lubricating compositions based on liposomes are described in U.S. Pat. No. 4,818,537 and U.S. Pat. No. 5,800,807, the disclosures of which are incorporated by reference herein.

In some embodiments, the additive can be one or more tonicity agents, which can be used to adjust the tonicity of the composition, for example, to the tonicity of natural
tears. Suitable tonicity agents include, by way of example and not limitation, dextrans (e.g., dextran 40 or 70), dextrose, glycerin, potassium chloride, propylene glycol, and sodium chloride. Equivalent amounts of one or more salts made up of cations, for example, such as potassium, ammonium and anions such as chloride, citrate, ascorbate, borate, phosphate, bicarbonate, sulfate, thiosulfate, bisulfate; the salts sodium bisulfate and ammonium sulfate can also be used. The amount of tonicity agent will vary, depending on the particular agent to be added.

The present disclosure is also directed to a kit comprising any of the ophthalmic compositions described herein. In certain embodiments, a kit can include one or more delivery systems, e.g., for delivering or administering an ophthalmic composition as provided above, and directions for use of the kit (e.g., instructions for treating a patient). In another embodiment, the kit can include a composition as described herein and a label that indicates that the contents are to be administered to a patient with diabetic retinopathy. In another embodiment, the kit can include a composition as described herein and a label that indicates that the contents are to be administered to a patient with one or more of ocular inflammation, corneal inflammation, diabetic retinopathy (DR), diabetic macular edema, macular degeneration (including, but not limited to, age related macular degeneration), uveitis, retinal inflammation, retinal vascular leakage, retinal neovascularization, cancer, and other inflammatory and neovascular disorders of the eye.

The present disclosure is also directed to a pharmaceutical composition comprising any of the ophthalmic compositions described herein and a pharmaceutically acceptable carrier. The pharmaceutical composition may further include a second therapeutic agent. In some embodiments, the second therapeutic agent has a synergistic effect with the ophthalmic composition. In some embodiments, a second therapeutic agent is selected from the group consisting of: an additional PPARα agonist, an anti-angiogenic agent, an anti-VEGF reagent, and a VEGF Trap. Non-limiting examples of a second therapeutic agent include: AVASTIN® (bevacizumab), LUCENTIS® (ranibizumab), KENALOG® (triamcinolone acetonide), ganciclovir, foscarnet, vancomycin, ceftazidime, amikacin, amphotericin B, dexamethasone, ACULAR® (ketorolac tromethamine), ACUVAIL® (ketorolac tromethamine), AK-CON-A®
(naphazoline), AKTEN® (lidocaine hydrochloride), ALAMAST® (pemirolast potassium), ALPHAGEN® (brimonidine), ALREX® (loteprednol etabonate), ASTEPRO® (azelastine hydrochloride), AZASITE® (azithromycin), BEPREVE® (bepotastine besilate), BESIVANCE® (besifloxacin), BETAXON® (levobetaxolol hydrochloride), COSOPT® (dorzolamide hydrochloride – timolol maleate), DUREZOL® (difluprednate), EYLEA® (aflibercept), LOTEMAX® (loteprednol etabonate), LUMIGAN® (bimataprost), MACUGEN® (pegaptanib), OCUFLOX® (ofloxacin), OCUHIST® (naphazoline with pheniramine), OZURDEX® (dexamethazone), QUIXIN® (levoflaxacin), RESCULA® (unoprostone isopropl), RESTASIS® (cyclosporine), SALAGEN® (pilocarpine hydrochloride), TRAVATAN® (travoprost), VALCYTE® (valganciclovir HCl), VIROPTIC® (trifluridine), VISTIDE® (cidofovir), VISUDYNE® (verteporfin), VITRAVENE® (fomivirsen), ZADITOR® (ketotifen), ZIOPTAN® (tafluprost), ZIRGAN® (ganciclovir), ZYMAXID® (gatifloxacin) and the like and combinations or derivatives thereof.

The present disclosure is further related to a method of inhibiting activation of the Wnt signaling pathway in an eye of a patient, the method comprising administering any of the ophthalmic/pharmaceutical compositions described in detail herein above to at least one eye of a patient.

The present disclosure is further related to a method of inhibiting enzyme activity and/or enzyme production of at least one angiogenic, inflammatory and/or fibrogenic factor of DR. Such factors include, but are not limited to, VEGF, ICAM-1, TNF-α, and CTGF. The method includes administering to at least one eye of a patient suffering from or predisposed to DR any of the ophthalmic/pharmaceutical compositions described in detail herein above.

The present disclosure is also directed to a method of treating at least one retinal condition selected from the group consisting of retinal leukostasis, inflammation, vascular leakage, fibrosis, abnormal neovascularization (such as, but not limited to, retinal neovascularization and/or choroidal neovascularization) and carcinogenesis in the retina. The method comprises administering to at least one eye of a subject suffering
from or predisposed to the at least one retinal condition any of the ophthalmic/pharmaceutical compositions described in detail herein above.

The present disclosure is further directed to a method of inhibiting and/or decreasing the occurrence and/or severity of at least one condition/disorder selected from the group consisting of ocular inflammation, corneal inflammation, diabetic retinopathy (DR), diabetic macular edema, macular degeneration (including, but not limited to, age related macular degeneration), uveitis, retinal inflammation, retinal vascular leakage, retinal neovascularization, cancer, and other inflammatory and neovascular disorders of the eye. The method includes administering an effective amount of any of the ophthalmic/pharmaceutical compositions described in detail herein above to at least one eye of a patient suffering from or predisposed to at least one of the above conditions, whereby the ophthalmic/pharmaceutical composition may inhibit activation of the Wnt signaling pathway, thereby inhibiting and/or decreasing the occurrence and/or severity of the condition/disorder.

The present disclosure is further directed to a method of inhibiting phosphorylation of low-density lipoprotein receptor-related protein 6 (LRP6). The method comprises the step of administering to at least one eye of a patient any of the ophthalmic/pharmaceutical compositions described in detail herein above.

In any of the methods described herein above, the method of administration may comprise injection of the composition into the vitreous of the eye of the patient and/or topical application of the composition to a surface of the eye of the patient.

Also, in any of the methods described herein above, if the ophthalmic/pharmaceutical composition does not include a second therapeutic agent, the method may further comprise the administration of a second therapeutic agent. In some embodiments, the second therapeutic agent has a synergistic effect with the ophthalmic/pharmaceutical composition (as described in detail herein above); the administration of the second therapeutic agent may be either simultaneously or sequentially with the administration of the ophthalmic/pharmaceutical composition. Administration of the second therapeutic agent may be by the same route (e.g., direct or local administration) or by a different route. In some embodiments, the
ophthalmic/pharmaceutical composition is administered topically and the second therapeutic agent is administered by injection (e.g., intravitreal injection).

Examples are provided herein below. However, the present disclosure is to be understood to not be limited in its application to the specific experimentation, results and laboratory procedures. Rather, the Examples are simply provided as one of various embodiments and are meant to be exemplary, not exhaustive. However, it is to be understood that the information contained therein is provided for the purpose of description, and the present disclosure is not limited to such exemplary information contained therein. The present disclosure is capable of other embodiments or of being practiced or carried out in various ways.

EXAMPLES

Example 1. Production of fenofibrate nanoemulsions

A. Materials and Methods

Table 1.

<table>
<thead>
<tr>
<th>Chemicals &amp; Reagents</th>
<th>Supplier</th>
<th>Catalogue #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerin</td>
<td>Sigma</td>
<td>G2289-500ML</td>
</tr>
<tr>
<td>Castor Oil</td>
<td>Sigma</td>
<td>C9606-500ML</td>
</tr>
<tr>
<td>Soybean lecithin</td>
<td>Alfa Aesar</td>
<td>36486</td>
</tr>
<tr>
<td>Pluronic F68</td>
<td>Sigma</td>
<td>P1300-500G</td>
</tr>
<tr>
<td>Alpha tocopherol</td>
<td>Sigma</td>
<td>T3251-25G</td>
</tr>
<tr>
<td>Carboxymethylcellulose high vis.</td>
<td>Calbiochem</td>
<td>217274</td>
</tr>
<tr>
<td>Fenofibrate</td>
<td>Charlesson</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.
Materials and Equipment

<table>
<thead>
<tr>
<th>Materials</th>
<th>Supplier</th>
<th>Catalogue #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab-Pack Eye Dropper LDPE</td>
<td>Nalgene</td>
<td>7097734010</td>
</tr>
<tr>
<td>Disposable Graduated Transfer Pipets</td>
<td>VWR</td>
<td>414004-017</td>
</tr>
<tr>
<td>500 ml Glass Bottles</td>
<td>VWR</td>
<td>89000-238</td>
</tr>
</tbody>
</table>

B. Protocol

Formulations were made up in 500 ml glass bottles. The chemicals were weighed into the appropriate bottles and the weights were recorded (Table 3).

Table 3.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>%</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerin</td>
<td>2.2</td>
<td>11</td>
</tr>
<tr>
<td>Castor Oil</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>Soybean lecithin</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>Pluronic F68</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Alpha tocopherol</td>
<td>0.002</td>
<td>0.01</td>
</tr>
<tr>
<td>1% CMC/Water</td>
<td>76.798</td>
<td>383.99</td>
</tr>
<tr>
<td>Drug (Fenofibrate)</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>500</td>
</tr>
</tbody>
</table>

After the addition of all chemicals (including the drug), bottles were mixed well by shaking. The bottle was then autoclaved at 120°C on a 20 minute liquid cycle. The autoclaved solution was cooled and then homogenized using a C3 high pressure homogenizer at 20 kPSI for 5 passes.

Example 2. Evaluation of fenofibrate in the treatment of DR
A. Materials and Methods

Animals: Brown Norway (BN) rats, male Akita mice and their age-matched wild-type (wt) littermates (C57BL/6 mice), and Pparα−/− mice were purchased from Jackson Laboratory (Bar Harbor, MA). Rodents were kept on a 12-hour light–dark cycle with an ambient light intensity of 85 ± 18 lux. Care, use and treatment of the animals were in strict agreement with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and local ethics committee approval was obtained.

Oral fenofibrate administration: Fenofibrate (Sigma-Aldrich, St. Louis, MO) was given as a 0.25% or 0.15% admixture with rodent chow (5001, LabDiet®/TestDiet®, Ft.Worth, TX). The diabetic or non-diabetic rodents were fed chow with or without fenofibrate for 7 weeks.

Eyedrop formulation. The formulation described in Example 1 was used throughout these experiments.

Intravitreal injection of fenofibrate: Briefly, animals were anesthetized with a 50:50 mix of ketamine (100 mg/mL) and xylazine (20 mg/mL), and pupils were dilated with topical phenylephrine (2.5%) and tropicamide (1%). A sclerotomy was created approximately 0.5 mm posterior to the limbus with a blade, and a glass injector (~33 gauge) connected to a syringe filled with 1 μl (for mice), 3 μl (for OIR rats) or 5 μl (for STZ-diabetic rats) of 125 μM fenofibrate in 10% rat serum, 0.1% DMSO and 0.9% NaCl, and the same volumes of the vehicle as control into the contralateral eye. The tip of the injector was introduced into the vitreous through a peripheral retinotomy, and the formulation was slowly introduced into the eye.

Western blot analysis: For in vivo studies, the eyes were enucleated, and the corneas and lenses removed. The two eyecups from each rodent were combined and homogenized, and protein concentration measured by the Bradford method (Sharma, SK and Babitch, JA, J Biochem Biophys Methods 1980; 2:247-250). The same amount (50 μg) of total protein from each rodent was resolved by SDS-PAGE and electrophoresed onto a polyvinylidene fluoride (PVDF) membrane, as described previously (Chen, YMet al., FEBS Lett 2006; 580:4200-4204). The membrane was blotted with primary antibodies followed by secondary antibodies (detailed below), and the signal was
detected using the enhanced chemiluminescence (ECL) system (GE Healthcare, Piscataway, NJ).

Primary antibodies were: rabbit anti-ICAM-1 antibody (Abcam, Cambridge, MA) and rabbit anti-VEGF antibody (Santa Cruz, CA), and rabbit anti-CTGF antibody (Abcam, Cambridge, MA).

**The OIR model and analysis of retinal NV:** The OIR model was induced in BN rats as described previously (Ricci B, *Doc Ophthalmo 1990*; 74:171-177). BN rats at postnatal day 7 (P7) were placed in a 75% oxygen chamber until day P12. Fluorescein retinal angiography and quantification of pre-retinal vascular cells were performed at day P18 as described by Smith et al. (Smith, LE et al., *Invest Ophthalmo Vis Sci* 1994; 35:101-111). The retinal angiograph was then examined under a fluorescence microscope (Axioplan2 Imaging, Carl Zeiss, Jena, Germany).

**STZ-induced diabetic rats:** Experimental diabetes was induced by an intraperitoneal (i.p.) injection of streptozocin (STZ) (50 mg/kg in 10 mmol/l of citrate buffer; pH 4.5) into anesthetized BN rats (8 weeks old) after an overnight fast. Age-matched control rats received an injection of citrate buffer alone. Blood glucose levels were measured 24 hours after the STZ injection and monitored weekly thereafter. Only animals with consistently elevated glucose levels >350 mg/dl were considered diabetic. No exogenous insulin treatment was given.

**Retinal angiography with high molecular-weight fluorescein:** Rats at postnatal day 18 were anesthetized and perfused with fluorescein via cardiac intraventricular injection of 50 mg/ml $2\times10^6$-molecular-weight fluorescein isothiocyanate-dextran (Sigma, St. Louis, MO) as described by Smith et al. (Smith, LE et al., *Invest Ophthalmo Vis Sci* 1994; 35:101-111). The animals were immediately euthanized. The eyes were enucleated and fixed with 4% paraformaldehyde in PBS for 10 min. The retina was then separated from the eyecup and fixed with 4% paraformaldehyde for three hours. Several incisions were made to the retina, which was flat-mounted on a gelatin-coated slide. The vasculature was then examined under a fluorescence microscope (Axioplan2 Imaging, Carl Zeiss, Jena, Germany) by an operator masked to treatment allocation.
For quantification of pre-retinal vascular cells, eyes were fixed, sectioned and stained as described previously (Chen, Y et al., *Am J Pathol* 2009; 175:2676-2685). The pre-retinal nuclei were counted by an operator masked to therapy, averaged, and compared.

**Retinal vascular permeability assay:** Retinal vascular permeability was quantified by measuring Evans blue dye leakage from blood vessels into the retina following a documented method (Zhang, SX et al., *Diabetologia* 2004; 47:124-131) with modifications. Animals were anesthetized, and Evans blue (Sigma, St. Louis, MO) injected through the femoral vein (10 mg/kg body weight) under microscopic inspection. After injection, the animals were kept on a warm pad for two hours to ensure complete dye circulation. The chest cavity was then opened, and blood collected through the right atrium. The mice were perfused via the left ventricle with PBS (pH 7.4), which was pre-warmed to 37°C to prevent vasoconstriction. Immediately after perfusion, the eyes were enucleated, and the retinae carefully dissected under an operating microscope. Evans blue dye was extracted by formamide. The Evans blue dye intensity from the supernatant and from serum was measured. Retinal protein levels were measured by A$_{280}$. Evans blue dye levels in the retina were normalized by serum Evans blue dye concentration and total retinal protein concentrations.

**Retinal vascular leukostasis assay:** The assay followed a documented protocol (Ishida, S. et al., *Invest Ophthalmol Vis Sci* 2003; 44:2155-2162). Briefly, anesthetized rats were perfused with PBS to remove non-adherent leukocytes in vessels. The adherent leukocytes in the vasculature and vascular endothelial cells were stained with FITC-conjugated concanavalin-A (Con-A, 40 μg/ml). The retinae were then flat-mounted and adherent leukocytes in the vasculature were counted under a fluorescence microscope by an operator masked to treatment allocation.

**ELISA for retinal soluble ICAM-1:** The retinae were homogenized and centrifuged. The total protein concentration in the supernatant was measured using the bicinchoninic acid protein assay reagent kit (Pierce, Rockford, IL). Soluble ICAM-1 levels (R&D Systems Inc., Minneapolis, MN) were measured using ELISA according to
the manufacturers’ instructions and normalized by total protein concentration in the retina.

**Bioanalysis of fenofibrate and fenofibric acid tissue distribution by Liquid chromatography-mass spectrometry**

*Tissue Extraction preparation*  Freshly prepared sample, blank, or internal standard (IS) was mixed and incubated with cold acetonitrile on ice for 30 min and centrifuged at 5,000 rpm for 5 min at 4 °C. 200 μL of supernatant was dried and reconstituted in a buffer with formic acid, trifluoroacetic acid (TFA), and acetonitrile and loaded on an HPLC-MS system.

*HPLC-MS system setting for fenofibrate and fenofibric acid:* Two solvent-delivery systems included four LC-10AD VP pumps (Shimadzu, Columbia, MD) and an autoinjector (CTC Analytics, Zwingen, Switzerland). The loading volume of the injector loop was 40μL and the injection volume was set at 10 μL. Chromatographic separations were performed with a Magic MS column (C18, 5 μm, 100A, 0.5x150mm) and connected to a guard column (BDS Hypersyl C8, 2.1x20 mm, 5 μm). Two mobile phases were used in the chromatography. Mobile phase A was 0.09% formic acid, 0.01% TFA, 2% CH₃CN, 97.9% water, and mobile phase B was 0.09% formic acid, 0.0085% TFA, 95% CH₃CN, and 4.9% water. The mobile phase gradients for the analytical column and the guard column was 30% B to 60% in 1 min, 60% to 90% in 10 min, hold 1 min. The Valco valve was set up with a target mass of 300 m/z. Analyses were performed on a Bruker Daltonics HCT Ultra Ion trap. MRM transitions monitored were: BZFB used as IS, m/z 362.0→276, fenofibric acid m/z 319.1→233.1, and fenofibrate m/z 361.1→233.1.

**Retinal endothelial cell (REC) tube formation:** Primary cultures of bovine RECs were grown in Medium 200 supplemented with low serum growth supplement (Invitrogen, Carlsbad, CA). Cells at passage five were used throughout the study. After pooling of 50 μl ice cold Matrigel into 12-well plates at 37°C for 30 min to solidify, RECs were overlaid onto the Matrigel with or without 50 μm fenofibrate (Sigma, St. Louis, MO) in the medium, and tube formation was examined at six hours.

**Endothelial cell scratch wound assay:** Eighty percent confluent RECs were wounded by drawing a line with a 200 μl pipette tip (VWR international, West Chester,
PA) across the monolayer surface. After washing with PBS, the cells were cultured in medium 200 (Invitrogen, Camarillo, CA) with or without 50 μM fenofibrate for 24 hours. The average linear migration rate was calculated by tracing the border of the cell monolayer on both sides of the wound at 0 and 24 hours, measuring the cell-free area over a fixed length along the wound, subtracting the areas observed at 0 and 24 hours, and dividing the resulting difference in areas by the length of the region measured. All data were averaged over at least four individual wound zones.

**Transwell inserts cell migration assay:** The undersurfaces of Transwell motility chamber inserts of a 96-well Transwell (Neuro Probe, Inc., Gaithersburg, MD) were coated with or without 10 mg/ml mouse cellular fibronectin and Dil (1,1'-dioctadecyl-3,3,3',tetramethylindocarbocyanine perchlorate) labeled REC were cultured in the upper chamber inserts in the presence of fenofibrate at various concentrations. After six hours incubation, the cells on the upper surface of the membrane were removed, the fluorescence in the cell monolayer was determined and the inhibition of the maximal signal was calculated.

**Statistical analysis:** Quantitative data were analyzed and compared between fenofibrate treatment groups and control groups, or between the cultured cells treated with fenofibrate or the control. Statistical analysis was performed by Student’s t test for studies of two groups and by one-way ANOVA for studies of more than two groups. Statistical significance in multiple groups was determined by Tukey’s Post-hoc analysis and statistical significance was set at $p<0.05$.

**B. Results**

**Characterization of Fenofibrate (FBN) and Fenofibric Acid (FBA) in MS/MS Spectrum**

The bioavailability of topical fenofibrate in the retina was tested. Analysis of fenofibrate by HPLC/MS showed an abundant ion peak around $m/z$ 361.1±0.3, representing that the $m/z$ 361.1 ion corresponding of fenofibrate. In the MS spectrum,
internal standard (bezafibrate) and fenofibric acid obtained on an ion trap mass spectrometer was observed in an abundant peak at m/z 362.0 and m/z 319.1, respectively.

**Tissue distribution of fenofibrate in animals following topical application of eye drop.** The concentrations of fenofibrate and its activated metabolite, fibric acid, were measured using standard HPLC-MS analysis in tissue homogenates from mice, rats, and rabbits administrated oral fenofibrate or fenofibrate eye drops. As shown in Figure 1, fenofibric acid and fenofibrate administered in the eye drop formulation were only detected in the retina, and were undetectable in the liver and serum. In animals fed with oral fenofibrate, only fenofibric acid was detected, fenofibrate itself was un-detectable in all tissue extractions. In oral fenofibrate-treated mice and rats, the concentration of fenofibric acid in the serum and liver was over 100 times higher than that detected in the retina. These results demonstrate that topical application of fenofibrate eye drops allows for significant penetration of the drug into the retina, but would not likely produce the systemic adverse effects associated with the drug in the serum and liver.

**Topical application of fenofibrate reduces retinal vascular leakage in rats having oxygen induced retinopathy (OIR) rats.** Increases of retinal vascular leakage is responsible for diabetic edema in DR, and the ischemia induced retinopathy rat model is a widely accepted experimental model to evaluate treatment of proliferative diabetic retinopathy (PDR). As fenofibrate eye drops penetrate well into the retina and local intraocular injection of fenofibrate significantly reduced retinal vascular leakage in oxygen induced retinopathy (OIR) (Hahn. SE and Goldberg, DM, *Biochem Pharmacol 1992; 43:625-633*), topical application of fenofibrate was tested to determine whether it also alleviated vascular leakage in OIR rats. Consistent with previous studies, five days post-topical application of fenofibrate eye drops significantly reduced retinal vascular leakage, compared to subjects treated with vehicle only (Fig. 2).

**Topical ocular fenofibrate attenuates retinal neovascularization (NV) in OIR rats.** It has been previously shown that both oral and local fenofibrate prevented retinal neovascularization (NV) in OIR rats. In the present study, the effect of topical fenofibrate eye drops on ischemia-induced retinal NV in ischemia induced retinopathy rats was evaluated. As shown by fluorescein angiography in figure 3, the fenofibrate-treated eyes
developed less severe retinal NV compared to the control eyes treated with the vehicle only. Quantification of non-perfusion retinal area showed significantly fewer non-perfusion areas in the retinas treated with fenofibrate eye drops, relative to the control eyes treated with vehicle, supporting the conclusion that an inhibitory effect of fenofibrate on ischemia-induced NV can be achieved by topical administration.

As VEGF plays a critical role in the pathogenesis of breaking the blood-retinal barrier, vascular leakage, and retinal neovascularization (Witmer, A.N. et al., *Prog Retin Eye Res* 2003; 22:1-29), the direct effects of fenofibrate eye drops on retinal VEGF over-expression induced by ischemia was evaluated. Fenofibrate was topically applied on the cornea of OIR rats at P12, and vehicle served as a control. As shown in figure 4, fenofibrate eye drops greatly reduced retinal VEGF levels in the OIR rats, compared to vehicle control, indicating that topical administration of fenofibrate prevents ischemia induced retinal angiogenesis.

**Fenofibrate attenuates retinal vascular permeability in type 1 diabetic models.** Clinical trials and previous studies have shown that fenofibrate significantly reduced retinal edema in diabetic patients and diabetic animals. To determine if topical ocular fenofibrate would have the same effects on retinal vascular leakage in diabetic models, STZ-induced diabetic rats at 6 weeks after diabetes onset were topically administrated fenofibrate eye drops for four weeks. Controls were age-matched non-diabetic rats and diabetic rats treated with vehicle only. Retinal vascular leakage was evaluated using Evans blue dye as a tracer with normalization to total retinal protein concentration. Treatment with fenofibrate eye drops significantly reduced retinal vascular leakage in STZ-diabetic rats, compared to the vehicle-treated diabetic rats, to a level similar to that in non-diabetic rats (Fig. 5).

**Fenofibrate eye drops reduces retinal vascular leukostasis and attenuates overexpression of inflammatory factors in type 1 diabetic rats.** As retinal inflammation is an early pathological change in DR, fenofibrate effects on leukocyte adherence (leukostasis) in the retinal microvasculature in STZ-induced diabetic rats was examined. Diabetic and non-diabetic rats were topically administrated fenofibrate eye drops for four weeks, and retinal leukostasis was examined. Unlike the retinal vasculature in non-
diabetic rats (Fig. 6A), multiple adherent leukocytes were observed in the retinal vasculature of diabetic rats treated with vehicle, (Fig. 6B), but there were fewer leukocytes in the topical fenofibrate-treated diabetic rats (Fig. 6C). There were significantly fewer adherent leukocytes in the fenofibrate-fed diabetic rats compared to that in untreated diabetic rats (P<0.001), to a level comparable to that in the retinae of non-diabetic rats (Fig. 6D).

Intercellular adhesion molecule 1 (ICAM-1), induced by interleukin-1 and tumor necrosis factor alpha (TNFα), mediates cell-cell adhesions and recruits inflammatory cytokines and thus plays an important role in early stage of inflammation in DR (Kowalski, J. et al., Int J Clin Pharmacol Ther 2003; 41:241-247). The effects of topical ocular fenofibrate on retinal inflammatory markers in STZ diabetic rats was evaluated by measurement of the levels of ICAM-1. As shown in Figure 7, topical ocular fenofibrate significantly reduced retinal ICAM-1 levels in STZ diabetic rats. These data suggest that topical fenofibrate eye drops have a therapeutic effect on the retinal inflammation in diabetes.

**Topical ocular fenofibrate does not causes abnormalities to the cornea and retinal function.**

The effect of topical application fenofibrate eye drops was evaluated to determine whether such administration causes any retinal function abnormalities or cornea stimulation by using ERG, histological section and slit lamp examination. Hematoxylin and eosin stain (H&E) staining the histological structure of the retina and cornea in fenofibrate–treated rats appeared indistinguishable from those of vehicle-treated rats. In addition, rats treated with fenofibrate demonstrated that fenofibrate did not affect cornea transparent or abnormalities of iridocorneal angle tissues compared with vehicle-treated rats. Electroretinography (ERG) examination of fenofibrate eye drop-treated rats and vehicle treated littermates demonstrated that one month of fenofibrate eye drops treatments did not cause any significant retinal abnormalities (FIG. 8). These data suggest that topical application of fenofibrate eye drops could be a safe and efficient route for treatment of DR.
Fenofibrate inhibits REC tube formation and migration. Effects of fenofibrate on endothelial cell migration were evaluated using primary REC, as endothelial cell migration is an important step in retinal NV. In the tube formation assay, REC cultured on Matrigel in the absence of fenofibrate aggregated to form a tube-like pattern, while REC exposed to 50 μM fenofibrate did not form the tube-like structure (FIG. 9A-B).

REC migration was also evaluated using the scratch wound healing assay in primary REC monolayer, which showed that REC treated with fenofibrate had substantially decreased motility as measured at 24 hours after scratch wounding (FIG. 9C-D). Additionally, the Transwell assay of cell migration demonstrated that the number of REC that migrated through the filter to the other side of the transwell, induced by fibronectin, was significantly decreased by fenofibrate, compared to control treated with the vehicle only (FIG. 9E-G). Taken together, these results suggest that fenofibrate inhibits retinal endothelial cell migration.

Example 3. Therapeutic effect of fenofibrate drops in oxygen-induced retinopathy (OIR) and type-1 diabetes mellitus (TIDM) retinopathy models

A. Materials and Methods

Cell lines and cell culture. The human umbilical vein vascular endothelial cell line (HUVEC) was purchased from American Type Culture Collection (Manassas, Virginia, USA). HUVEC cells were maintained in Medium 200 (GIBCO) supplemented with low serum growth supplement (LSGS, GIBCO, USA) at 37°C in a humid atmosphere (5% CO₂-95% air). Medium was changed every other day during culturing. Passage 3-6 of HUVEC cells were used for experiment.

Hypoxia and high glucose mimicking. For chemically mimicked hypoxia, cobalt chloride (CoCl₂, Sigma, MO, US) was used at a final concentration of 400 μM. HUVEC cells were treated with CoCl₂ and 20, 40, 80 and 160 μM of fenofibrate (Sigma, MO, US) for 8 hours at the same time. Then the cells were harvested with cell lysis buffer (Cell Signal, MA, US), homogenized by a sonicator and denatured in 95°C. The treated samples were used for Western blot assay. For high glucose induction, HUVEC cells were treated with 30 mM D-glucose (Sigma, MO, US) and 30 mM D-Mannitol
as control, also different doses of fenofibrate as mentioned above for 48 hours. Cell harvest and sample treatment for Western Blot were the same with hypoxia mimicking above.

**Scratch Assay**

**Western blot analysis.** After the eyeballs were enucleated, the retinas were dissected and homogenized. The protein concentrations were measured by the Bradford method (Sharma, SK and Babitch, JA, *J Biochem Biophys Methods* 1980 2:247-250) and equalized. An equal amount (60 µg) of total retina protein from each individual was electrophoresed by SDS-PAGE gels and transferred onto a polyvinylidene fluoride (PVDF) membrane via mini transfer tank (Invitrogen), as described previously (Chen, Y et al., *FEBS Lett* 2006 580:4200-4204). The membrane was incubated with primary antibodies at 4°C overnight followed by a 1-hour secondary antibodies incubation at room temperature. Signals were detected using the Enhanced Chemiluminescence (ECL) system (GE Healthcare, Piscataway, NJ).

**Antibodies.** Antibodies against Vascular Endothelial Growth Factor (VEGF), Connective Tissue Growth Factor (CTGF) and Intercellular Adhesion Molecule (ICAM-1) were both purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and used as 1:250 dilution. TNF-α was from Abcam (Cambridge, MA, USA) and used as 1:500 dilution.

**Eyedrop formula.** The formula described in Example 1 was used throughout these experiments.

**HPLC/ MS.** Two solvent-delivery systems included four LC-10AD VP pumps (Shimadzu, Columbia, MD) and an autoinjector (CTC Analytics, Zwingen, Switzerland). The loading volume of the injector loop was 40 µL and the injection volume was set at 10 µL. Chromatographic separations were performed with a Magic MS column (C18, 5 µm, 100A, 0.5x150 mm) and connected to a guard column (BDS Hypersyl C8, 2.1x20 mm, 5 µm). Two mobile phases were used in the chromatography. Mobile phase A was 0.09% formic acid, 0.01% TFA, 2% CH₃CN, 97.9% water; and mobile phase B was 0.09% formic acid, 0.0085% TFA, 95% CH₃CN, and 4.9% water. The mobile phase
gradients for the analytical column and the guard column was 30% B to 60% in 1 min, 60% to 90% in 10 min, hold 1 min. The Valco valve was set up with a target mass of 300 m/z. Analyses were performed on a Bruker Daltonics HCT Ultra Ion trap. MRM transitions monitored were: BZFB used as IS, m/z 362.0→276, fenofibric acid m/z 319.1→233.1, and fenofibrate m/z 361.1→233.1.

**Animals.** Brown Norway rats and their age-matched wild-type (wt) were purchased from Jackson Laboratories (Bar Harbor, MA). Rodents were kept on a 12-hour light–dark cycle with an ambient light intensity of 85 ± 18 lux. Care, use and treatment of the animals were in strict agreement with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**STZ-induced diabetic models.** Experimental type 1 diabetes rats were induced by intraperitoneal (i.p.) injection of streptozocin (58 mg/kg in 10 mmol/l of citrate buffer; pH 4.5) into anesthetized BN rats (7-week old) after an overnight fast. Age-matched control rats received an injection of citrate buffer alone. Blood glucose levels were measured 48 hours after the STZ injection and monitored every other week thereafter. Only the rats with blood glucose levels consistently > 350 mg/dl were considered T1DM models. No insulin treatment was given.

**Oxygen-induced retinopathy models and analysis of retinal NV.** The OIR model was induced in BN rats as described previously (Smith, LE et al., *Invest Ophthal Mol Vis Sci* 1994 35:101-111). Rats at postnatal day 18 were anesthetized with 10 mg/kg xylazine plus 75 mg/kg ketamine i.p. and perfused with fluorescein via left ventricle injection of 50 mg/ml 2×10^6-molecular-weight fluorescein isothiocyanate-dextran (Sigma, St. Louis, MO) as described by Smith et al. The animals were immediately killed. The eyes were enucleated and fixed with 4% paraformaldehyde in PBS for two hours. The retina was then isolated from the eyecup and cut with 4-6 peripheral incisions, then flat-mounted on a gelatin-coated slide. The vasculature was then examined under a fluorescence microscope (Axioplan2 Imaging, Carl Zeiss, Jena, Germany). Quantification of neovascularization was performed according to the method described by Smith et al. Briefly, eyes were fixed, sectioned and stained in hematoxylin
and cosin (HE) stain and the preretinal nuclei were counted, average and compared using the Student’s t test.

**Retinal vascular permeability assay.** Retinal vascular permeability was quantified by measuring Evans blue dye leakage from vessels into retinas following a method previously described (Lip, PL et al., *Ophthalmology* **2001** 108:705-710) with modification (26). After anesthetization, the animals were injected with Evans blue (Sigma, St. Louis, MO) through the femoral vein (30 mg/kg) and kept on warm pads for 2 hours to ensure the complete in vivo circulation of Evans blue. Then the chest cavity was opened, the rats were perfused via the left ventricle with 37 °C pre-warmed PBS (pH 7.4), eyeballs were enucleated and the retinas were carefully dissected under microscope immediately. Evans blue was extracted from retina by formamide and the solution was centrifuged with 70,000 rpm for 30 min. The concentrations in both the supernatant of retina-formamide solution and the serum of blood collected from the right atrium were measured simultaneously. Evans blue levels in the retina was normalized by the total retinal protein concentration.

**Retinal vascular leukostasis assay.** The procedure was carried on according to the previous description with minor modification (Kinoshita, N et al. *Diabetologia* **2002** 45:735-739). Anesthetized rats were perfused with 100 ml/kg body weight of phosphate buffered saline (PBS) over two minutes to remove non-adherent leukocytes in vessels. The adherent leukocytes in the vasculature and vascular endothelial cells were stained with 278 μg/ml FITC-conjugated concanavalin A lectin (Con-A: Vector Labs, Burlingame, CA) immediately. The retinas were flat-mounted and the total adherent leukocytes in retina vasculature were counted under the fluorescence microscope (Olympus, Tokyo, Japan).

**ELISA for retina soluble ICAM-1.** Retinas were homogenized by sonication and centrifuged at 14 rpm for 10 min at 4 °C. The total protein concentration in the supernatant was measured by Bradford assay. Soluble ICAM-1 expression levels were measured using ELISA kits (R&D Systems Inc., Minneapolis, MN) according to the manufacturer’s instructions and normalized by the total protein concentration in the retina.
**Statistical analysis.** Quantitative data were described as the mean ± SE (standard error) and compared between the normal diet group and the group with oral administration of fenofibrate, or between the cells treated with and without fenofibrate using Student’s *t* tests. The FA grading results were analyzed by $\chi^2$ test. Statistical significance criterion was set at $p<0.05$.

**B. Results**

**Fenofibrate blocked expression of ICAM-1, VEGF and CTGF in HUVEC cells under both hypoxic and high glucose conditions.** As shown in Fig 10A, to find out whether fenofibrate has direct effect on endothelial cells, HUVEC cells were exposed in conditions of hypoxia or high glucose (induced as described above), ICAM-1, VEGF and CTGF were all overexpressed but dose-dependently inhibited by fenofibrate. This result demonstrates that fenofibrate directly suppresses the expression of inflammatory and angiogenesis markers in endothelial cells.

**3% fenofibrate formula efficiently delivered to rat and rabbit retina.** To evaluate the efficiency of a fenofibrate eye drop formulation to deliver the drug to the retina, P12-16 OIR rats (n=4) were administered drops five times/day. Eyeballs were enucleated and retinas were dissected and stored in -80 °C. Wild-type rabbits were given the drops four times/day for seven days. Retinas were collected as described above. (n=3×2). Fenofibrate and fenofibric acid were detected by HPLC/MS/MS methods. In OIR rats, the average amount detected by HPLC/MS/MS was 1.6±0.3 ng fenofibrate and 10.2±7.5 ng fenofibric acid per retina (60-80 μg total protein). In rabbits, the average amount detected was 16±18.5 ng of fenofibrate and 70.3±58.2 ng of fenofibric acid per retina (450-600 μg total protein). These results suggest that the eye drop formulations provides for efficient delivery of fenofibrate.

**Topical administration of 3% fenofibrate reduced retinal leakage in STZ-induced diabetic rats and OIR rats.** To determine the effect of a fenofibrate eye drop formulation on retinal leakage, 3% fenofibrate topical treatment was administered 5 times/day in P12-16 in OIR models and 4 times/day for 8 weeks in STZ-induced diabetic rats. As is known, retinal leakage and blood-retinal barrier break-down are pathological
features of DR and also OIR. Retinal vascular leakage was measured using the Evans Blue-albumin leakage method and compared with the rats treated with a control formula (vehicle alone). The results showed that the rats topically treated with fenofibrate had significantly lower vascular permeability than those treated with the control, which suggests that the topical administration of fenofibrate ameliorates retinal vascular leakage in both OIR and STZ-induced diabetic retinopathy models. \( P<0.01 \), OIR \( N(\text{drug})=4, N(\text{vehicle})=4 \); T1DM \( N(\text{drug})=4, N(\text{vehicle})=4 \) Fig 11A and B.

**Fenofibrate ameliorated leukocyte adhesion in retinas in a T1DM models and blocked expression of ICAM-1 in OIR and STZ-induced diabetic rats.** To observe the change in inflammation in fenofibrate topical treated animal models, STZ-induced diabetic rats and ischemia-induced retinopathy rats were treated with topical administration of fenofibrate as described above. As shown in Figure 12, the total number of leukocytes that adhered to the retinal endothelial cells were increased in diabetic rats treated with control drops (vehicle only) \( 98.7\pm9.8 \) compared with those in non-diabetic control rats \( 15.1\pm2.8, P<0.01 \). The total number of leukocytes that adhered to the retinal endothelial cells were, however, reduced in the diabetic rats treated with fenofibrate drops. \( 41\pm8.8 \) cells/retina, \( P<0.01 \) Furthermore, as shown in figure 13, consistent with the leukostasis results, a Western blot indicated that retina ICAM-1 expressions were dramatically induced by oxygen in control P16 OIR rats, but expression was significantly decreased following 5-day topical administration of 3% fenofibrate eye drop formulation. SICAM-1 expression in retinas of control T1DM rat models were significantly increased, but this expression was also inhibited by 8-week topical administration of a 3% fenofibrate eye drop formulation as measured by ELISA methods. \( P<0.01, n=3 \) These data demonstrate that retinal inflammation in diabetic rats and OIR rats was significantly inhibited by topical administration of fenofibrate.

**Fenofibrate drops inhibited ischemia-induced retinal neovascularization and VEGF expression.** To further investigate the anti-angiogenesis effect of the local fenofibrate treatment, OIR rats were treated as described above. Fluorescent angiography pictures of the retinas were taken following flat-mounting at D18. Central 1/3 non-vessel area semi-quantification was significantly decreased in P18 OIR rats following topical
administration of fenofibrate for 7 days. (FIGs. 14A, B and C) Preretinal neovascularization was also quantified by counting nuclei numbers growing into the vitreous space (preretinal vascular cells) on sixteen noncontinuous cross-sections from each eye following an established method (Smith, LE et al., *Invest Ophthalmol Vis Sci* 1994 35:101-111). The results showed a significant decrease in OIR rats treated with fenofibrate compared with those treated only with vehicle. Western blot results indicated that vascular endothelial growth factor (VEGF) expression was dramatically induced by relative hypoxia after 5 days in a 70% oxygen chamber but decreased by treatment for five days with the fenofibrate eye drop formulation. (FIG. 14D, each lane represents one rat). These results suggest that topical administration of fenofibrate ameliorates angiogenesis in OIR models.

**Toxicity of fenofibrate eye drop formula was not detected on cornea or photoreceptors in retina.** Histology and functional examination of corneas and retinas were used to detect the potential toxicity of the fenofibrate eye drop formulation following topical administration. HE staining of the cornea showed no histological change in normal rats treated with the formulation 4 times/day for 5 days. Surface pictures of the STZ rats treated with the eye drop formulation 4 times/day for 8 weeks did not show macroscopic change. ERG examination also did not show pathological abnormalities in the STZ rats (FIG. 15A and B). These results indicate that the 3% fenofibrate eye drop formulation did not lead to detectable abnormalities in either histology or function of the treated corneas or retinas.

Thus, in accordance with the present disclosure, there has been provided ophthalmic compositions comprising PPARα agonists, as well as methods for producing and using same. Although the present disclosure has been described in conjunction with the specific drawings and language set forth above, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the present disclosure.
WHAT IS CLAIMED IS:

1. An ophthalmic composition comprising at least one PPAR-alpha agonist.

2. The ophthalmic composition of claim 1, wherein at least one PPAR-alpha agonist is fenofibrate.

3. The ophthalmic composition of claim 1 or 2, wherein the ophthalmic composition is formulated as an eye drop.

4. The ophthalmic composition of any of claims 1-3, wherein the concentration of PPAR-alpha agonist in the ophthalmic composition is in a range selected from the group consisting of a range of from about 0.0001% to about 95%, a range of from about 0.001% to about 50%; a range of from about 0.005% to about 40%; a range of from about 0.01% to about 35%; a range of from about 0.05% to about 30%; a range of from about 0.1% to about 25%; a range of from about 0.1% to about 20%; a range of from about 0.1% to about 15%; and a range of from about 1% to about 10%.

5. The ophthalmic composition of any of claims 1-4, further comprising at least one delivery agent that assists in the penetration of the surface of the eye.

6. The ophthalmic composition of claim 5, wherein the delivery agent assists in delivery to the cornea of the eye.

7. The ophthalmic composition of claim 5, wherein the delivery agent assists in delivery of the composition to the retina of the eye.

8. The ophthalmic composition of any of claims 1-7, wherein the ophthalmic composition comprises an emulsion.
9. The ophthalmic composition of any of claims 1-8, further comprising at least one lipid.

10. The ophthalmic composition of any of claims 1-9, further comprising at least one of glycerin, castor oil, soybean lecithin, polyoxyethylene-polyoxypropylene block copolymer, alpha tocopherol, and carboxymethyl cellulose.

11. The ophthalmic composition of claim 10, further comprising at least two of glycerin, castor oil, soybean lecithin, polyoxyethylene-polyoxypropylene block copolymer, alpha tocopherol, and carboxymethyl cellulose.

12. The ophthalmic composition of claim 11, further comprising at least three of glycerin, castor oil, soybean lecithin, polyoxyethylene-polyoxypropylene block copolymer, alpha tocopherol, and carboxymethyl cellulose.

13. The ophthalmic composition of claim 12, further comprising at least four of glycerin, castor oil, soybean lecithin, polyoxyethylene-polyoxypropylene block copolymer, alpha tocopherol, and carboxymethyl cellulose.

14. The ophthalmic composition of claim 13, further comprising at least five of glycerin, castor oil, soybean lecithin, polyoxyethylene-polyoxypropylene block copolymer, alpha tocopherol, and carboxymethyl cellulose.

15. The ophthalmic composition of claim 10, further comprising glycerin, castor oil, soybean lecithin, polyoxyethylene-polyoxypropylene block copolymer, alpha tocopherol, and carboxymethyl cellulose.

17. A pharmaceutical composition comprising the ophthalmic composition of any of claims 1-15 and a pharmaceutically acceptable carrier.

18. The pharmaceutical composition of claim 17, further comprising a second therapeutic agent.

19. The pharmaceutical composition of claim 18, wherein the second therapeutic agent is at least one of an anti-angiogenic agent or an anti-VEGF reagent.

20. The pharmaceutical composition of claim 18, wherein the second therapeutic agent is selected from the group consisting of: AVASTIN® (bevacizumab), LUCENTIS® (ranibizumab), KENALOG® (triamcinolone acetonide), ganciclovir, foscarnet, vancomycin, ceftazidime, amikacin, amphotericin B, dexamethasone, ACULAR® (ketorolac tromethamine), ACUVAIL® (ketorolac tromethamine), AK-CON-A® (naphazoline), AKTEN® (lidocaine hydrochloride), ALAMAST® (pemirolast potassium), ALPHAGEN® (brimonidine), ALREX® (loteprednol etabonate), ASTEPRO® (azelastine hydrochloride), AZASITE® (azithromycin), BEPREVE® (beopotastine besilate), BESIVANCE® (besifloxacin), BETAXON® (levobetaxolol hydrochloride), COSOPT® (dorzolamide hydrochloride – timolol maleate), DUREZOL® (dipropionate), EYLEA® (afibercept), LOTEMAX® (loteprednol etabonate), LUMIGAN® (bimatoprost), MACUGEN® (pegaptanib), OCUFLOX® (ofloxacin), OCUHIST® (naphazoline with pheniramune), OZURDEX® (dexamethazone), QUIXIN® (levofloxacin), RESCULA® (unoprostone isopropyl), RESTASIS® (cyclosporine), SALAGEN® (pilocarpine hydrochloride), TRAVATAN® (travoprost), VALCYTE® (valganciclovir HCl), VIROPTIC® (trifluridine), VISTIDE® (cidofovir), VISUDYNE® (verteporfin), VITRAVENE® (fomiviren), ZADITOR® (ketotifen), ZIOPTAN® (tafluprost), ZIRGAN® (ganciclovir), ZYMAXID® (gatifloxacin), and combinations thereof.
21. A method of inhibiting enzyme activity and/or enzyme production of at least one angiogenic, inflammatory, or fibrogenic factor of diabetic retinopathy (DR), said method comprising administering to at least one eye of a patient suffering from or predisposed to DR at least one of the ophthalmic composition of any of claims 1-15 or the pharmaceutical composition of any of claims 17-20.

22. The method of claim 21, wherein the factor of DR is selected from the group consisting of VEGF, ICAM-1, TNF-α, and CTGF.

23. A method of treating at least one retinal condition selected from the group consisting of retinal leukostasis, inflammation, vascular leakage, fibrosis, abnormal neovascularization and carcinogenesis in the retina, said method comprising the step of administering to at least one eye of a patient suffering from or predisposed to the at least one retinal condition at least one of the ophthalmic composition of any of claims 1-15 or the pharmaceutical composition of any of claims 17-20.

24. A method of inhibiting and/or decreasing the occurrence and/or severity of at least one condition/disorder selected from the group consisting of ocular inflammation, corneal inflammation, diabetic retinopathy, diabetic macular edema, macular degeneration, uveitis, retinal inflammation, retinal vascular leakage, and retinal neovascularization, said method comprising administering to at least one eye of a patient suffering from or predisposed to the at least one condition at least one of the ophthalmic composition of any of claims 1-15 or the pharmaceutical composition of any of claims 17-20.

25. A method of inhibiting activation of Wnt signaling pathway in an eye of a patient, comprising administering to at least one eye of a patient at least one of the ophthalmic composition of any of claims 1-15 or the pharmaceutical composition of any of claims 17-20.
26. A method of inhibiting phosphorylation of low-density lipoprotein receptor-related protein 6 (LRP6), comprising administering to at least one eye of a patient at least one of the ophthalmic composition of any of claims 1-15 or the pharmaceutical composition of any of claims 17-20.

27. The method of any of claims 21-26, further comprising the step of administering a second therapeutic agent to the patient.

28. The method of claim 27, wherein the second therapeutic agent is at least one of an anti-angiogenic agent or an anti-VEGF reagent.

29. The method of claim 27, wherein the second therapeutic agent is selected from the group consisting of: AVASTIN® (bevacizumab), LUCENTIS® (ranibizumab), KENALOG® (triamcinolone acetonide), ganciclovir, foscarnet, vancomycin, ceftazidime, amikacin, amphotericin B, dexamethasone, ACULAR® (ketorolac tromethamine), ACUVAIL® (ketorolac tromethamine), AK-CON-A® (naphazoline), AKTEN® (lidocaine hydrochloride), ALAMAST® (pemrolast potassium), ALPHAGEN® (brimonidine), ALREX® (loteprednol etabonate), ASTEPRO® (azelastine hydrochloride), AZASITE® (azithromycin), BEPREVE® (bepotastine besilate), BESIVANCE® (besifloxacin), BETAXON® (levobetaxolol hydrochloride), COSOPT® (dorzolamide hydrochloride – timolol maleate), DUREZOL® (difluprednate), EYLEA® (afibercept), LOTEMAX® (loteprednol etabonate), LUMIGAN® (bimatoprost), MACUGEN® (pegaptanib), OCUFLOX® (ofloxacin), OCUHIST® (naphazoline with pheniramine), OZURDEX® (dexamethasone), QUIXIN® (levofloxacin), RESCULA® (unoprostone isopropyl), RESTASIS® (cyclosporine), SALAGEN® (pilocarpine hydrochloride), TRAVATAN® (travoprost), VALCYTE® (valganciclovir HCl), VIROPTIC® (trifluridine), VISTIDE® (cidofovir), VISUDYNE® (verteporfin), VITRAVENE® (fomiviren), ZADITOR® (ketotifen), ZIOPTAN® (tafluprost), ZIRGAN® (ganciclovir), ZYMAXID® (gatifloxacin), and combinations thereof.
30. The method of any of claims 21-26, wherein the step of administering is further defined as injecting the composition into the vitreous of the eye of the patient.

31. The method of any of claims 21-26, wherein the step of administering is further defined as topically applying the composition to a surface of the eye of the patient.
FIG. 2

Retinal vascular permeability

vehicle

Fenofibrate

Diabetes

**
FIG. 3A  OIR + Vehicle

FIG. 3B  OIR + Feno

FIG. 3C

non-vessel area (pixels)

vehicle  Fenofibrate

**
FIG. 4A

FIG. 4B
FIG. 5
FIG. 6A
Normal

6/16

DM+Vehicle

FIG. 6B

DM+Fenofibrate

FIG. 6C

FIG. 6D

Retinal leukocytes

0 20 40 60 80 100 120

NDM Vehicle Feno

**

DM
FIG. 7
**FIG. 14D**

- **Normoxia**
- **OIR vehicle**
- **OIR fenofibrate**

**VEGF**

**β-actin**

**Relative VEGF expression**

- **normoxia**
- **vehicle**
- **fenofibrate**

* * *

*
FIG. 15A

ERG comparison of STZ rats eydoprop vehicle with 3% fenofibrate

<table>
<thead>
<tr>
<th>Group(n)</th>
<th>A wave</th>
<th></th>
<th>B wave</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rod</td>
<td>Cone</td>
<td>Rod</td>
<td>Cone</td>
</tr>
<tr>
<td>Vehicle (5)</td>
<td>480.5±81.6</td>
<td>27.6±24.6</td>
<td>1032.5±189.6</td>
<td>173.9±21.2</td>
</tr>
<tr>
<td>Fenofibrate (4)</td>
<td>524.1±75.4</td>
<td>20.9±18.5</td>
<td>1151.6±234.6</td>
<td>200.6±41.3</td>
</tr>
<tr>
<td>P-value</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

FIG. 15B
INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/57995

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61F 9/00 (2012.01)
USPC - 424/78.02, 424/78.04, 514/20.8, 514/954

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) - A61F 9/00 (2012.01)
USPC - 424/78.02, 424/78.04, 514/20.8, 514/954

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest, PatBase (USPTO, EPO, JPO, WIPO), GoogleScholar (PL, NPL), FreePatentsOnline (USPTO, EPO, JPO, WIPO, NPL);
search terms: ophthalmic, eye drop, PPAR, Fenofibrate, Proctofofen, Ankebin, Antara, Elasterate, Elasterin, Fenobrate, Fenofibrato, Fenofibratrum, Fenogal, Fenoglide, Fenotard, Lipantyl, Lipantil, Lipidex, Lipidil, Lipifan...

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>US 2007/0066883 A1 (Ferrito et al.) 22 March 2007 (22.03.2007), para [0024], [0027], [0034], [0078], [0083]</td>
<td>1-3</td>
</tr>
<tr>
<td>X</td>
<td>US 2002/1077265 A1 (Chen et al.) 30 August 2002 (08.08.2002), [0029], [0063], [0086]</td>
<td>1-3</td>
</tr>
</tbody>
</table>

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
06 November 2012 (06.11.2012)

Date of mailing of the international search report
30 NOV 2012

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. 571-272-3301

Authorized officer:
Lee W. Young
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)
INTERNATIONAL SEARCH REPORT

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.: 4-31
   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:

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.: 4-31.

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.:

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.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)