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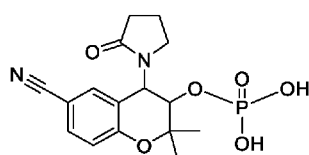
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Declarations under Rule 4.17:

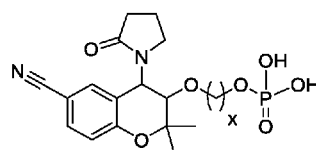
- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

(54) Title: IMPROVED METHODS AND COMPOSITIONS FOR CROMAKALIM PRODRUG THERAPY

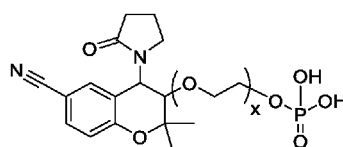


CKLP1

Formula I



Formula II



Formula III

FIG. 12

(57) Abstract: New medical uses for the compounds of Formula (I), (II), or (III) or a pharmaceutically acceptable salt thereof. New methods and compositions for the use of certain cromakalim prodrugs and their pharmaceutically acceptable salts.



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IMPROVED METHODS AND COMPOSITIONS FOR CROMAKALIM PRODRUG THERAPY

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 63/134,042, filed January 5, 2021, U.S. Provisional Application No. 63/120,604, filed December 2, 2020, and U.S. Provisional Application No. 62/971,752, filed February 7, 2020. The application is incorporated by reference for all purposes.

10 STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under Grant No. EY021727 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

15 This application is in the field of medical therapy and provides new methods and compositions for the use of certain cromakalim prodrugs and their pharmaceutically acceptable salts.

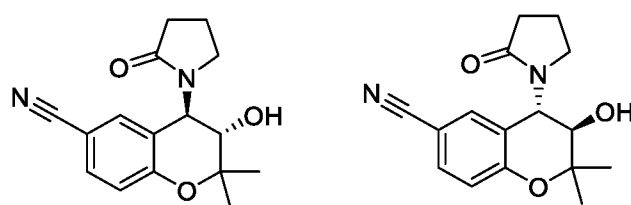
BACKGROUND OF THE INVENTION

Cromakalim and its use as an anti-hypertensive was first described in European Patent EP
20 0120428B1 assigned to the Beecham Group, Inc. Disclosures of cromakalim's effects on intraocular pressure and glaucoma were reported in PCT Application WO 89/10757; Lin *et al.*, "Effects of Cromakalim and Nicorandil on Intraocular Pressure after Topical Administration in Rabbit Eyes" *Journal of Ocular Pharmacology and Therapeutics*, 1995, 11, 195; and, Roy Chowdhury *et al.*, "Ocular Hypotensive Effects of the ATP-Sensitive Potassium Channel Opener
25 Cromakalim in Human and Murine Experimental Model Systems" *PLOS One*, 2015, 10, e0141783.

Cromakalim and diazoxide were reported to lower blood pressure in Quast, U. *et al. J Pharmacol Exp Ther* 1989, 250, 261. Additionally, publications by Chowdhury *et al.* and Roy Chowdhury *et al.* describe the use of diazoxide and nicorandil ("ATP-Sensitive Potassium (KATP)
30 Channel Openers Diazoxide and Nicorandil Lower Intraocular Pressure" *IOVS*, 2013, 54, 4894

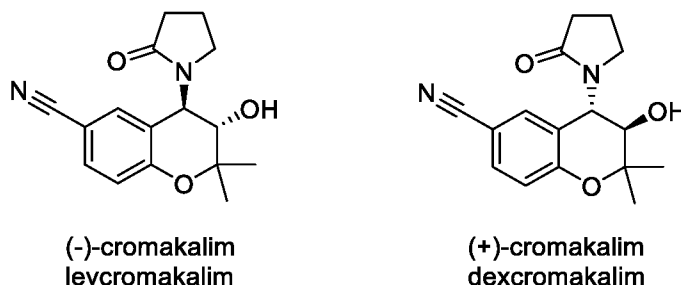
and “ATP-Sensitive Potassium (K_{ATP}) Channel Activation Decreases Intraocular Pressure in the Anterior Chamber of the Eye” *IOVS*, 2011, 52, 6435). Cromakalim placed in membrane patches from rabbit mesenteric arterial smooth muscle cells increases the open-state probability (P_{open}) of single K_{ATP} channels more than 9-fold in the presence of ATP (Brayden, J.E. *et al.*, *Blood Vessels*, 1991, 28, 147). Other ATP-sensitive potassium channel openers include pinacidil and minoxidil sulfate, which act as vasodilators *in vitro* and *in vivo*.

Cromakalim exists as a mixture of diastereomers in the trans-configuration (a mixture of (3*R*,4*S*) and (3*S*,4*R*) diastereomers):



Cromakalim (mixture of trans-diastereomers)

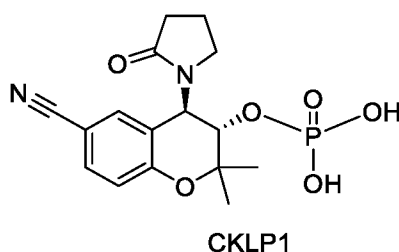
The (3*S*,4*R*)- diastereomer is also referred to as (-)-cromakalim or levromakalim and the (3*R*,4*S*)- diastereomer is also referred to as (+)-cromakalim or dexromakalim:



The majority of cromakalim's reported activity stems from the (3*S*,4*R*)-diastereomer, levromakalim (Ashwood *et al.* Synthesis and Antihypertensive Activity of 4-(Cyclic Amido)-2H-1-benzopyrans” *J. Med. Chem.* 1986, 29, 2194 and Attwood *et al.* “Synthesis of Homochiral Potassium Channel Openers: Role of the Benzopyranyl 3-Hydroxyl Group in Cromakalim and Pyridine N-Oxides in Determining the Biological Activities of Enantiomers” *Bioorg. Med. Chem. Lett.* 1992, 2, 229).

While cromakalim has established activity as a potassium channel opener and vasodilator, it is substantially insoluble in water. The lipophilicity of cromakalim has limited its usefulness for certain *in vivo* applications. Cromakalim is often solubilized with DMSO or cremophor, which is also used for the non-water-soluble drug taxol. Cremophor in particular has toxic side effects.

In response to the need to create a cromakalim formulation that has appropriate properties for administration into aqueous environments *in vivo*, Mayo Foundation for Medical Education and Research and Regents of the University of Minnesota created the phosphate ester prodrug CKLP1, also reported as a sodium salt:



CKLP1 provides the improvement of increased water solubility for ease of administration in combination with hydrolysis *in vivo* to the parent levcromakalim. See WO 2015/117024 filed by Mayo Foundation for Medical Education and Research and the Regents of The University of Minnesota.

Roy Chowdhury *et al.* “Analogues of the ATP-Sensitive Potassium (KATP) Channel Opener Cromakalim with *in Vivo* Ocular Hypotensive Activity” *J. Med. Chem.* 2016, 59, 6221, reported that the phosphate prodrug is more water soluble than cromakalim and was reported to lower intraocular pressure (IOP) in a normotensive (i.e., normal IOP) mouse model, however, the drug was only administered for 7 days. The article also reported the efficacy of increasing doses of certain cromakalim derivatives in rabbit eyes over an 8-day period. While these reported results were interesting, they had the weakness that the tests were only carried out in normotensive animal models (i.e., mice and rabbits with normal IOP to begin with), and only over a short time period with constant therapy.

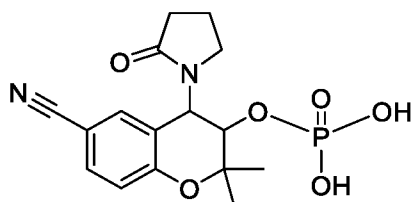
The effect of CKLP1 on episcleral venous pressure and distal outflow resistance was described in Roy Chowdhury *et al.* “Effect of Cromakalim Prodrug 1 (CKLP1) on Aqueous Humor Dynamics and Feasibility of Combination Therapy with Existing Ocular Hypotensive Agents”

IOVS, 2017, 58, 5731. Pharmacokinetic parameters in rabbits following topical and intravenous administration was described in Roy Chowdhury *et al.* "Pharmacological and Pharmacokinetic Profile of the Novel Ocular Hypotensive Prodrug CKLP1 in Dutch-belted Pigmented Rabbits" *PLoS One*, 2020, 15, e0231841). The synthesis of CKLP1 and the corresponding (3*R*,4*S*)-
 5 enantiomer is described in Roy Chowdhury *et al.* (*J. Med. Chem.* 2016, 59, 6221).

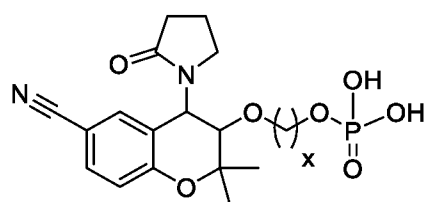
Given the potential unexplored benefits of cromakalim, it would be beneficial to have additional methods and compositions for medical therapy.

SUMMARY OF THE INVENTION

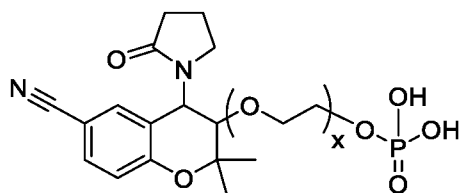
10 The present invention provides new medical uses for cromakalim prodrugs and pharmaceutically acceptable salts thereof of Formula I, II or III:



CKLP1
Formula I

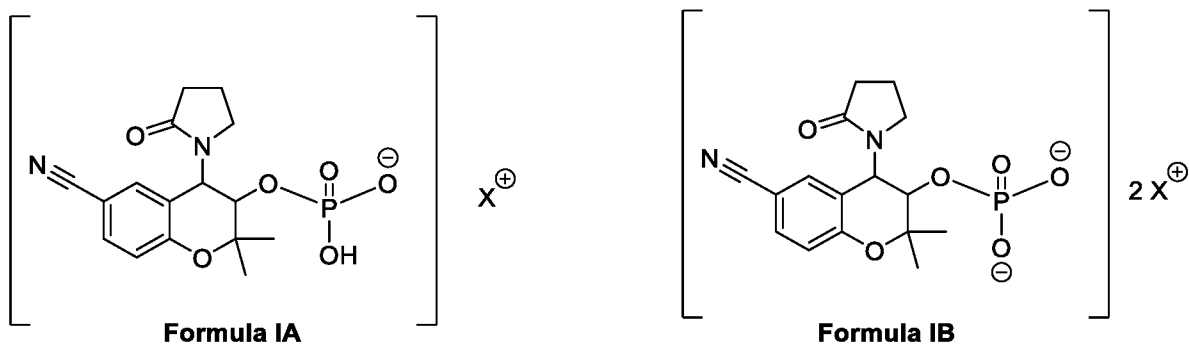


Formula II

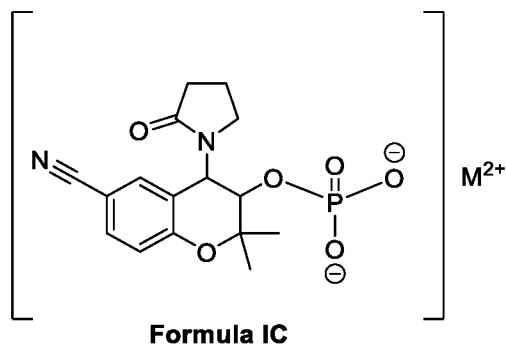


Formula III

Pharmaceutically acceptable salts of CKLP1 (Formula I) include:



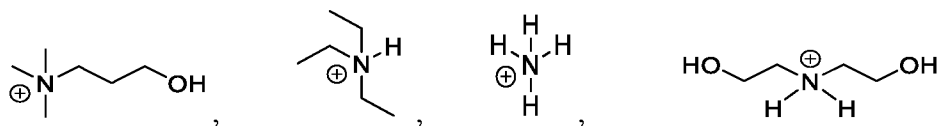
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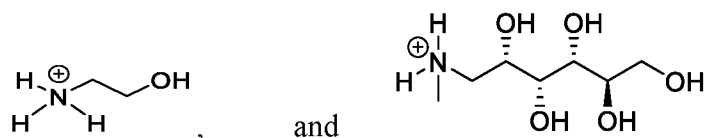


wherein X^+ and M^{2+} can be any pharmaceutically acceptable cation that achieves the desired results.

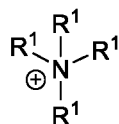
In certain embodiments, the cation is selected from sodium, potassium, aluminum, calcium, magnesium, lithium, iron, zinc, arginine, chlorprocaine, choline, diethanolamine, ethanolamine, lysine, histidine, meglumine, procaine, hydroxyethyl pyrrolidine, ammonium, tetrapropylammonium, tetrabutylphosphonium, methyldiethanamine, and triethylamine.

In one embodiment, X^+ is Na^+ or K^+ . In one embodiment, X^+ is Li^+ . In one embodiment, X^+ is Cs^+ . In one embodiment, X^+ is an ammonium ion with a net positive charge of one. Non-limiting examples of ammonium ions with a net positive charge of one include:





In an alternative embodiment, the ammonium ion with a net positive charge of one has the formula below:



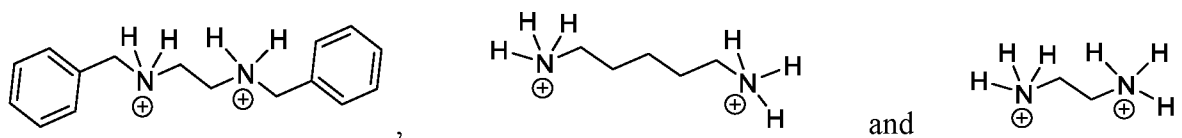
5 wherein R^1 is C_1 - C_6 alkyl, for example, but not limited to, methyl, ethyl, propyl, isopropyl, butyl, tbutyl, sec-butyl, isobutyl, $-CH_2C(CH_3)_3$, $-CH(CH_2CH_3)_2$, and $-CH_2CH(CH_2CH_3)_2$, cyclopropyl, CH_2 -cyclopropyl, cyclobutyl, and CH_2 -cyclobutyl, or aryl, for example, phenyl or naphthyl wherein the C_1 - C_6 alkyl or aryl can be optionally substituted, for example with a hydroxyl group. In one embodiment, the ammonium ion is



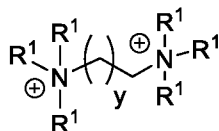
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M^{2+} , for example, may be, but is not limited to an alkaline earth metal cation (magnesium, calcium, or strontium), a metal cation with an oxidation state of +2 (for example, zinc or iron), or an ammonium ion with a net positive charge of two (for example, benzathine, hexamethyl diammonium, and ethylenediamine). In one embodiment, M^{2+} is Mg^{2+} . In one embodiment, M^{2+} is Ca^{2+} . In one embodiment, M^{2+} is Sr^{2+} . In one embodiment, M^{2+} is Zn^{2+} . In one embodiment, M^{2+} is Fe^{2+} . In one embodiment, M^{2+} is an ammonium ion with a net positive charge of two. Non-limiting examples of ammonium ions with a net positive charge of two include:

15



In an alternative embodiment, the ammonium ion with a net positive charge of two has the formula below:



wherein

R^1 is C_1 - C_6 alkyl, for example, but not limited to, methyl, ethyl, propyl, isopropyl, butyl, tbutyl, sec-butyl, isobutyl, $-\text{CH}_2\text{C}(\text{CH}_3)_3$, $-\text{CH}(\text{CH}_2\text{CH}_3)_2$, and $-\text{CH}_2\text{CH}(\text{CH}_2\text{CH}_3)_2$, cyclopropyl, CH_2 -cyclopropyl, cyclobutyl, and CH_2 -cyclobutyl, or aryl, for example, phenyl or naphthyl wherein the C_1 - C_6 alkyl or aryl can be optionally substituted, for example with a hydroxyl group; and,

y is an integer selected from 1, 2, 3, 4, 5, 6, 7, and 8.

Importantly, it has been discovered that the compounds of the present invention are particularly useful for controlled drug delivery applications because they exhibit unique and unexpected pharmacokinetics. The prodrugs act as internal control release devices in that they convert to the active cromakalim, and in one embodiment, levcromakalim, slowly. In one embodiment, the prodrugs are stored in tissues, including ocular tissues, and are slowly released over time. This slow conversion to the active moiety in combination with storage and slow release from tissues leads to long-term, continuous, and controlled dosing of active cromakalim, and in one embodiment, levcromakalim, following administration of CKLP1. These unexpected pharmacokinetic properties could not have been predicted in advance.

Therefore, in one embodiment, the present invention provides the controlled delivery of levcromakalim via the administration of a cromakalim prodrug of Formula I, Formula II, or Formula III or a pharmaceutically acceptable salt thereof to a host, including a human, in need thereof. In one embodiment, the controlled delivery of levcromakalim to the eye is achieved by the topical administration of a compound of the present invention wherein the compound is converted to levcromakalim optionally via alkaline phosphatase, which is found in the tissues and aqueous humor of the eye. In select embodiments of the present invention, a compound of Formula I, Formula II, or Formula III or a pharmaceutically acceptable salt thereof is administered to the eye, for example, as a topical drop, and is converted to levcromakalim in the eye, for example in the sclera, optic nerve, cornea, iris, ciliary body, trabecular meshwork, and/or the retina.

As discussed in the non-limiting Example 2, *in vitro* studies have shown that when exposed to alkaline phosphatase, CKLP1 is converted in a concentration-dependent manner to levromakalim, which in turn promotes cell hyperpolarization through ATP-sensitive potassium channels. In a non-limiting embodiment, the use of CKLP1 as a controlled delivery device to deliver levromakalim lowers IOP, for example by lowering episcleral venous pressure.

As discussed in Example 4 and as a non-limiting exemplary illustration of the present invention, CKLP1 was administered to hound dogs. Following once-daily topical CKLP1 administration in hound dogs, CKLP1 and levromakalim concentrations were measured in plasma and select tissues. Surprisingly, it was discovered that CKLP1 metabolizes slowly to levromakalim and that the concentration of CKLP1 was high in certain tissues, including ocular tissues such as the ocular nerve, anterior segment, the trabecular network, and the cornea.

It was also surprisingly discovered that following CKLP1 administration in dogs, it takes an extended period of time for IOP levels to return to baseline (FIG. 7A). This same effect was observed in African green monkeys (FIG. 8A). This is suggestive of a CKLP1 tissue depot in the eye that allows for slow release of CKLP1 because the half-life of levromakalim is only 2 hours. If there were no depot, 98% of levromakalim might be metabolized by 12 hours, but instead a slow return to baseline is observed (greater than 24 hours). In one embodiment, topically administered CKLP1 is stored in tissues, including, but not limited to, the trabecular meshwork, and then slowly released to the distal outflow pathway where it is converted to levromakalim to induce an IOP-lowering effect. In one embodiment, the return to IOP baseline following one or more (e.g., 2 or 3) dosage forms of a cromakalim prodrug of Formula I-Formula III in a host in need thereof, including a human, is at least about 12 hours, at least about 24 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, or at least about 72 hours.

Controlled-release delivery that leads to long-term delivery of the active metabolite requires less frequent dosing, which is important for patient compliance, adherence, and better outcomes. A compound with internal control release device capability is also advantageous because its administration does not require a vehicle, such as an implant or polymeric carrier, to provide the controlled release.

For these reasons, CKLP1 is well-tolerated as a topical dose. CKLP1 is also safe as evidenced by the detailed analysis of tissue histology in hound dogs wherein no observable toxicity

caused by the treatment was noted, nor were any substantial changes in blood chemistries. Topical dosing of CKLP1 also did not lead to significant changes in blood pressure (Example 4).

Furthermore, the effect of levromakalim on selected biomarkers for hyperemia and perturbations to vessel integrity have been established (Example 7). Levromakalim had no significant impact on the expression of the measured proteins that are indicative of tissue and vessel integrity. The effect of levromakalim was compared to Y-27632, a Rho kinase inhibitor, which is a class of drugs (exemplified by Rhopressa) that have been shown to have significant side effects caused by perturbations in vessel integrity (e.g., leakiness and vasodilation causing hyperemia, as well as vessel rupture leading to petechia and subconjunctival hemorrhages). Unlike Y-27632, levromakalim did not significantly alter the protein expression or distribution of these proteins. Therefore, in one embodiment, the use of a cromakalim prodrug of Formula I, II or III or a pharmaceutically acceptable salt thereof does not cause significant hyperemia in a patient in need thereof when used during therapy as described further herein, and in some embodiments, over long-term therapy, for example at least one, two, three, four, five, six, or more months. Alternatively, the administration of a compound of Formula I, Formula II, or Formula III does not significantly induce the expression of at least one protein independently selected from CD31 and VE-Cadherin.

CKLP1 was developed as a water-soluble alternative to levromakalim, but pharmacokinetic studies have now shown that it is also surprisingly advantageous due to its slow conversion to the active metabolite and potential for storage and slow release from tissues. This slow metabolism to levromakalim in combination with potential storage and slow release from tissues are advantageous pharmacokinetic properties that unexpectedly lead to controlled, long-term delivery of levromakalim. Furthermore, in addition to the unique pharmacokinetics of CKLP1, the active metabolite, levromakalim, has also been shown to be unexpectedly and advantageously safe in terms of the tissue and vessel integrity.

The cromakalim prodrugs or pharmaceutically acceptable salt of Formula I, Formula II, or Formula III can include a cromakalim moiety that is either the (-) (3*S*,4*R*)-enantiomer (levromakalim) or the (+) (3*R*,4*S*)-enantiomer (dexromakalim) or any mixture thereof. The CKLP1 prodrugs can be used as the free acid or a fully or partially neutralized acid. In one embodiment, the pH of the pharmaceutical formulation that includes the cromakalim prodrugs or

pharmaceutically acceptable salt of Formula I, Formula II, or Formula III is adjusted using a pharmaceutically acceptable base to the desired pH level for pharmaceutical administration, often between about 5.5 or 6.5 and 8.5, and more typically between 6.5 and 8.

At physiological pH, a compound of the present invention with a free acid will exist in equilibrium with the fully ionized or, in one embodiment, the partially ionized form. For example, the pH of the eye is approximately 7.4-7.6 and is mostly composed of water. Therefore, the free hydroxyls of the compounds of the present invention will exist in the body as the corresponding ionized form (due to the natural equilibrium in a slightly basic solution). This ionized form will then degrade to cromakalim, and in one embodiment, levromakalim.

The present invention also provides new medical uses for CKLP1 prodrugs, including blood vessel disorders, cardiovascular disorders, lymphatic diseases, and erectile dysfunction. In addition to exhibiting therapeutic efficacy for ocular disorders, it has been surprisingly discovered that CKLP1 when administered systemically can induce peripheral vasodilation, for example in dogs (Example 5) and rats (Example 7). This is a surprisingly beneficial side effect that can treat blood vessel disorders, such as Raynaud's disease, ischemic limb syndrome, pulmonary arterial hypertension, or sexual disorders, such as erectile dysfunction. Therefore, in one embodiment, CKLP1 is administered to a host in need thereof, for example a human, for the treatment of Raynaud's disease. In another embodiment, CKLP1 is administered to a host in need thereof, for example a human, for the treatment of erectile dysfunction.

The invention includes at least the following aspects:

- (i) New medical uses that administer an effective amount of a compound of Formula I (CKLP1) or a compound of Formula II or III or a pharmaceutically acceptable salt thereof to treat a disorder in a host in need thereof;
- (ii) Long term medical therapy, including ocular therapy (*i.e.*, for at least 6 weeks, 7 weeks, or at least 2, 3, 4, 5, or 6 months or indefinitely for the duration of the therapy) to a host in need thereof for, including but not limited to, normal tension glaucoma, that includes the administration of an effective amount of CKLP1 or other cromakalim prodrugs of Formula II or III as described herein or a pharmaceutically acceptable salt thereof in a manner that does not create significant

tachyphylaxis (i.e., loss of activity over time), or alternatively, which does not induce tolerance;

- (iii) Once-daily (QD) human dosing using an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof to treat glaucoma associated with elevated intraocular pressure, including but not limited to primary open angle glaucoma (POAG), primary angle closure glaucoma (also known as chronic open angle glaucoma, chronic simple glaucoma and glaucoma simplex), pediatric glaucoma, pseudo-exfoliative glaucoma, pigmentary glaucoma, traumatic glaucoma, neovascular glaucoma, irido corneal endothelial glaucoma (ICE), and in an alternative embodiment, uveitic glaucoma, steroid induced glaucoma, and acute glaucoma resulting from advanced cataracts and/or from intravitreal injections;
- (iv) Ocular therapy using an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof that does not result in significant hyperemia (which can result in “red eye”, vascular congestion, small bleeds, small punctate bleeds or microhemorrhages) to a host in need thereof;
- (v) An effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof either as primary or secondary or adjunctive treatment as part of the protocol for MIGS (Minimally Invasive Glaucoma Surgery), including but not limited to miniature versions of trabeculectomy (microtrabeculectomies), trabecular bypass surgeries, totally internal or suprachoroidal shunts, milder/gentler versions of laser cyclo photocoagulation, and in an alternative embodiment, Schlemm’s canal stents that dilate Schlemm’s canal, goniotomies, canaloplasties, and laser trabeculoplasties;
- (vi) Formulations for topical delivery that include an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof for ocular therapy to a host in need thereof;
- (vii) Formulations that include an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable

salt thereof for dermatological or transdermal applications for a host in need thereof;

(viii) Formulations that include an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof for enteral and parenteral delivery of CKLP1 to treat systemic disorders for a host in need thereof;

(ix) The administration of an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof to treat a cardiovascular disorder in a host such as high blood pressure, congestive heart failure, transient ischemic attack, heart attack, acute myocardial infarction, acute and chronic myocardial ischemia, unstable angina or associated chest pain, arrhythmias, or pulmonary arterial hypertension (PAH), a cardioprotective agent in a host experiencing a heart attack or undergoing heart surgery, a cardioprotective agent for the preservation of heart prior to organ donation, microvascular dysfunction, or endothelial dysfunction;

(x) The administration of an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof to treat a blood vessel disorder, such as Raynaud's disease, peripheral artery disease, including chronic and acute limb ischemia as well as chronic cold hands and/or feet, in a host in need thereof;

(xi) The administration of an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof to treat an endocrine disorder such as hypoglycemia, hyperinsulinism or diabetes in a host;

(xii) The administration of an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof to treat a skeletal muscle disorder such as skeletal muscle myopathy in a host;

(xiii) The administration of an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof

to treat a urology disorder such as erectile dysfunction or female sexual arousal disorder;

(xiv) The administration of an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof to treat a dermatology disorder such as hypotrichosis (failure to have normal eyelash growth) or baldness in a host in need thereof;

(xv) The administration of an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof to treat a neurological disorder such as neuropathic pain or neurodegenerative disease (for example Parkinson's disease and Huntington's disease) in a host in need thereof;

(xvi) The administration of an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof to treat a lymphatic disease such as lymphedema, lymphangitis, lymphadenitis, lymphangiomatosis, Castleman's disease, or a cancer of the lymph system, including Hodgkin's lymphoma, non-Hodgkin's lymphoma, or lymphangiomatosis, in a host in need thereof;

(xvii) The administration of an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof to treat an ocular lymphatic disease selected from conjunctival myxoma, dry eye, conjunctival lymphangiectasia, chemosis, mustard gas keratitis, corneal inflammation, orbital cellulitis, chalazion, dermatochalasis, and blepharochalasis;

(xviii) The administration of an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof to treat tumor hypoperfusion or hypoxia in a host in need thereof;

(xix) The administration of an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof to treat a mitochondrial disorder;

(xx) The administration of an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof

to treat an ocular disorder in a host such as Graves' ophthalmopathy, thyroid-associated orbitopathy (TAO), Graves' orbitopathy (GO), retrobulbar tumors, cavernous sinus thrombosis, orbital vein thrombosis, episcleral/orbital vein vasculitis, superior vena cava obstruction, superior vena cava thrombosis, carotid cavernous sinus fistula, dural cavernous sinus shunts, orbital varices, central retinal vein occlusion (CRVO), branch retinal vein occlusion (BRVO), artery occlusive/embolic and or hypoperfusion diseases, optic nerve damage due to ischemia (posterior and anterior ischemic optic neuropathy (NAION);

(xxi) A method of providing cellular protection and/or neuroprotection comprising administering an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof to a host in need thereof;

(xxii) The administration of an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof to treat Sturge-Weber Syndrome, including but not limited to Sturge-Weber Syndrome-induced glaucoma in a host in need thereof; and

(xxiii) A pharmaceutical composition comprising an effective amount of CKLP1 or other cromakalim prodrug of Formula II or III as described herein or a pharmaceutically acceptable salt thereof to treat any one of the disorders or diseases described in embodiments (i)-(xxii).

BRIEF DESCRIPTION OF FIGURES

FIG. 1A is a graph of the levromakalim induced hyperpolarization in HEK-Kir6.2/SUR2B cells showing the averaged FLIPR traces of membrane potential response to 0.3 μ M, 3 μ M, and 30 μ M levromakalim compared to the assay buffer control as described in Example 1. Arrows indicate point of either compound or 10 μ M glibenclamide, a KATP channel inhibitor, addition (in continued presence of test agent). Bar indicates the time range data that was exported for EC₅₀ calculation in Table 1. The x-axis is time measured in seconds and the y-axis is average relative fluorescence response (RFU).

FIG. 1B is a dose-response curve of levcromakalim-induced hyperpolarization in HEK-Kir6.2/SUR2B cells. The data has been averaged across replicate testing days as described in Example 1. Data points for individual batches are mean $\pm$ SEM (standard error of the mean) for 4-6 replicates recorded across two separate experimental days. Data points for combined experiments is mean $\pm$ SEM of all replicates regardless of batch. Fitted EC₅₀ values are summarized in Table 1. The x-axis is compound concentration measured in μ M and the y-axis is the percent activation of the KATP potassium channel.

FIG. 2A is a graph of the CKLP1 induced hyperpolarization in HEK-Kir6.2/SUR2B cells showing averaged FLIPR traces of membrane potential response to 100 μ M CKLP1 compared to the assay buffer control (100 μ M pinacidil control included as reference) as described in Example 1. Arrows indicate point of either compound or 10 μ M glibenclamide (a KATP channel inhibitor) addition (in continued presence of test agent). Bar indicates time range data that was exported for EC₅₀ calculation. The x-axis is time measured in seconds and the y-axis is average relative fluorescence response (RFU).

FIG. 2B is a dose-response curve of CKLP1 induced hyperpolarization in HEK-Kir6.2/SUR2B cells. The data has been averaged across replicate testing days as described in Example 1. Data points for individual batches are mean $\pm$ SEM for 4-6 replicates recorded across two separate experimental days. Data points for combined data is mean $\pm$ SEM of all replicates regardless of batch. Fitted EC₅₀ values are summarized in Table 1. The x-axis is compound concentration measured in μ M and the y-axis is the percent activation of the KATP potassium channel.

FIG. 3 is a dose-response curve of cromakalim- and pinacidil-induced hyperpolarization in HEK-Kir6.2/SUR2B cells. The data has been averaged across replicate testing days as described in Example 1. Data points for individual batches are mean $\pm$ SEM for 4-6 replicates recorded across two separate experimental days. Data points for combined data is mean $\pm$ SEM of all replicates regardless of batch. Fitted EC₅₀ values are summarized in Table 1. The x-axis is compound concentration measured in μ M and the y-axis is the percent activation of the KATP potassium channel.

FIG. 4A is a graph showing the conversion of CKLP1 to levcromakalim *in vitro* in the presence of decreasing concentrations (0.2 U/100 μ L, 0.02 U/100 μ L, 0.002 U/100 μ L, and 0.0002

U/100 μ L) of alkaline phosphatase over the course of 60 minutes as described in Example 2. The x-axis is the time measured in minutes and the y-axis is the percent conversion of levromakalim.

FIG. 4B is a graph showing the conversion of CKLP1 to levromakalim *in vitro* in the presence of decreasing concentrations (0.2 U/100 μ L, 0.02 U/100 μ L, 0.0020 U/100 μ L, and 0.00020 U/100 μ L) of alkaline phosphatase over the course of 72 hours as described in Example 2. The x-axis is the time measured in minutes and the y-axis is the percent conversion of levromakalim.

FIG. 5A is a graph showing the conversion of CKLP1 to levromakalim *in vitro* over the course of 60 minutes. As described in Example 2, the concentration of CKLP1 (0.01 mM, 0.1 mM, 1 mM, 10 mM, 20 mM, and 40 mM) was varied and the concentration of alkaline phosphate was kept constant. The x-axis is the time measured in minutes and the y-axis is the percent conversion of levromakalim.

FIG. 5B is a graph showing the conversion of CKLP1 to levromakalim *in vitro* over the course of 72 hours. As described in Example 2, the concentration of CKLP1 was varied (0.01 mM, 0.1 mM, 1 mM, 10 mM, 20 mM, and 40 mM) and the concentration of alkaline phosphate was kept constant. The x-axis is the time measured in minutes and the y-axis is the percent conversion of levromakalim.

FIG 6 is a dose response of CKLP1 in hound dogs as described in Example 4. Dose response studies with CKLP1 show that all concentrations lowered IOP significantly compared to baseline. Statistically, both 10 mM and 15 mM concentrations had the greatest reduction in IOP, although no difference was noted between the two concentrations. Therefore, the 10 mM concentration was selected for all subsequent experiments. The x-axis is the concentration of CKLP1 measured in mM and the y-axis is the change in IOP compared to baseline measured in mmHg.

FIG. 7A is a graph of the extended-dose study discussed in Example 4. Once daily treatment of CKLP1 at 10 mM caused sustained IOP reduction over a treatment period of 61 consecutive days with excellent tolerability and no observable ocular side effects. Time of treatment with CKLP1 is indicated along the x-axis, while pre- and post-treatment is indicated by the shaded boxes. The x-axis is time measured in days and the y-axis is the change in IOP compared to vehicle control measured in mmHg.

FIG. 7B is a graph showing the systolic and diastolic blood pressure of hound dogs following once daily topical 10 mM CKLP1 treatment as discussed in Example 4. CKLP1 treatment did not cause any significant changes in average systolic and diastolic blood pressures when compared to baseline values. The x-axis is labelled with systolic or diastolic blood pressure and the y-axis is blood pressure measured in mmHg.

FIG. 8A is a graph of IOP measurement in African green monkeys following topical CKLP1 treatment as discussed in Example 4. Once daily treatment with 10 mM CKLP1 lowered IOP in African green monkeys. IOP returned to near baseline following withdrawal of treatment. No contraindicative side effects were observed during the course of the treatment. Time of treatment with CKLP1 is indicated along the x-axis, while pre- and post-treatment is indicated by the shaded boxes. The x-axis is time measured in days and the y-axis is the change in IOP compared to vehicle control measured in mmHg.

FIG. 8B is a graph showing the systolic and diastolic blood pressure of African green monkeys following topical CKLP1 treatment as discussed in Example 4. Daily treatment with 10 mM CKLP1 for a period of 7 days had no significant effect on systolic or diastolic blood pressure in African green monkeys. The x-axis is labelled with systolic or diastolic blood pressure and the y-axis is blood pressure measured in mmHg.

FIG. 9A is a graph of the concentration of CKLP1 and levromakalim in blood collected from hound dogs at eight different time points on day 1 of the study as described in Example 4. The hound dogs were treated with 50 μ L topical ocular administration of 10 mM CKLP1 in both eyes once daily for eight days and FIG. 9A is a graph of time points from day 1. The graph indicates conversion of CKLP1 to levromakalim along with characteristic absorption and elimination profiles of the drugs. Pharmacokinetic parameters from the analysis of the data from FIG. 9A is provided in Table 2A and Table 2B. The x-axis is time measured in hours and the y-axis is concentration measured in ng/mL.

FIG. 9B is a graph of the concentration of CKLP1 and levromakalim in blood collected from hound dogs at eight different time points on day 4 of the study as described in Example 4. The hound dogs were treated with 50 μ L topical ocular administration of 10 mM CKLP1 in both eyes once daily for eight days and FIG. 9B is a graph of time points from day 4. The graph indicates conversion of CKLP1 to levromakalim along with characteristic absorption and

elimination profiles of the drugs. Pharmacokinetic parameters from the analysis of the data from FIG. 9B is provided in Table 2A and Table 2B. The x-axis is time measured in hours and the y-axis is concentration measured in ng/mL.

FIG. 9C is a graph of the concentration of CKLP1 and levcromakalim in blood collected from hound dogs at eight different time points on day 8 of the study as described in Example 4. The hound dogs were treated with 50 μ L topical ocular administration of 10 mM CKLP1 in both eyes once daily for eight days and FIG. 9C is a graph of time points from day 8. The graph indicates conversion of CKLP1 to levcromakalim along with characteristic absorption and elimination profiles of the drugs. Pharmacokinetic parameters from the analysis of the data from FIG. 9C is provided in Table 2A and Table 2B. The x-axis is time measured in hours and the y-axis is concentration measured in ng/mL.

FIG. 10 is a graph of the distribution of CKLP1 and levcromakalim in various ocular and systemic hound dog tissues and fluids following a 50 μ L topical once daily ocular administration of 10 mM CKLP1 for 12-13 days as described in Example 4. CKLP1 was identified in low concentrations in the heart and liver, and in higher concentrations in all ocular tissues analyzed. Trabecular meshwork, optic nerve and cornea showed the highest levels of CKLP1 and levcromakalim (ng per gram of tissue). Both drugs were excreted in the urine. TM = trabecular meshwork; AH = aqueous humor; VH = vitreous humor. The x-axis is labelled with the tissue and the y-axis is the concentration of CKLP1 or levcromakalim measured in ng/g. The concentration of CKLP1 was measured in ng/g with the exception of the vitreous humor, the aqueous humor, and the urine which were measured in ng/mL.

FIG. 11A is a representative hematoxylin and eosin-stained tissue specimen of trabecular meshwork and aqueous vessel plexus from a hound dog treated once daily with a 50 μ L topical ocular administration 10 mM CKLP1 for 12-13 days as described in Example 4. The tissue selection was devoid of any pathological findings, indicating good tolerability of the CKLP1 in these animals. The Scale bar is 50 μ m.

FIG. 11B is a representative hematoxylin and eosin-stained tissue specimen of retina from a hound dog treated once daily with a 50 μ L topical ocular administration of 10 mM CKLP1 for 12-13 days as described in Example 4. The tissue selection was devoid of any pathological findings, indicating a good tolerability of the CKLP1 in these animals. The Scale bar is 50 μ m.

FIG. 11C is a representative hematoxylin and eosin-stained tissue specimen of kidney from a hound dog treated once daily with a 50 μ l topical ocular administration of 10 mM CKLP1 for 12-13 days as described in Example 4. The tissue selection was devoid of any pathological findings, indicating a good tolerability of the CKLP1 in these animals. The Scale bar is 50 μ m.

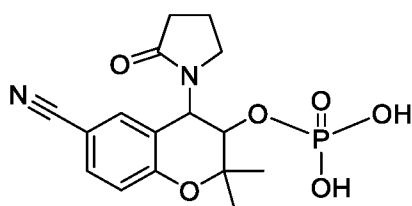
FIG. 11D is a representative hematoxylin and eosin-stained tissue specimen of liver from a hound dog treated once daily with a 50 μ l topical ocular administration of 10 mM CKLP1 for 12-13 days as described in Example 4. The tissue selection was devoid of any pathological findings, indicating a good tolerability of the CKLP1 in these animals.

FIG. 12 are images of Formula I, Formula II, and Formula III of the present invention. CKLP1 is Formula I.

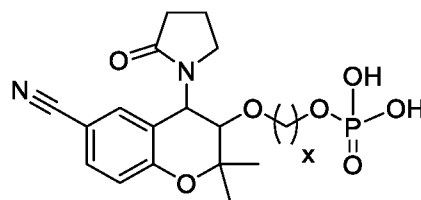
DETAILED DESCRIPTION OF THE INVENTION

I. Cromakalim Phosphate and other Prodrugs and their Pharmaceutically Acceptable Salts for Medical Uses as Described Herein

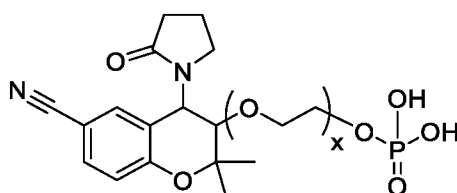
In one aspect, the invention is new medical uses for cromakalim prodrugs and pharmaceutically acceptable salts thereof of Formula I, II or III:



CKLP1
Formula I



Formula II

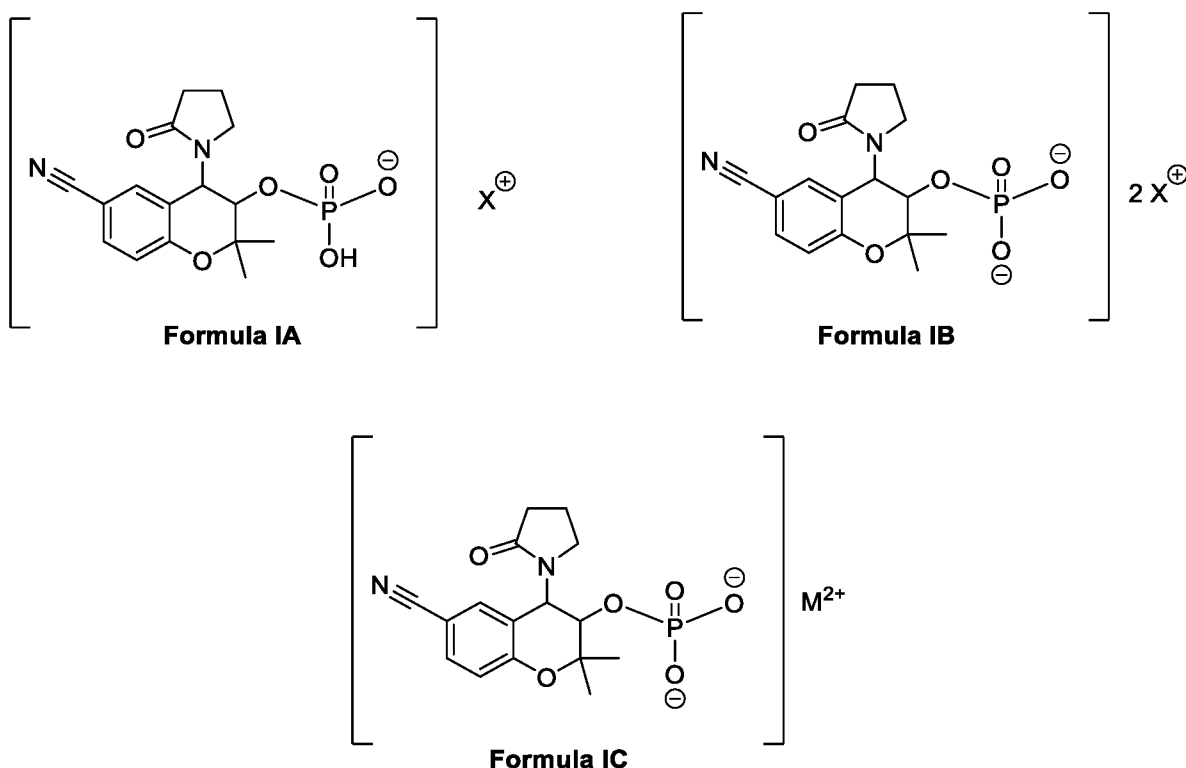


Formula III

It has been surprisingly discovered that the prodrugs of the present invention exhibit unexpected pharmacokinetic properties that lead to long-term, controlled delivery of cromakalim, and in one embodiment, levromakalim. The prodrugs act as internal control release devices in

that they convert to the active cromakalim or levromakalim slowly, and in one embodiment, are stored in tissues, including ocular tissues, and slowly released over time. This could not have been predicted in advance and affords unexpected continuous and controlled delivery of the active moiety.

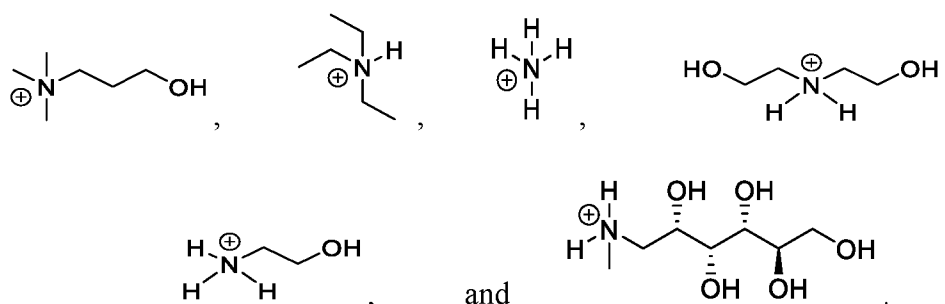
5 Pharmaceutically acceptable salt of CKLP1 (Formula I) include:



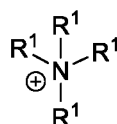
10 wherein X^+ and M^{2+} can be any pharmaceutically acceptable cation that achieves the desired results.

In certain embodiments, the cation is selected from sodium, potassium, aluminum, calcium, magnesium, lithium, iron, zinc, arginine, chlorprocaine, choline, diethanolamine, ethanolamine, lysine, histidine, meglumine, procaine, hydroxyethyl pyrrolidine, ammonium,
 15 tetrapropylammonium, tetrabutylphosphonium, methyldiethanamine, and triethylamine.

In one embodiment, X^+ is Na^+ or K^+ . In one embodiment, X^+ is Li^+ . In one embodiment, X^+ is Cs^+ . In one embodiment, X^+ is an ammonium ion with a net positive charge of one. Non-limiting examples of ammonium ions with a net positive charge of one include:



In an alternative embodiment, the ammonium ion with a net positive charge of one has the formula below:



5

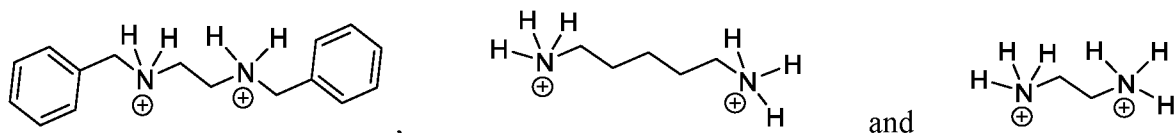
wherein R^1 is C_1 - C_6 alkyl, for example, but not limited to, methyl, ethyl, propyl, isopropyl, butyl, tbutyl, sec-butyl, isobutyl, $-CH_2C(CH_3)_3$, $-CH(CH_2CH_3)_2$, and $-CH_2CH(CH_2CH_3)_2$, cyclopropyl, CH_2 -cyclopropyl, cyclobutyl, and CH_2 -cyclobutyl, or aryl, for example, phenyl or naphthyl wherein the C_1 - C_6 alkyl or aryl can be optionally substituted, for example with a hydroxyl group. In one embodiment, the ammonium ion is

10

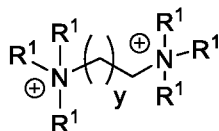


M^{2+} , for example, may be, but is not limited to an alkaline earth metal cation (magnesium, calcium, or strontium), a metal cation with an oxidation state of +2 (for example, zinc or iron), or an ammonium ion with a net positive charge of two (for example, benzathine, hexamethyl diammonium, and ethylenediamine). In one embodiment, M^{2+} is Mg^{2+} . In one embodiment, M^{2+} is Ca^{2+} . In one embodiment, M^{2+} is Sr^{2+} . In one embodiment, M^{2+} is Zn^{2+} . In one embodiment, M^{2+} is Fe^{2+} . In one embodiment, M^{2+} is an ammonium ion with a net positive charge of two. Non-limiting examples of ammonium ions with a net positive charge of two include:

15



In an alternative embodiment, the ammonium ion with a net positive charge of two has the formula below:

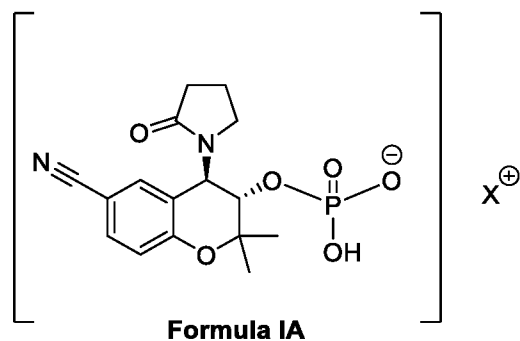
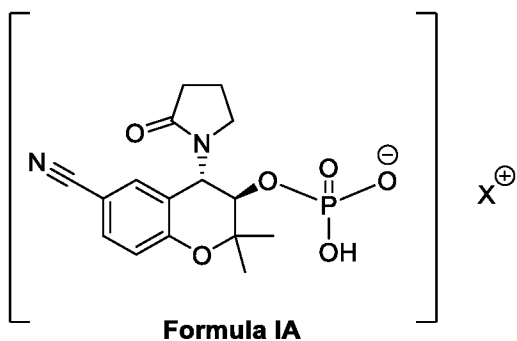
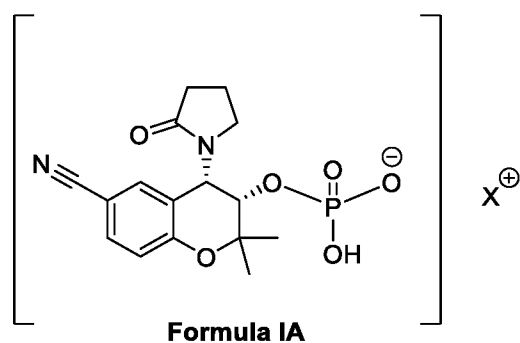
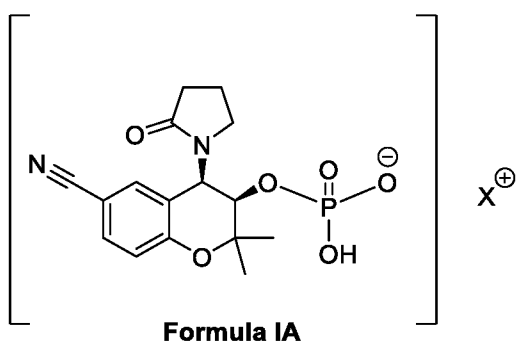


wherein

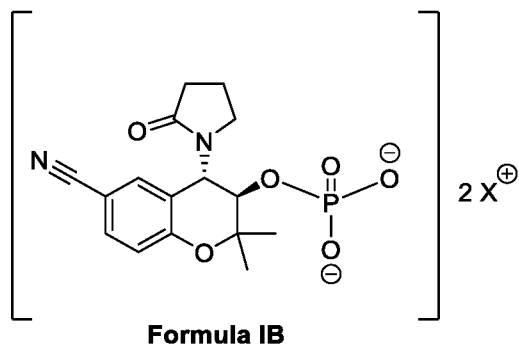
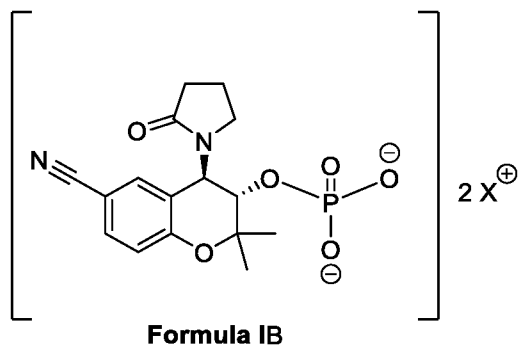
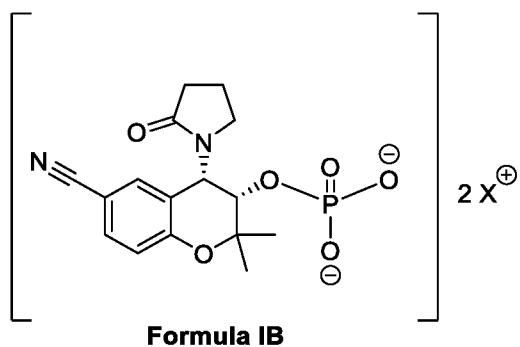
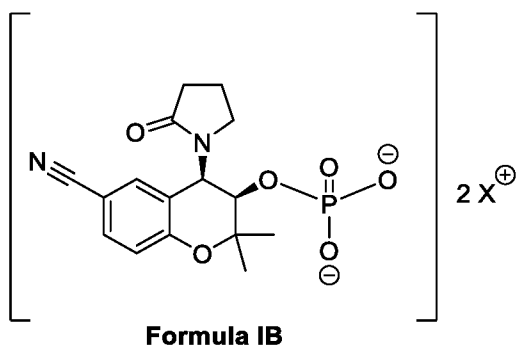
- 5 R^1 is C_1 - C_6 alkyl, for example, but not limited to, methyl, ethyl, propyl, isopropyl, butyl, tbutyl, sec-butyl, isobutyl, $-\text{CH}_2\text{C}(\text{CH}_3)_3$, $-\text{CH}(\text{CH}_2\text{CH}_3)_2$, and $-\text{CH}_2\text{CH}(\text{CH}_2\text{CH}_3)_2$, cyclopropyl, CH_2 -cyclopropyl, cyclobutyl, and CH_2 -cyclobutyl, or aryl, for example, phenyl or naphthyl wherein the C_1 - C_6 alkyl or aryl can be optionally substituted, for example with a hydroxyl group; and,
- y is an integer selected from 1, 2, 3, 4, 5, 6, 7, and 8.

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Non-limiting examples of a compound of Formula IA include:

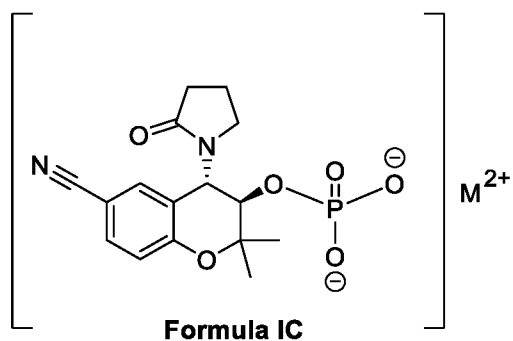
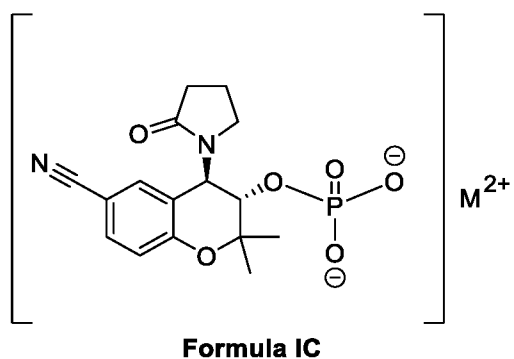


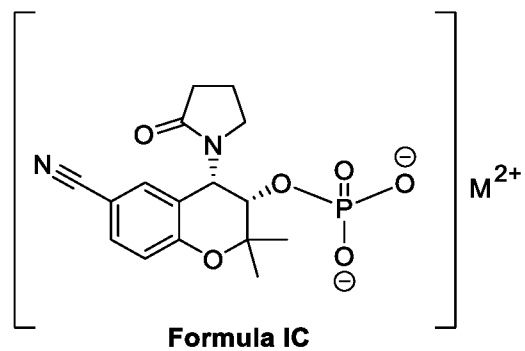
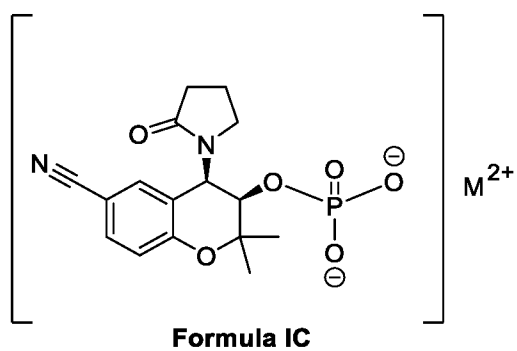
Non-limiting examples of a compound of Formula IB include:



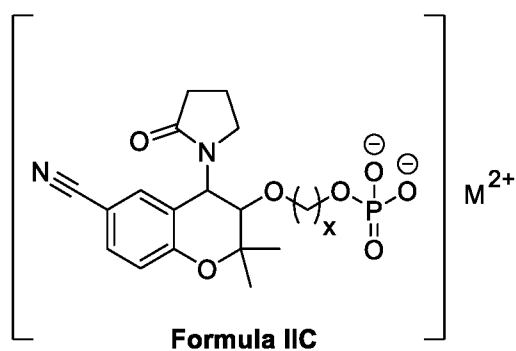
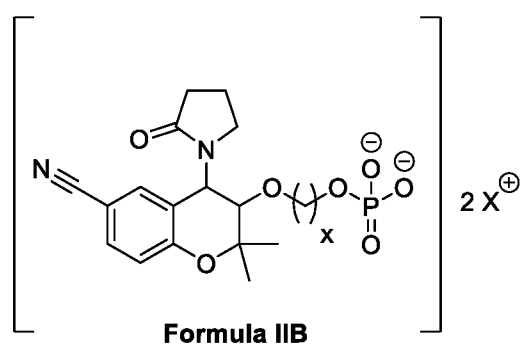
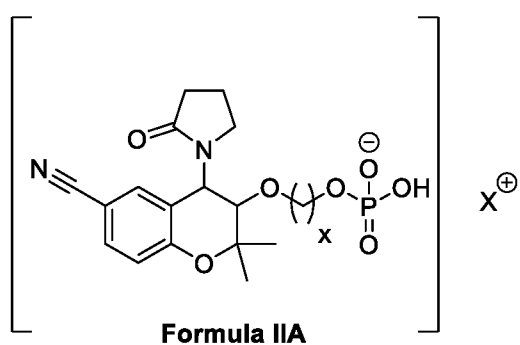
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Non-limiting examples of a compound of Formula IC include:



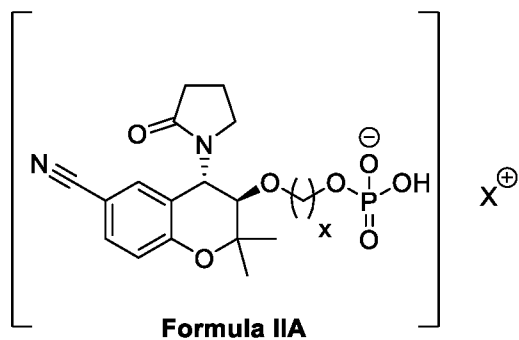
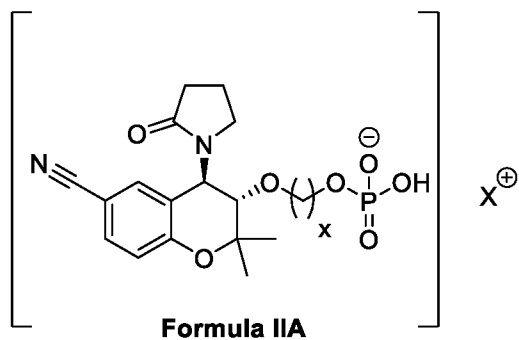
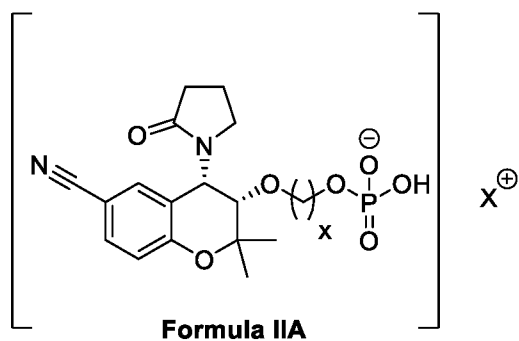
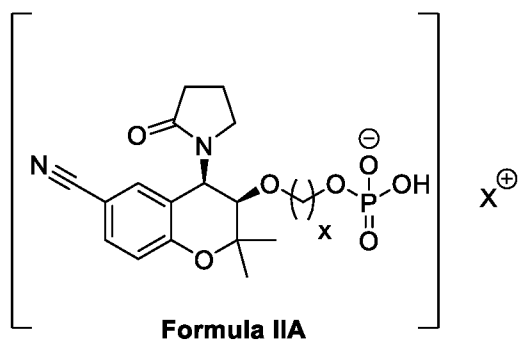


Pharmaceutically acceptable salt of Formula II include:



wherein X^+ and M^{2+} are as defined above; and
 x is an integer selected from 1, 2, 3, 4, or 5.

Non-limiting examples of a compound of Formula IIA include:



In one embodiment of Formula IIA, x is 1.

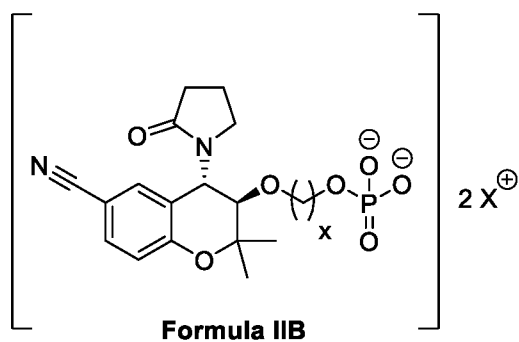
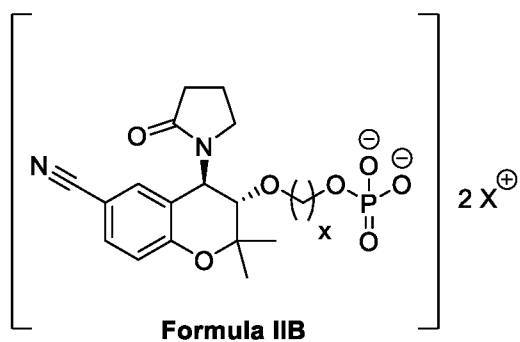
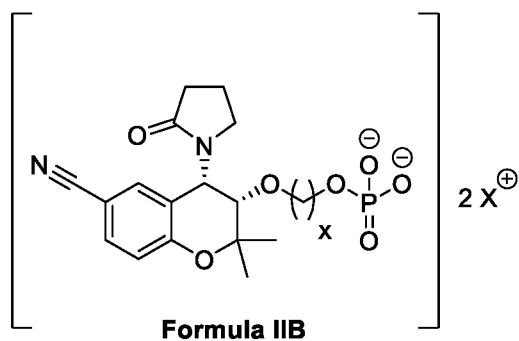
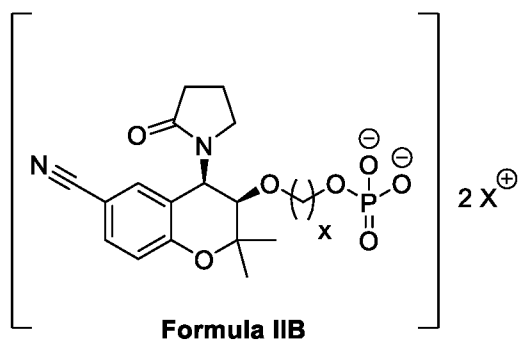
In one embodiment of Formula IIA, x is 2.

In one embodiment of Formula IIA, x is 3.

In one embodiment of Formula IIA, x is 4.

In one embodiment of Formula IIA, x is 5.

Non-limiting examples of a compound of Formula IIB include:



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In one embodiment of Formula IIB, x is 1.

In one embodiment of Formula IIB, x is 2.

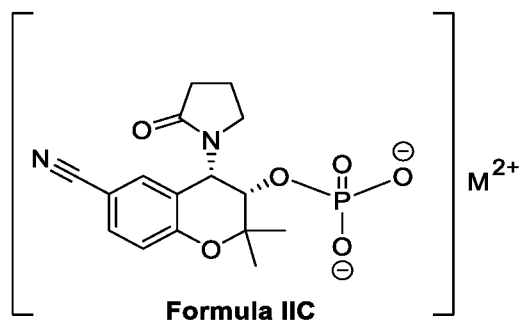
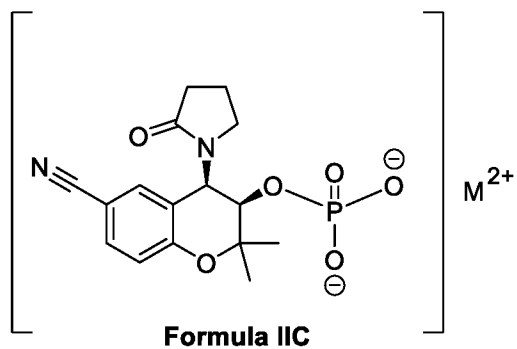
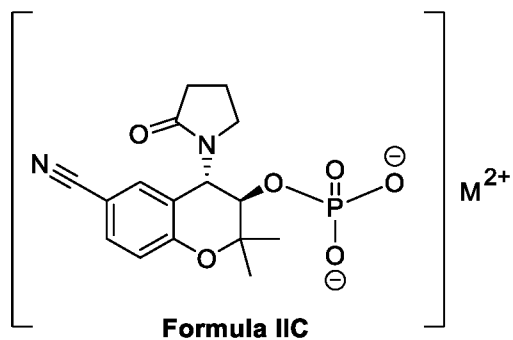
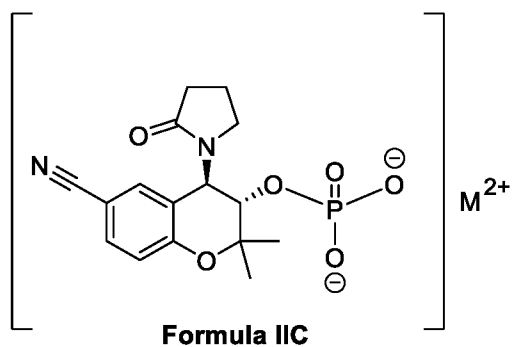
In one embodiment of Formula IIB, x is 3.

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In one embodiment of Formula IIB, x is 4.

In one embodiment of Formula IIB, x is 5.

Non-limiting examples of a compound of Formula IIC include:



In one embodiment of Formula IIC, x is 1.

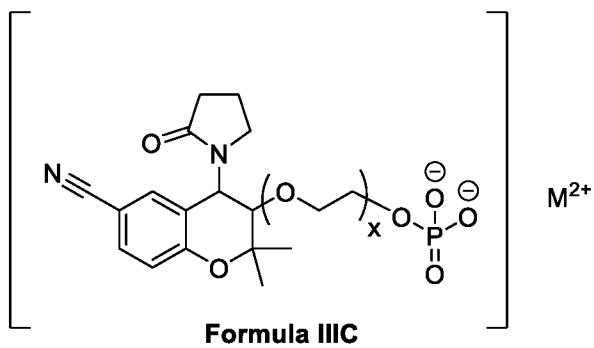
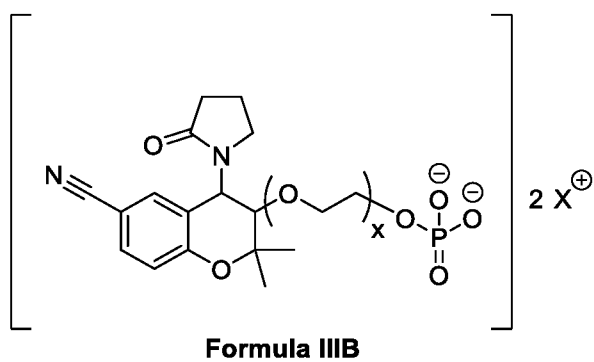
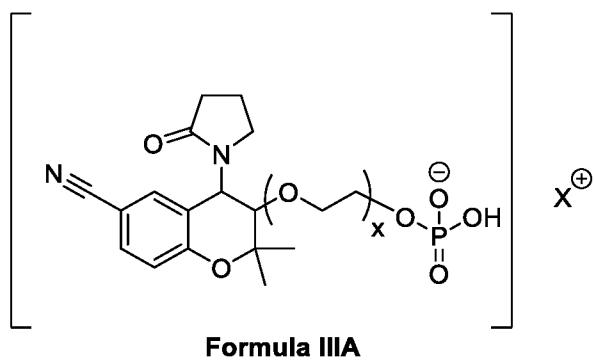
In one embodiment of Formula IIC, x is 2.

In one embodiment of Formula IIC, x is 3.

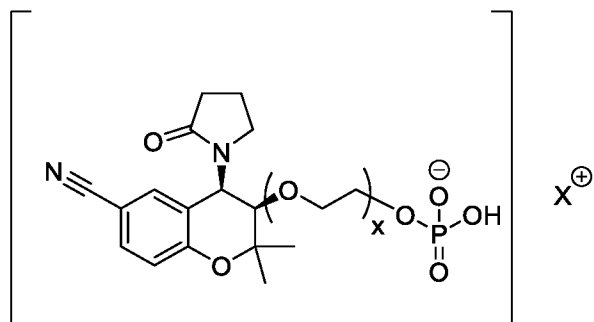
In one embodiment of Formula IIC, x is 4.

In one embodiment of Formula IIC, x is 5.

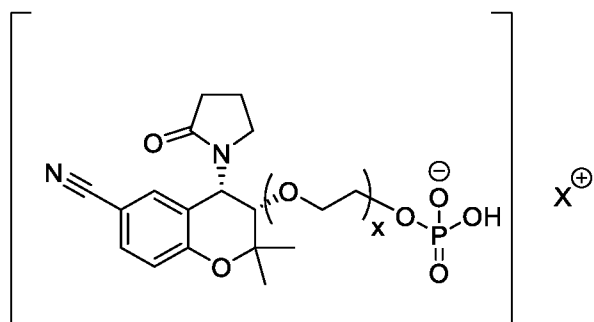
Pharmaceutically acceptable salt of Formula III include:



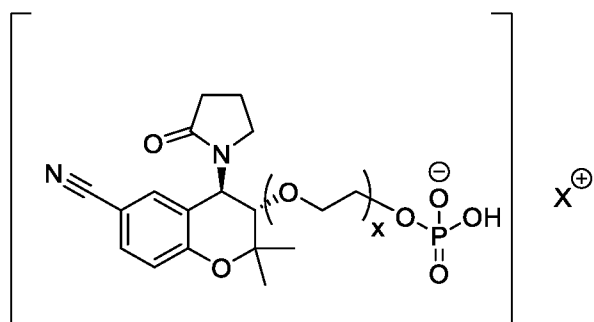
Non-limiting examples of a compound of Formula IIIA include:



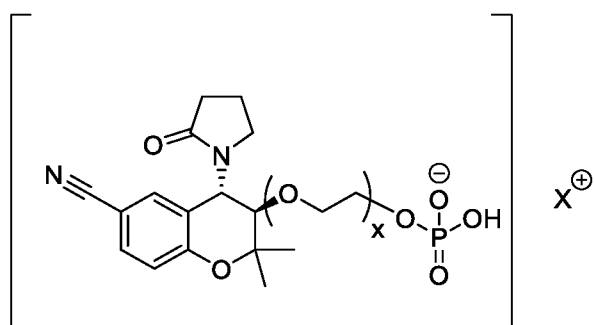
Formula IIIA



Formula IIIA



Formula IIIA



Formula IIIA

5

In one embodiment of Formula IIIA, x is 1.

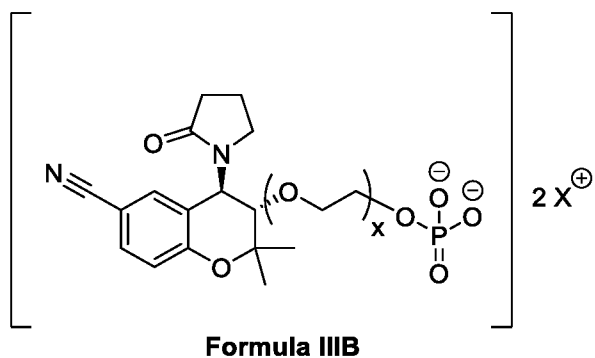
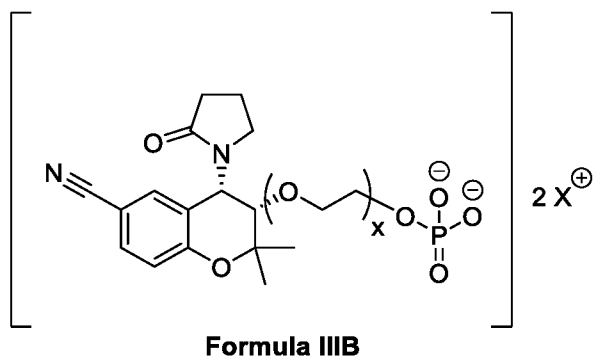
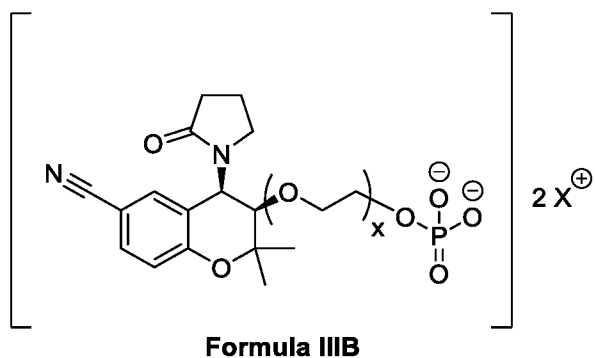
In one embodiment of Formula IIIA, x is 2.

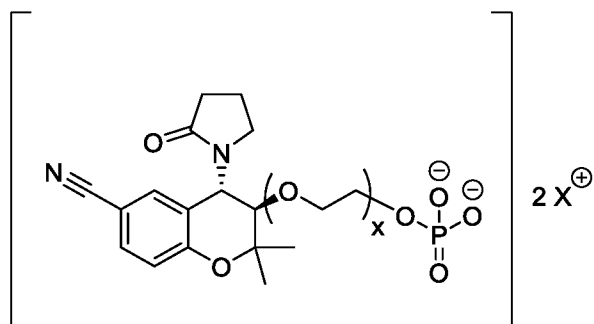
In one embodiment of Formula IIIA, x is 3.

In one embodiment of Formula IIIA, x is 4.

In one embodiment of Formula IIIA, x is 5.

5 Non-limiting examples of a compound of Formula IIIB include:



**Formula IIIB**

In one embodiment of Formula IIIB, x is 1.

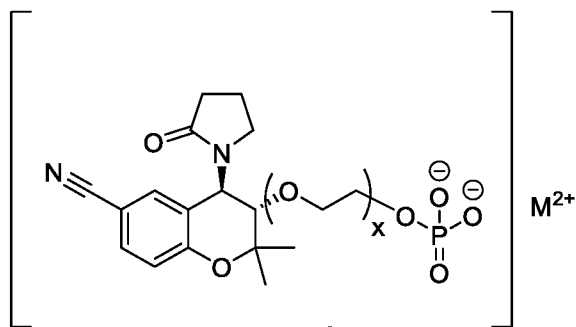
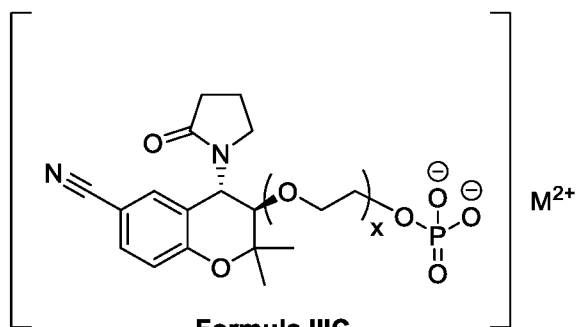
In one embodiment of Formula IIIB, x is 2.

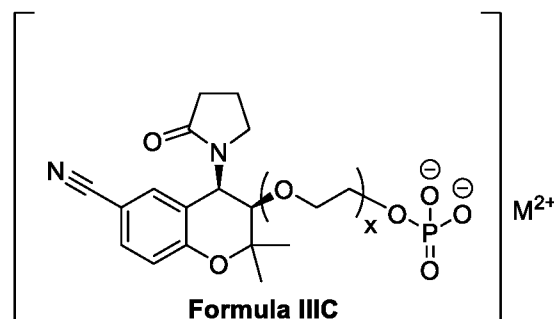
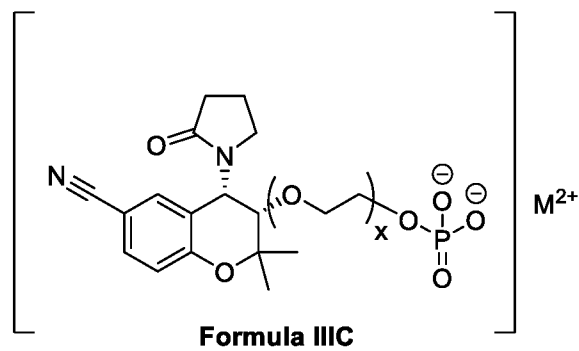
In one embodiment of Formula IIIB, x is 3.

5 In one embodiment of Formula IIIB, x is 4.

In one embodiment of Formula IIIB, x is 5.

Non-limiting examples of a compound of Formula IIIC include:

**Formula IIIC****Formula IIIC**



In one embodiment of Formula IIIC, x is 1.

5 In one embodiment of Formula IIIC, x is 2.

In one embodiment of Formula IIIC, x is 3.

In one embodiment of Formula IIIC, x is 4.

In one embodiment of Formula IIIC, x is 5.

10 It is part of the invention described herein that a selected pharmaceutically acceptable salt
such as described above is useful in medical treatments that are based on cromakalim or
levcromakalim activity. In general, a pharmaceutically acceptable salt can increase or decrease the
effectiveness or toxicity of a drug or can change its pharmacokinetics or its distribution in the body
through tissues. For example, one pharmaceutically acceptable salt may concentrate in an organ,
15 and another salt may concentrate in a different organ. As another example, increased water
solubility alone does not guarantee that a compound will penetrate the eye, reach the relevant site
of action, achieve sufficient *in vivo* concentrations, or have a beneficial pharmacologic effect. For
ocular topical dosing, drugs have to reside on the surface of the eye long enough to penetrate the
eye. This requires traversing multiple layers of the ocular surface, including the tear film, the

cornea, the conjunctiva, and the sclera, which all have varying degrees of hydrophilicity and hydrophobicity due to cell membranes, cell junctions, and the aqueous, lipid, and protein components of the tear film. Topical dosing is made more complicated by the constant renewing and washing of the ocular surface via the tear that in turn drain through the nasolacrimal (tear) ducts. For a compound to enter the eye, it must be able to penetrate before it is washed out.

An aspect of the present invention is that the disclosed pharmaceutically acceptable salts are able to achieve a useful pharmaceutical effect, and in particular can enter relevant tissues or chambers of the eye in an effective amount to achieve efficacy, for example, by entering into the anterior chamber, reaching the trabecular meshwork, into the vitreous humor, or reaching the retina. Therefore, another aspect of the present invention is that the compound itself or its pharmaceutically acceptable salts of Formulas I, II and III described herein, and in particular CKLP1, can be delivered through multiple tissues for topical or systemic delivery generally, as further disclosed herein, in a therapeutic amount in a manner that is consistent over a sufficient length of time to provide a pharmacologic effect on the target tissue to modify the disorder of interest.

II. Medical Uses of Compounds of Formulas I, II and III, and in particular CKLP1, or their pharmaceutically acceptable salts

The present invention provides new methods of use and compositions to deliver an effective amount of a cromakalim phosphate or other prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1 or its salt. The invention includes at least the following aspects.

A “patient” or “host” or “subject”, as used herein, is typically a human and the method is for human therapy. In appropriate circumstances, the scope may include a non-human animal in need of treatment or prevention of any of the disorders as specifically described herein, for example, a mammal, primate (other than human), cow, sheep, goat, horse, dog, cat, rabbit, rat, mice, bird or the like.

Long Term Therapy without Significant Tachyphylaxis or Tolerance

In one embodiment, the invention includes long term medical therapy, including ocular therapy (i.e., for at least 6 weeks, 7 weeks, or at least 2, 3, 4, 5, or 6 months or indefinitely for the duration of the therapy) using a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, in a manner that does not create significant tachyphylaxis (i.e., loss of activity over time) or tolerance, including but not limited to normal tension glaucoma. Tachyphylaxis is the decrease in response to a drug that occurs over time. It can occur after an initial dose or after a series of doses. Tolerance is the requirement to increase the dose of a drug to produce a given response.

The present invention provides a method for the use of a cromakalim prodrug of Formulas I, II or III or a pharmaceutically acceptable salt thereof, including CKLP1 or a salt thereof, for long-term therapy in a manner that does not induce significant tachyphylaxis or alternatively, tolerance. The loss of activity over time has been noted with a number of drugs, including for ocular therapy. For example, tachyphylaxis is a common effect of over-the-counter ocular allergy medications and is also observed using several drugs for other ophthalmic conditions, including glaucoma. Tachyphylaxis has a number of causes, including the increased or decreased expression of receptors or enzymes. This phenomenon has been noted in particular with beta adrenergic antagonists and with histamine.

The dose can be once a day or several times a day in the best judgement of the physician, and as further described herein. In one aspect, it is delivered as a topical drop for glaucoma, including normal tension glaucoma or for any form of high-pressure glaucoma, including as otherwise listed herein by example. It is advantageous to the patient to be able to take a stable dose of the drug over a lengthy period without having to change medications or dosage strength. While each patient is unique, and patients may exhibit different results based on their genetics or disease, in general, the long-term therapy using an effective amount of the cromakalim prodrug of Formulas I, II or III or a pharmaceutically acceptable salt thereof in a suitable delivery system for the disorder to be treated is achievable according to this invention.

Once Daily Dosing

In another embodiment, once-daily (QD) human dosing to treat elevated IOP glaucoma, including but not limited to primary open angle glaucoma (POAG), primary angle closure
5 glaucoma, pediatric glaucoma, pseudo-exfoliative glaucoma, pigmentary glaucoma, traumatic glaucoma, neovascular glaucoma, iridocorneal endothelial glaucoma (primary open angle glaucoma is also known as chronic open angle glaucoma, chronic simple glaucoma and glaucoma simplex) is provided. In an alternative embodiment, once-daily (QD) human dosing is used to treat
10 acute high-pressure glaucoma resulting from advanced cataracts. In a further alternative embodiment, once-daily (QD) human dosing is used to treat acute high-pressure glaucoma resulting from steroid induced glaucoma, uveitic glaucoma, or post-intravitreal injections. An aspect of the present invention is the ability to treat glaucoma with once-daily dosing in humans, without (or alternatively with) a controlled release formulation (for example, a gel or microparticle or nanoparticle). In a typical embodiment, it is administered without a controlled release
15 formulation, including for example, in a simple formulation such as phosphate buffered saline or citrate buffer, optionally with an ocular excipient, including but not limited to, mannitol or another osmotic agent.

Patient compliance and adherence are serious issues, and the fewer times per day that dosing is required, the more likely compliance is achieved. Once-a-day human dosing for
20 glaucoma is advantageous to maintain the ocular pressure in the desired range to minimize optic nerve damage, while also optimizing compliance and adherence. Many of the treatments for glaucoma must be used multiple times a day for effective therapy or must be formulated in a gel or controlled delivery material to achieve once a day dosing. However, the cromakalim prodrug of Formula I, II or III or its pharmaceutically acceptable salt thereof, including CKLP1, in the selected
25 effective dosage in certain embodiments can be administered once a day in a topical drop or other convenient manner.

Hyperemia

In yet another embodiment, ocular therapy using an effective amount of a cromakalim
30 prodrug of Formula I, II or III or its pharmaceutically acceptable salt thereof, including CKLP1,

that does not result in significant hyperemia is provided. Hyperemia is an excess and or prominence of blood in vessels supplying an organ. Ocular hyperemia, also called “red eye”, can include or result in vascular congestion, excessive vascular vasodilation, small bleeds, small punctate bleeds and/or micro hemorrhages. Ocular hyperemia can have a variety of causes, including but not limited to, exogenous irritants, contact lens, inflammation, vessel disruption, conjunctivitis (including infectious or allergic), trauma, endogenous ocular insults, subconjunctival hemorrhage, conjunctival hemorrhage, blepharitis, anterior uveitis, glaucoma, or irritating drugs and environmental irritants (i.e., sun and wind).

Certain ocular drugs either do not address hyperemia or actually cause hyperemia. According to the present invention, the use of a cromakalim prodrug of Formula I, II or III or its pharmaceutically acceptable salt thereof, including CKLP1, does not cause significant hyperemia in the patient when used during therapy, and in one embodiment, over long-term therapy as described herein. Significant hyperemia in one embodiment is that which causes enough discoloration or discomfort to the patient that the patient considers it an adverse effect of the treatment, which can, if significant enough, lead to poor compliance and even discontinuation of therapy. The present invention can result in an advance in the art by assisting patient compliance and comfort. In one embodiment, the administration of a compound of Formula I, Formula II, or Formula III does not significantly induce the expression of at least one protein independently selected from CD31 and VE-Cadherin.

In one embodiment, the administration of a compound of Formula I, Formula II, or Formula III does not significantly induce the expression of at least one protein independently selected from endothelin, fibronectin, α -SMA, phospho-eNOS, and total eNOS.

Another aspect of the present invention is the treatment of glaucoma associated with Sturge Weber Syndrome, which is a congenital disorder that affects the skin, neurological system and sometimes the eyes. It is sometimes referred to as a neurocutaneous disorder. Sturge Weber Syndrome can result in Sturge Weber Syndrome-induced glaucoma, which affects 30-70% of the patients with ocular improvement. Managing Sturge Weber Syndrome-induced glaucoma can be complex, and a number of patients need surgery or a drainage device. According to the invention, Sturge Weber Syndrome-induced glaucoma can be treated by administering an effective amount of a cromakalim prodrug of Formulas I, II or III or a pharmaceutically acceptable salt thereof,

including CKLP1, optionally in a pharmaceutically acceptable carrier, as described herein. The patient can remain on long-term therapy under the care of a physician.

Hypoglycemia, Hyperinsulinism, and Diabetes

5 Hypoglycemia is a condition caused by low levels of glucose in the blood. Glucose is the human body's main source of energy, and if the level of glucose in the blood is lower than what the body needs to support its energy demands, a number of symptoms occur. For example, the patients' blood sugar level may drop to 3.9 millimoles per liter or less. Initial symptoms of hypoglycemia include an irregular heart rhythm, fatigue, pale skin, shakiness, anxiety, sweating,
10 hunger, irritability, a tingling sensation around the mouth, and/or crying out during sleep. As sugar levels get even lower these symptoms worsen to include confusion, visual disturbances, seizures, and a loss of consciousness. If sugar levels drop too low, death may result.

Hypoglycemia can be caused by a disorder of the endocrine system where the body no longer naturally regulates blood sugar levels appropriately. Treatment with a cromakalim prodrug
15 or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, can help stabilize the endocrine system and thus reduce the onset or maintenance of hypoglycemia.

In one embodiment, the endocrine system abnormality causing hypoglycemia that is treated by an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is hyperinsulinism. Hyperinsulinism occurs when the body has an above
20 normal level of insulin in the blood, for example more than 175 picomoles per liter while fasting or more than 1600 picomoles per liter after eating. Insulin breaks down glucose so when its levels are too high hypoglycemia and the symptoms thereof may occur.

Diabetes is a condition in which a person's blood sugar level is too high. Diabetes is generally split into two types. Type 1 diabetes is a form of autoimmune disease which occurs when
25 the patient's immune system attacks and destroys insulin-producing cells in the pancreas leaving the patient with little or no natural insulin. In Type 2 diabetes, the patient's cells become resistant to insulin and the pancreas is unable to make enough insulin to overcome this resistance. Regardless of the type of diabetes, the possible symptoms include increased thirst, frequent urination, extreme hunger, unexplained weight loss, presence of ketones in urine, fatigue,
30 irritability, blurred vision, slow-healing sores, and frequent infections.

An aspect of the present invention is the ability to administer an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, to a patient in need thereof to treat diabetes. In one embodiment, the compound is used to treat Type 1 diabetes. In another embodiment the compound is used to treat Type 2 diabetes.

5 In one embodiment, the cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III is administered in an effective amount in a parenteral dosage form for the treatment of hypoglycemia, hyperinsulinism, or diabetes. In one embodiment, the prodrug of Formula I-III or pharmaceutically acceptable salt thereof is administered continuously throughout the day via an infusion and a pump. In an alternative embodiment, the prodrug of Formula I-III or pharmaceutically acceptable salt thereof is administered via an oral dosage form, such as a pill, tablet, or capsule. In one embodiment, the prodrug of Formula I-III or pharmaceutically acceptable salt thereof is administered at least once, twice, or three times a day.

10 In one embodiment, a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, is administered in combination or alternation with a treatment for diabetes, including metformin, sulfonylureas (glyburide (DiaBeta, Glynase), glipizide (Glucotrol) and glimepiride (Amaryl)), meglitinides (repaglinide (Prandin) and nateglinide (Starlix)), DPP-4 inhibitors (sitagliptin (Januvia), saxagliptin (Onglyza) and linagliptin (Tradjenta)), GLP-1 receptor agonists (Exenatide (Byetta, Bydureon), liraglutide (Victoza) and semaglutide (Ozempic)), SGLT2 inhibitors (canagliflozin (Invokana), dapagliflozin (Farxiga) and empagliflozin (Jardiance)), or insulin.

Skeletal Muscle Myopathy

25 Skeletal muscle myopathies (also known as myofibrillar myopathies) are disorders in which the skeletal muscle fibers contain defects that result in muscle weakness. For example, the muscle fibers may have defective sarcomeres, which are necessary for muscle contraction and are normally composed of rod-like structures called Z-disks. Z-disks link neighboring sarcomeres together to form myofibrils, the basic unit of muscle fibers. The defective sarcomeres may form clumps in the muscle fibers, significantly reducing muscle fiber strength.

30 An aspect of the present invention is the ability to administer an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including

CKLP1, to a patient in need thereof to treat a skeletal muscle myopathy. In one embodiment, an effective amount of the prodrug of Formula I-III is administered parenterally, orally, or topically for the treatment of a skeletal muscle myopathy. In one embodiment, the prodrug is administered intravenously. In one embodiment, the prodrug is administered in combination or alternation with
5 a corticosteroid drug (prednisone), immunosuppressant drugs (azathioprine, methotrexate, cyclosporine A, cyclophosphamide, mycophenolate mofetil, and tacrolimus), adrenocorticotrophic hormone or other biological therapeutics such as rituximab or tumor necrosis factor (TNF) inhibitors (infliximab or etanercept).

In one embodiment the patient has a mutation in the desmin (*DES*) gene. In another
10 embodiment, the patient has a mutation in the myotilin (*MYOT*) gene. In another embodiment, the patient has a mutation in the LIM-domain binding 3 (*LDB3*) gene. In another embodiment, the patient does not have a mutation in *DES*, *MYOT*, or *LDB3*.

In one embodiment, the myopathy is acquired. Acquired myopathies can be further subclassified as inflammatory myopathies, toxic myopathies, and myopathies associated with
15 systemic conditions. In one embodiment, the inflammatory myopathy is selected from polymyositis, dermatomyositis, and inclusion body myositis (IBM). Toxic myopathies are myopathies that are drug-induced and are a side effect observed with the use of cholesterol-lowering drugs, HIV therapy, antiviral therapy, rheumatologic agents, and antifungal agents (Valiyil *et al. Curr Rheumatol Rep.* 2010, 12, 213). Therefore, in one embodiment, an effective
20 amount of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, is administered for the treatment of a toxic myopathy induced by a medication. Non-limiting examples of medications that induce toxic myopathy include steroids, cholesterol-lowering medications (for example, statins, fibrates, niacin, and ezetimibe), propofol, amiodarone, colchicine, chloroquine, antivirals and protease inhibitors, omeprazole, and tryptophan.

25 In an alternative embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, is administered for the treatment of a myopathy associated with systemic conditions. Non-limiting examples of

systemic diseases include endocrine disorders, systemic inflammatory diseases, electrolyte imbalance, critical illness myopathy, and amyloid myopathy.

In one embodiment, the myopathy is inherited. Inherited myopathies can be further subclassified as muscular dystrophies, congenital myopathies, mitochondrial myopathies, and metabolic myopathies. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, is administered for the treatment of muscular dystrophy, including dystrophinopathy (Duchenne muscular dystrophy), myotonic dystrophy 1 and 2, facioscapulohumeral muscular dystrophy, oculopharyngeal muscular dystrophy, or limb girdle muscular dystrophy. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, is administered for the treatment of congenital myopathy, including nemaline myopathy or central core myopathy. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, is administered for the treatment of a metabolic myopathy, including acid maltase or acid alpha-1,4-glucosidase deficiency (Pompe's disease), glycogen storage disorders 3-11, carnitine deficiency, fatty acid oxidation defects, or carnitine palmitoyl transferase deficiency. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, is administered for the treatment of a mitochondrial myopathy, including Kearns-Sayre syndrome (KSS), mitochondrial DNA depletion syndrome (MDS), mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes (MELAS), maternally inherited deafness and diabetes (MIDD), mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), myoclonus epilepsy with ragged red fibers (MERRF), neuropathy ataxia, and retinitis pigmentosa (NARP), or Pearson syndrome.

Erectile Dysfunction and Female Sexual Arousal Disorder due to Blood Flow

Erectile dysfunction is a disorder characterized by a persistent difficulty having and/or maintaining an erection. Erectile dysfunction can be caused by a variety of factors including psychological, emotional, and physical problems. An aspect of the present invention is the administration of an effective amount of a cromakalim prodrug or its pharmaceutically acceptable salt of Formula I-III, including CKLP1, to a patient in need thereof to treat erectile dysfunction. In

one embodiment, the patient with erectile dysfunction has low blood flow to their pubic area. Therefore, in one aspect cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, or a pharmaceutically acceptable salt thereof increases blood flow to the pubic area.

5 Female sexual arousal disorder is a disorder characterized by a persistent difficulty having and/or maintaining sexual arousal. Female sexual arousal disorder can be caused by a variety of factors including psychological, emotional, and physical problems. An aspect of the present invention is the ability to administer an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, to a patient in need
10 thereof to treat Female sexual arousal disorder. In one embodiment, the patient with Female sexual arousal disorder has low blood flow to her pubic area. Therefore, in one embodiment, the cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, increases blood flow to the pubic area.

In one embodiment, the prodrug of Formula I-III is administered in an effective amount
15 orally as needed for the treatment of erectile dysfunction or Female sexual arousal disorder. In one embodiment, the prodrug can be administered topically in an effective amount as a cream, gel, or ointment, taken as needed, for the treatment of erectile dysfunction or Female sexual arousal disorder. In certain embodiments the prodrug of Formula I-III, for example CKLP1, is formulated as an active agent in a lubricant for treatment of erectile dysfunction and/or Female sexual arousal
20 disorder.

In certain embodiments, the cromakalim prodrug of Formula I-III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered in an effective amount in combination or alternation with one or more additional treatments for erectile dysfunction, including but not limited to a phosphodiesterase inhibitor (for example, sildenafil, dildenafil citrate, vardenafil, vardenafil HCl, tadalafil, avanafil), testosterone therapy, a penile injection (for example, ICI or
25 intracavernosal alprostadil), intraurethral medication (for example, IU or alprostadil), penile implants, a combination of therapies (for example, bimix or trimix) or surgery.

In certain embodiments, an effective amount of the compound of Formula I-III or its pharmaceutically acceptable salt, for example CKLP1, is administered in combination with one or
30 more additional treatments for Female sexual arousal disorder, including but not limited to

estrogen therapy, an estrogen receptor modulator (for example, ospemifene), androgen therapy, an antidepressant (for example, flibanserin), or melanocortin agonist (for example, bremelanotide).

Hypotrichosis and Baldness

5 Hypotrichosis of the eyebrows and eyelashes is a disorder in which there is little to no growth of hair, or an insufficient amount of hair, on the eyebrows and/or eyelashes at the edge of eyelids.

An aspect of the present invention is the ability to administer an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including
10 CKLP1, to a patient in need thereof to treat hypotrichosis. In one embodiment the patient has a genetic mutation that causes hypotrichosis. In another embodiment the patient does not have a genetic mutation that causes hypotrichosis.

In one embodiment, the prodrug of Formula I-III is administered as a topical dosage form applied to the upper eyelid margin at the base of the eyelashes. In one embodiment, the prodrug
15 is administered at least once a day or twice a day.

In certain embodiments, the compound of the present invention is provided in an effective amount in combination or alternation with a prostaglandin analog (for example, bimatoprost).

Baldness is hair loss or the absence of hair, most typically on the scalp. Common types of baldness include male or female pattern baldness, alopecia areata, telogen effluvium (the loss of
20 hair after a stressful situation), and anagen effluvium (abnormal hair loss during the first phase of the hair growth cycle). In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, is administered to a patient in need thereof to treat baldness. In one embodiment, the baldness is male or female pattern baldness. In one embodiment, the baldness is alopecia areata. In one embodiment, the baldness is
25 telogen effluvium. In one embodiment, the baldness is anagen effluvium.

Neuropathic Pain and Neurodegenerative diseases (for example Parkinson's disease and Huntington's disease)

Neuropathic pain is a disorder in which nerve damage or a malfunctioning nervous system
30 causes shooting or burning pain. Neuropathic pain can be acute or chronic and may be caused by

a variety of factors including alcoholism, amputation, chemotherapy, diabetes, facial nerve problems, AIDS, multiple myeloma, multiple sclerosis, nerve or spinal cord compression, herniated disk, arthritis, shingles, spine surgery, syphilis, or thyroid problems. Patients with neuropathic pain may experience a shooting and burning pain or a tingling or numbness sensation.

5 An aspect of the present invention is the ability to administer an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, to a patient in need thereof to treat neuropathic pain.

10 In one embodiment, an effective amount of the compound of Formula I-III or its pharmaceutically acceptable salt is administered orally, enterally, or parenterally for the treatment of neuropathic pain. The prodrug can be administered once, twice, or three times a day according to the instructions of the healthcare provider, for as long as necessary.

15 In one embodiment, for the treatment of neuropathic pain, an effective amount of a compound of Formula I-III or its pharmaceutically acceptable salt is used in combination or alternation with a calcium channel $\alpha 2$ -delta ligand (for example, pregabalin or gabapentin), a tricyclic antidepressant (for example, amitriptyline, nortriptyline, or desipramine), an SNRI antidepressant (for example, duloxetine or venlafaxine), or an opioid (for example, tramadol or tapentadol).

20 Neurodegenerative diseases are those that cause or result from the degeneration of the patient's nerves. This cellular process includes a neuroinflammatory reaction that involves the activation of glial cells, including microglia and astrocytes. A neurodegenerative disease may make it difficult for the patient to balance, move, talk, breathe, or remember. Neurodegenerative diseases include amyotrophic lateral sclerosis (ALS), Friedreich's ataxia, Huntington's disease, Lewy body disease, Parkinson's disease, and spinal muscular atrophy.

25 An aspect of the present invention is the administration of an effective amount of a compound of the present invention, for example CKLP1, or its pharmaceutically acceptable salt to a patient in need thereof to treat a neurodegenerative disease. In one embodiment the neurodegenerative disease is Parkinson's disease. In another embodiment the neurodegenerative disease is Huntington's disease. In an alternative embodiment the neurodegenerative disease is Alzheimer's disease.

The therapy for a neurodegenerative disease includes combination or alternation therapy with an effective amount of a compound as disclosed herein. Drugs for Parkinson's disease include amantadine, nilotinib, zonisamide, selegiline, methylphenidate, and salbutamol. Drugs for Huntington's disease include tetrabenazine, tiapride, clozapine, olanzapine, risperidone, 5 quetiapine, and memantine. Drugs for amyotrophic lateral sclerosis (ALS) include mastinib, dolutegravir, abacavir, lamivudine, retigabine, and tamoxifen. Drugs for Lewy body disease include donepezil, galantamine, and rivastigmine. Drugs for spinal muscular atrophy include Nusinersen and Onasemnogene abeparvovec.

Following ischemia, stroke, convulsions, or trauma, neuroprotective drugs are often 10 administered to prevent damage to the brain and/or spinal cord. In one embodiment, an effective amount of a compound of Formula I-III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered as a neuroprotective agent. In one embodiment, the compound is administered following ischemia, stroke, convulsions, or trauma. In one embodiment, an effective amount of a compound of Formula I-III or its pharmaceutically acceptable salt thereof, including 15 CKLP1, is administered as a cellular protective agent.

Tumor Hypoperfusion and Hypoxia

In one aspect an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered to a patient to treat tumor 20 hypoperfusion or tumor hypoxia. Tumor hypoperfusion refers to reduced blood flow in the tumor. Tumor hypoxia refers to a reduced level of oxygen in the tumor cells. There can be overlap between the two.

When a tumor is in a state of hypoperfusion, perhaps because it is growing quickly, it does not have sufficient blood flow to allow tumor therapeutics to have access to the tumor cells. This 25 can create resistance to chemotherapeutic treatment. In one embodiment, a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III or a pharmaceutically acceptable salt thereof, including CKLP1, is administered to a patient with hypoperfusion of a tumor so that the tumor is more easily treated with anti-tumor medication such as chemotherapy.

In another embodiment, the cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered to a patient with hypoperfusion of non-tumor cells, for example as a result of trauma.

When a tumor is hypoxic, it is in a low oxygen state due to the lack of oxygen in the cell.

5 Tumors that are hypoxic can be more likely to exhibit metastatic behavior. Therefore, in one aspect, a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, is administered in an effective amount to a patient to treat tumor hypoxia, optionally in combination or alternation with chemotherapy or other anti-tumor treatment.

10 In another embodiment, an effective amount of the compound of the present invention or its pharmaceutically acceptable salt is administered to treat hypoxia or hypoperfusion optionally in combination with a vascular endothelial growth factor (VEFG) therapy.

In an alternative embodiment, an effective amount of the compound of Formula I-III or its pharmaceutically acceptable salt is used in combination or alternation with oxygen therapy (for example, an oxygen mask or a small tube clipped under the nose to provide supplemental oxygen)
15 or an asthma medication (for example, fluticasone, budesonide, mometasone, beclomethasone, ciclesonide, montelukast, zafirlukast, zileuton, salmeterol, formoterol, vilanterol, albuterol, levalbuterol, prednisone, methylprednisone, omalizumab, mepolizumab, benralizumab, or reslizumab).

20 *Selected Cardiovascular Disorders*

Unstable angina is a condition in which the heart does not get enough blood and oxygen from the narrowing of coronary arteries, causing unexpected chest pain and discomfort. The most common cause of the condition is coronary artery disease due to atherosclerosis. Angina can be treated with angioplasty and stent placement or enhanced external counterpulsation. Several
25 medications can also improve symptoms and these include aspirin, nitrates, beta blockers, statins, and calcium channel blockers. Many of these drugs have unwanted side effects. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, is administered to a patient with unstable angina and the associated chest pains.

In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, for example CKLP1, is administered in combination with angioplasty, stent placement, and/or enhanced external counterpulsation. In another embodiment, a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, for example
5 CKLP1, is administered in combination or alternation with aspirin, nitrate, a beta blocker, a statin, or a calcium channel blocker.

Congestive heart failure (CHF) is a chronic progressive condition in which the ventricles of the heart are not capable of pumping enough blood volume to the rest of the body. The most typical form of CHF is left-sided CHF where the left ventricle does not properly pump blood, and
10 this often progresses to the right-side. The four stages of CHF are indicative of the severity of the disease and also determine various treatment options. If left untreated, blood and other fluids can back up inside the lungs, abdomen, liver, and the lower body and can be life-threatening. Medications for CHF include ACE inhibitors, beta-blockers, and diuretics. Each of these medications have associated side effects. For example, ACE inhibitors have the potential to raise
15 potassium levels in the blood and cannot be tolerated in some patients. For this reason, in one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, is administered to a patient with CHF. The heart failure may be in Stage 1, Stage 2, Stage 3, or Stage 4.

Chronic or acute myocardial ischemia is the inability of blood flow to reach the heart,
20 which prevents the heart from receiving enough oxygen. Myocardial ischemia can be caused by atherosclerosis, a blood clot, or a coronary artery spasm. Myocardial ischemia can cause serious abnormal heart rhythms or even lead to a heart attack. Current treatment for myocardial ischemia may include the administration of an aspirin, nitrate, beta-blocker, ACE inhibitor, or cholesterol-lowering medication, each of which has side effects and efficacies of various degrees. In one
25 embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered to a patient with chronic or acute myocardial ischemia.

Microvascular dysfunction (or coronary microvascular disease) is a type of non-obstructive coronary artery disease that causes the small blood vessels feeding the heart muscle to not work.
30 Patients with microvascular dysfunction do not have plaque buildup in the coronary artery blood

vessels, but have damage to the inner walls of the blood vessels that can lead to spasms and decrease blood flow to the heart muscle. In an alternative embodiment of the invention, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is provided in an effective amount for the treatment of microvascular dysfunction.

5 Coronary artery disease is the buildup of plaque in the walls of coronary arteries, vessels that supply the heart with blood. This plaque narrows the arteries, slowing blood flow, and if a piece of plaque breaks off and lodges in an artery, it can block blood flow completely. The blockage of blood flow to the heart by a plaque and/or blood clot is referred to as acute myocardial infarction, often referred to as a heart attack. Symptoms vary, but often include pressure or
10 tightness in the chest and arms, shortness of breath, and/or sudden dizziness. Emergency medical assistance is typically required. The patient may be administered one or a variety of drugs, including aspirin, a thrombolytic, an antiplatelet agent, a blood-thinning medication, a nitroglycerin, a beta blocker, ACE inhibitor, or statin. Potential surgical procedures include angioplasty or bypass surgery. Following a heart attack, cardiac rehabilitation is required that
15 includes medication to prevent another heart attack and subsequent complications.

 Given the life-threatening nature of a heart attack, it is advantageous to have a number of potential therapeutic agents as possible treatment options. Therefore, in one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered to a patient experiencing a heart attack and/or as a therapy in
20 cardiac rehabilitation. The drug is administered for a time period determined by the health care provider, including but not limited to at least two weeks, one month, two months, three months, or more. In one embodiment, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, acts as a cardioprotective agent during the heart attack. In one embodiment, an effective amount of the cromakalim prodrug or a pharmaceutically acceptable salt
25 of Formula I-III, including CKLP1, is used as a cardioprotective agent in a host undergoing heart surgery. In one embodiment, the host is undergoing a cauterization procedure. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, is administered for the treatment of left ventricular failure after an acute myocardial infarction (AMI) or heart attack. In an alternative embodiment of the present
30 invention, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt

thereof of Formula I-III, including CKLP1, is administered for the treatment of coronary artery disease.

In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, for example CKLP1, is administered in combination or
5 alternation with an ACE inhibitor, beta-blocker, aspirin, nitrate, a cholesterol-lowering medication, statin, or diuretic.

In one embodiment, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is provided in an effective amount for the preservation of the heart prior to organ donation.

10 Arrhythmia is the improper (too fast, too slow, or irregular) beating of the heart, which can be caused by a variety of medical conditions, including coronary artery disease, high blood pressure, electrolyte imbalances, or injury from a heart attack. Arrhythmia is very common, affecting 3 million people in the US every year. The majority of arrhythmia may be harmless, however very abnormal arrhythmia can cause serious or fatal symptoms. If left untreated,
15 arrhythmia can affect the heart, the brain, and other organs because not enough blood is able to reach the organs. Implantable devices for the treatment of arrhythmias include a pacemaker or an implantable cardioverter-defibrillator (ICD). In one embodiment, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered to a patient with arrhythmia. In one embodiment, a cromakalim prodrug or a pharmaceutically acceptable salt
20 of Formula I-III, including CKLP1, is provided in an amount effective to treat or prevent arrhythmias and/or ventricular fibrillation associated with AMI in a host in need thereof.

In one embodiment, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, for example CKLP1, is administered in combination with a pacemaker or ICD.

25 The endothelial layer is a layer of cells lining all blood vessels and is responsible for proper dilation and constriction of blood vessels. Endothelial tone is the balance between constriction and dilation and largely determines a person's blood pressure. Endothelial dysfunction is the failure of the endothelial layer to regulate dilation/constriction. Endothelial dysfunction is a well-established response to cardiovascular risk factors and in turn, often precedes the development of atherosclerosis. Treatments include ACE inhibitors and statin drugs, but studies for additional
30 drugs are underway. In one embodiment, an effective amount of a cromakalim prodrug or a

pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, is administered to a patient with endothelial dysfunction.

A transient ischemic attack (TIA) is similar to a stroke, but only lasts a few minutes and leaves no permanent damage. Like a stroke, a clot in the blood supply travels to the brain. The signs of a TIA include weakness, numbness, paralysis, slurred speech, dizziness, blindness, and/or a sudden, severe headache. Following a diagnosis of a TIA, it is important to try to prevent another TIA or a stroke. Typical medications include anti-platelet drugs, anticoagulants, and thrombolytic agents. Alternatively, angioplasty is often recommended. Anti-platelet drugs and anticoagulants have to be taken with caution since they increase the risk of bleeding. For this reason, vasodilators represent an alternative medication for TIA. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered to a patient diagnosed with a transient ischemic attack.

Carotid artery disease is the buildup of plaque in the carotid arteries that run along either side of the neck and supply blood to the brain, face, and neck. If a piece of plaque breaks off and causes a clot in a blood vessel leading to the brain, the clot can cause a stroke. In an alternative embodiment of the invention, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered to a patient diagnosed with a stroke.

In one embodiment, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, for example CKLP1, is administered in combination or alternation with an anti-platelet drug, anticoagulant, or thrombolytic agent.

High blood pressure is a condition where the force of blood flowing through the blood vessels is consistently high. This can often lead to many conditions, including heart conditions discussed herein and stroke. In an alternative embodiment of the invention, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered to a patient with high blood pressure as a treatment to lower blood pressure.

Blood Vessel Disorders

Raynaud's disease is a rare disorder of blood vessels in which fingers and toes feel numb in response to cold temperature or stress. This can induce a color change (usually white and then

blue) of fingers and toes accompanied by a feeling of numbness. This is caused by arteries in fingers and toes undergoing vasospasms when exposed to cold or stress and this then narrows vessels and temporarily limits blood supply. In one embodiment an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered to a patient for the treatment of Raynaud's disease, which may be via topical, enteric or parenteral delivery.

Peripheral artery disease (PAD) is a disease in which plaque builds up in arteries that carry blood to limbs, the heart, and other organs. This causes narrowed arteries that reduce blood flow from the heart. PAD can cause an embolism or thrombosis, which can lead to acute limb disease. Acute limb disease is treatable, but if left untreated (a delay of 6-12 hours), it can result in amputation and/or death. Symptoms include pain, pallor, and/or paralysis. In one embodiment, an effective amount of a CKLP1 prodrug or a pharmaceutically acceptable salt thereof is administered for the treatment of acute limb ischemia.

Chronic limb ischemia is a type of advanced PAD that develops over time and includes muscular pain, patellofemoral pain, and eventual tissue loss due to poor perfusion and hypoxia. Chronic limb ischemia is associated with diabetes, smoking, and high blood pressure. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered for the treatment of chronic limb ischemia.

Thrombophlebitis is when a blood clot forms in a vein and slows down the blood flow in the vein. It most often affects legs but can also happen in arms or other veins in the body. Thrombophlebitis can happen right under the skin or deeper in legs or arms. Types of thrombophlebitis include superficial phlebitis or superficial thrombophlebitis that occur just below the surface of the skin; deep vein thrombosis (DVT) that occurs deep in the body; and, migratory thrombophlebitis (Trousseau's syndrome or thrombophlebitis migrans), which is when a clot comes back in a different part of the body. In an alternative embodiment of the invention, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered for the treatment of thrombophlebitis. In one embodiment, the

thrombophlebitis is superficial thrombophlebitis. In one embodiment, the thrombophlebitis is deep vein thrombosis. In one embodiment, the thrombophlebitis is migratory thrombophlebitis.

Chronic venous insufficiency (CVI) is a condition that occurs when the venous wall and/or valves in the leg veins are not working effectively, making it difficult for blood to return to the heart from the legs. CVI causes blood to “pool” or collect in these veins, and this pooling is called stasis. If CVI is not treated, the pressure and swelling increases until the tiniest blood vessels in the legs (capillaries) burst. When this happens, the overlying skin takes on a reddish-brown color and is very sensitive to being broken if bumped or scratched. In an alternative embodiment of the invention, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered for the treatment of chronic venous insufficiency.

Pulmonary arterial hypertension (PAH) is a rare disease that usually presents in young adulthood, predominantly in women. PAH is a progressive disorder of the pulmonary arteries leading to the lungs and is fatal despite currently available therapies. In one embodiment an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered to a patient for the treatment of pulmonary arterial hypertension. In one embodiment, cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III is administered in combination with a PDE-5 inhibitor (for example, sildenafil or tadalafil), a prostanoid vasodilators (for example, epoprostenol, treprostinil, or iloprost), a guanylate cyclase stimulators (for example, riociguat), or an endothelin receptor antagonist (for example, bosentan, ambrisentan, or macitentan).

Aspects of the invention include administering the drug as described herein in combination or alternation with a calcium channel blocker (for example nifedipine, amlodipine, felodipine, bepridil, diltiazem, nicardipine, nisoldipine, verapamil and isradipine) or another vasodilator (for example hydralazine, nitroglycerin, alprostadil, riociguat, nesiritide, nitroprusside, sildenafil, and minoxidil).

Lymphatic Diseases

The lymphatic system acts to rid the bodies of toxins and waste and its primary role is to transport lymph, a fluid containing white blood cells, throughout the body to fight infection. The system is primarily composed of lymphatic vessels that are connected to lymph nodes, which filter

lymph. K_{ATP} channels are expressed by lymphatic muscle cells and studies have shown that certain K_{ATP} channel openers dilate lymphatic vessels.

For example, as discussed in a recent study by Garner *et al.* (“KATP Channel Openers Inhibit Lymphatic Contractions and Lymph Flow as a Possible Mechanism of Peripheral Edema”,
5 *Journal of Pharmacology and Experimental Therapeutics*, October 25, 2020) rhythmic contractions of isolated rat mesenteric lymph vessels are progressively impaired when exposed to K_{ATP} channel openers, such as cromakalim, minoxidil sulfate, and diazoxide. Increasing concentrations of cromakalim ultimately abolished the contractions of the vessels and impaired flow through the vessels by attenuating the frequency and amplitude of the contractions. Similar
10 effects were observed with minoxidil sulfate and diazoxide when administered at clinically relevant concentrations.

Inflammation of the lymph vessels is known as lymphangitis and symptoms generally include swelling, redness, and/or pain in the infected area. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1,
15 is administered for the treatment of lymphangitis.

The lymph nodes can also become infected with a virus, bacteria, and/ or fungi and this is referred to as lymphadenitis. Symptoms of lymphadenitis also include redness or swelling around the lymph nodes. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered for the
20 treatment of lymphangitis, and in one embodiment, the cromakalim prodrug of Formula I-Formula III is administered in combination with an antibiotic or antifungal medication.

A common cancer of the lymph system is Hodgkin’s lymphoma, in which cancer originates from the white blood cells called lymphocytes. The cancer can begin in any part of the body and symptoms include non-painful enlarged lymph nodes in the neck, under the arm, or in the groin.
25 There are two major types of Hodgkin lymphoma: classical Hodgkin lymphoma and nodular lymphocyte-predominant Hodgkin lymphoma. Treatment for Hodgkin’s lymphoma includes chemotherapy and/or radiation, and the most common treatment is the monoclonal antibody rituximab (Rituxan). In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered for the

treatment of Hodgkin's lymphoma, in combination with chemotherapy and/or radiation. In one embodiment, the chemotherapy is rituximab.

Non-Hodgkin's lymphoma is caused when the body produces too many abnormal white blood cells called lymphocytes, which leads to tumors. A common subtype of Non-Hodgkin's lymphoma is B-Cell Non-Hodgkin's lymphoma. Symptoms include swollen lymph nodes, fever, and/or chest pain. Non-Hodgkin's lymphoma is treated with chemotherapy and/or radiation. A common treatment is a regimen known as R-CHOP that consists of cyclophosphamide, doxorubicin, vincristine, and prednisone, plus the monoclonal antibody rituximab (Rituxan). In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered for the treatment of Non-Hodgkin's lymphoma, in combination with chemotherapy and/or radiation. In one embodiment, the chemotherapy consists of cyclophosphamide, doxorubicin, vincristine, prednisone, and rituximab.

Castleman's disease is a group of lymphoproliferative disorders characterized by lymph node enlargement and there are at least three distinct subtypes: unicentric Castleman disease (UCD), human herpesvirus 8 associated multicentric Castleman disease (HHV-8-associated MCD), and idiopathic multicentric Castleman disease (iMCD). In UCD, enlarged lymph nodes are present in a single region and in iMCD, enlarged lymph nodes are present in multiple regions. HHV-8-Associated MCD is similar to iMCD in that enlarged lymph nodes are present in multiple regions, but the patient is also infected with human herpesvirus 8.

In one embodiment, an effective amount of a cromakalim prodrug of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of Castleman's disease, including unicentric Castleman disease (UCD), human herpesvirus 8 associated multicentric Castleman disease (HHV-8-associated MCD), and idiopathic multicentric Castleman disease (iMCD).

Lymphangiomatosis is a disease where cysts and/or lesions are formed from lymphatic vessels. The masses are not present in one single localized mass, but are widespread. It is a multi-system disorder where abnormally proliferating lymphatic channels expand and infiltrate surrounding tissues, bones, and organs. It is a rare disease that is most widespread in children and teenagers. There is no standard treatment and often treatments are only aimed at reducing symptoms. In one embodiment, an effective amount of a compound of Formula I-Formula III or

its pharmaceutically acceptable salt is administered for the treatment or the reduction of symptoms associated with lymphangiomatosis.

Lymphangiectasia, also known as "lymphangiectasis", is a pathologic dilation of lymph vessels. When it occurs in the intestines, it causes a disease known as "intestinal lymphangiectasia" that is characterized by lymphatic vessel dilation, chronic diarrhea, and loss of proteins such as serum albumin and globulin. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment or the reduction of symptoms associated with lymphangiectasia.

The eye is unique in that certain parts of the eye are lymphatic rich, while other parts of the eye are devoid of lymphatics. Parts of the eye, including the eyelids, lacrimal glands, conjunctiva, limbus, optic nerve sheath, extraocular muscles, connective tissues of the extraocular muscle cones, are lymphatic rich, while the cornea and retina are lymphatic-free. A number of lymphatic disorders have been identified in the eye. Ocular lymphatic disorders include, but are not limited to, conjunctival myxoma, dry eye, conjunctival lymphangiectasia, chemosis, mustard gas keratitis, corneal inflammation, orbital cellulitis, chalazion, dermatochalasis, and blepharochalasis. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of an ocular lymphatic disorders. In one embodiment, the ocular lymphatic disorder is selected from conjunctival myxoma, dry eye, conjunctival lymphangiectasia, chemosis, mustard gas keratitis, corneal inflammation, orbital cellulitis, chalazion, dermatochalasis, and blepharochalasis.

There is also evidence that lymphatic vessels, but not angiogenic vessels, are important for immune rejection after corneal transplantation (T. Dietrich et al., Journal of Immunology, 2010, 184, 2, 535–539). Therefore, in one embodiment, a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered following a corneal transplant to reduce the risk of immune rejection.

Mitochondrial Disorders

Mitochondrial diseases are long-term, genetic, and often inherited. The diseases are a clinically heterogeneous group of disorders that result from a dysfunction in the mitochondrial

respiratory chain. The mitochondrial respiratory chain is the essential final common pathway for aerobic metabolism, and tissues and organs that are highly dependent on aerobic metabolism are preferentially involved in mitochondrial disorders. While some mitochondrial disorders only affect a single organ, many involve multiple organ systems and often present with prominent neurologic and myopathic features. Mitochondria contain a potassium specific channel (mitoKATP channel) sensitive to ATP. The mitochondrial KATP channel plays an important role in the mitochondrial volume control and in regulation of the components of protonmotive force.

Mitochondria are unique in that they have their own DNA called mitochondrial DNA, or mtDNA. Mutations in this mtDNA or mutations in nuclear DNA (DNA found in the nucleus of a cell) can cause mitochondrial disorder. Environmental toxins can also trigger mitochondrial disease. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of a mitochondrial disorder.

Inside the mitochondrion is a group of proteins that carry electrons along four chain reactions (Complexes I-IV), resulting in energy production. This chain is known as the Electron Transport Chain. A fifth group (Complex V) churns out the ATP. Together, the electron transport chain and the ATP synthase form the respiratory chain and the process is known as oxidative phosphorylation or OXPHOS. Complex I, the first step in this chain, is the most common site for mitochondrial abnormalities, representing as much as one third of the respiratory chain deficiencies. Often presenting at birth or in early childhood, a Complex I deficiency is usually a progressive neuro-degenerative disorder and is responsible for a variety of clinical symptoms, particularly in organs and tissues that require high energy levels, such as brain, heart, liver, and skeletal muscles. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of a Complex I deficiency.

A number of specific mitochondrial disorders have been associated with Complex I deficiency including Leber's hereditary optic neuropathy, mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged red fibers (MERRF), and Leigh Syndrome.

Mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes (MELAS) is a progressive neurodegenerative disorder with typical onset between the ages of two and fifteen, although it may occur in infancy or as late as adulthood. Initial symptoms may include stroke-like episodes, seizures, migraine headaches, and recurrent vomiting. The stroke-like episodes, often accompanied by seizures, are the hallmark symptom of MELAS and cause partial paralysis, loss of vision, and focal neurological defects. The gradual cumulative effects of these episodes often result in the variable combinations of loss of motor skills (speech, movement, and eating), impaired sensation (vision loss and loss of body sensations), and mental impairment (dementia). MELAS patients may also suffer additional symptoms including muscle weakness, peripheral nerve dysfunction, diabetes, hearing loss, cardiac and kidney problems, and digestive abnormalities. Lactic acid usually accumulates at high levels in the blood, cerebrospinal fluid, or both. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of mitochondrial encephalomyopathy lactic acidosis and stroke-like episodes (MELAS).

Myoclonic epilepsy with ragged red fibers (MERRF) is a multisystem disorder characterized by myoclonus, which is often the first symptom, followed by generalized epilepsy, ataxia, weakness, and dementia. Symptoms usually first appear in childhood or adolescence after normal early development. In over 80% of cases, MERRF is caused by mutations in the mitochondrial gene called *MT-TK*. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of myoclonic epilepsy with ragged red fibers (MERRF).

Leigh syndrome is a rare, inherited neurodegenerative condition. It usually becomes apparent in infancy, often after a viral infection, and symptoms usually progress rapidly. Early symptoms may include poor sucking ability, loss of head control and motor skills, loss of appetite, vomiting, and, seizures. As the condition progresses, symptoms may include weakness and lack of muscle tone, spasticity, movement disorders, cerebellar ataxia, and, peripheral neuropathy. Leigh syndrome can be due to mutations in either mitochondrial DNA or nuclear DNA. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of Leigh syndrome.

Complex II deficiency, which can vary greatly from severe life-threatening symptoms in infancy to muscle disease beginning in adulthood, can be caused by mutations in the *SDHA*, *SDHB*, *SDHD*, or *SDHAF1* genes. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of Complex II deficiency.

Complex III deficiency is a severe, multisystem disorder that includes features such as lactic acidosis, hypotonia, hypoglycemia, failure to thrive, encephalopathy, and delayed psychomotor development. Involvement of internal organs, including liver disease and renal tubulopathy, may also occur. It is generally caused by mutations in nuclear DNA in the *BCS1L*, *UQCRLB* and *UQCRLQ* genes and inherited in an autosomal recessive manner. However, it may also be caused by mutations in mitochondrial DNA in the *MT-CYB* gene, which is passed down maternally or occurs sporadically and may result in a milder form of the condition. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of Complex III deficiency.

Complex IV deficiency, also known as Cytochrome C oxidase deficiency (COX deficiency), is a condition that can affect several parts of the body including the skeletal muscles, heart, brain and liver. There are four types of COX deficiency differentiated by symptoms and age of onset: benign infantile mitochondrial type, French-Canadian type, infantile mitochondrial myopathy type, and Leigh syndrome. Complex IV deficiency is caused by mutations in any of at least 14 genes and the inheritance pattern depends on the gene involved. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of Complex IV deficiency.

There are many other types of mitochondrial diseases. For example, dominant optic atrophy (DOA) is an inherited optic nerve disorder characterized by degeneration of the optic nerves that typically starts during the first decade of life. Affected people usually develop moderate visual loss and color vision defects. The severity varies and visual acuity can range from normal to legal blindness. Autosomal dominant optic atrophy plus syndrome (ADOA plus) is a rare syndrome that causes vision loss, hearing loss, and symptoms affecting the muscles. The syndrome is associated with optic atrophy. Other symptoms of ADOA plus include sensorineural hearing

loss and symptoms affecting the muscles such as muscle pain and weakness. ADOA plus is caused by mutations in the *OPA1* gene. Both DOA and ADOA are inherited in an autosomal dominant manner. In certain embodiments, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of dominant optic atrophy (DOA) or autosomal dominant optic atrophy plus syndrome (ADOA plus).

Alpers syndrome is a progressive neurologic disorder that begins during childhood and is complicated in many instances by serious liver disease. Symptoms include increased muscle tone with exaggerated reflexes (spasticity), seizures, and dementia. Most often Alpers syndrome is caused by mutations in the *POLG* gene. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of Alpers syndrome.

Barth syndrome is a metabolic and neuromuscular disorder occurring almost exclusively in males that primarily affects the heart, immune system, muscles, and growth. It typically becomes apparent during infancy or early childhood. The main characteristics of the condition include abnormalities of heart and skeletal muscle (cardiomyopathy and skeletal myopathy); low levels of certain white blood cells called neutrophils that help to fight bacterial infections (neutropenia); and, growth retardation that potential leads to short stature. Other signs and symptoms may include increased levels of certain organic acids in the urine and blood (such as 3-methylglutaconic acid) and increased thickness of the left ventricle of the heart due to endocardial fibroelastosis, which can cause potential heart failure. Barth syndrome is caused by mutations in the *TAZ* gene and is inherited in an X-linked recessive manner. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of Barth syndrome.

Mitochondrial fatty acid β -oxidation disorders (FAODs) are a heterogeneous group of defects in fatty acid transport and mitochondrial β -oxidation. They are inherited as autosomal recessive disorders and have a wide range of clinical presentations. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of a mitochondrial fatty acid β -oxidation disorders (FAOD). FAODs include CPT I deficiency, CACT deficiency, CPT II

deficiency, LCAD deficiency, LCHAD deficiency, VLCAD deficiency, MCAD deficiency, SCHAD deficiency, and SCAD deficiency.

Primary carnitine deficiency is a genetic condition that prevents the body from using certain fats for energy, particularly during periods of fasting. The nature and severity of signs and symptoms may vary, but they most often appear during infancy or early childhood and can include severe brain dysfunction (encephalopathy), cardiomyopathy, confusion, vomiting, muscle weakness, and hypoglycemia. The condition is caused by mutations in the *SLC22A5* gene and is inherited in an autosomal recessive manner. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of primary carnitine deficiency.

Guanidinoacetate methyltransferase deficiency is an inherited disease that affects the brain and muscles. People with this disease may begin showing symptoms from early infancy to age three. Signs and symptoms can vary, but may include mild to severe intellectual disability, recurrent seizures, problems with speech, and involuntary movements. GAMT deficiency is caused by mutations in the *GAMT* gene. The disease is inherited in an autosomal recessive manner. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of guanidinoacetate methyltransferase deficiency.

Primary coenzyme Q10 deficiency involves a deficiency of coenzyme Q10 and can affect many parts of the body, especially the brain, muscles, and kidneys. The mildest cases of primary coenzyme Q10 deficiency can begin as late as a person's sixties and often cause cerebellar ataxia, which refers to problems with coordination and balance due to defects in the cerebellum. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of primary coenzyme Q10 deficiency.

Chronic progressive external ophthalmoplegia (CPEO) is a condition characterized mainly by a loss of the muscle functions involved in eye and eyelid movement. Signs and symptoms tend to begin in early adulthood and most commonly include weakness or paralysis of the muscles that move the eye (ophthalmoplegia) and drooping of the eyelids (ptosis). Some affected individuals also have myopathy, which may be especially noticeable during exercise. CPEO can be caused by

mutations in any of several genes, which may be located in mitochondrial DNA or nuclear DNA. CPEO can occur as part of other underlying conditions, such as ataxia neuropathy spectrum and Kearns-Sayre syndrome (KSS). KSS is a slowly progressive multi-system mitochondrial disease that often begins with ptosis. Other eye muscles eventually become involved, resulting in paralysis of eye movement. Degeneration of the retina usually causes difficulty seeing in dimly lit environments. In certain embodiments, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of chronic progressive external ophthalmoplegia or Kearns-Sayre syndrome.

Congenital lactic acidosis (CLA) is caused by mutations in mitochondrial DNA (mtDNA) that cause too much lactic acid to build up in the body, a condition called lactic acidosis. Severe cases of CLA manifest in the neonatal period, while milder cases caused by mtDNA mutations may not manifest until as late as early adulthood. Symptoms in the neonatal period include hypotonia, lethargy, vomiting, and tachypnea. As the disease progresses, it causes developmental delay, cognitive disabilities, abnormal development of the face and head, and organ failure. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of congenital lactic acidosis (CLA).

Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL) is a rare neurological disease characterized by slowly progressive cerebellar ataxia (lack of control of the movements) and spasticity with dorsal column dysfunction (decreased position and vibration sense) in most patients. The disease usually starts in childhood or adolescence, but in some cases not until adulthood. Symptoms may include difficulty speaking, epilepsy, learning problems, cognitive decline, and reduced consciousness, neurologic deterioration, and fever following minor head trauma. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL).

Leber hereditary optic neuropathy (LHON) is a condition characterized by vision loss. Some affected individuals develop features similar to multiple sclerosis. LHON is caused by mutations in the *MT-ND1*, *MT-ND4*, *MT-ND4L*, and *MT-ND6* genes. In one embodiment, an

effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of Leber hereditary optic neuropathy.

Glutaric acidemia type II (GA2) is a disorder that interferes with the body's ability to break down proteins and fats to produce energy. Most often, GA2 first appears in infancy or early childhood as a sudden episode of a metabolic crisis that can cause weakness, behavior changes (such as poor feeding and decreased activity) and vomiting. GA2 is inherited in an autosomal recessive manner and is caused by mutations in the *ETFA*, *ETFB*, or *ETFDH* genes. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of Glutaric acidemia type II (GA2).

Mitochondrial enoyl CoA reductase protein associated neurodegeneration (MEPAN) is caused by 2 mutations in the gene *MECR* (which encodes the protein mitochondrial trans-2-enoyl-coenzyme A-reductase). Characteristics of MEPAN include optic atrophy and childhood-onset dystonia. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of mitochondrial enoyl CoA reductase protein associated neurodegeneration (MEPAN).

Mitochondrial DNA (mtDNA) depletion syndrome (MDS) is a clinically heterogeneous group of mitochondrial disorders characterized by a reduction of the mtDNA copy number in affected tissues without mutations or rearrangements in the mtDNA. MDS is phenotypically heterogeneous, and can affect a specific organ or a combination of organs, with the main presentations described being either hepatocerebral (i.e., hepatic dysfunction, psychomotor delay), myopathic (i.e., hypotonia, muscle weakness, bulbar weakness), encephalomyopathic (i.e., hypotonia, muscle weakness, psychomotor delay) or neurogastrointestinal (i.e., gastrointestinal dysmotility, peripheral neuropathy). There are generally four classes of MDDS: 1) a form that primarily affects muscle associated with mutations in the *TK2* gene; 2) a form that primarily affects the brain and muscle associated with mutations in the genes *SUCLA2*, *SUCLG1*, or *RRM2B*; 3) a form that primarily affects the brain and the liver associated with mutations in *DGUOK*, *MPV17*, *POLG*, or *TWNK* (also called *PEO1*); and, 4) a form that primarily affects the brain and the gastrointestinal tract associated with mutations in *ECGF1* (also called *TYMP*). In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable

salt thereof, including CKLP1, is administered for the treatment of a mitochondrial DNA (mtDNA) depletion syndrome (MDS).

Mitochondrial neurogastrointestinal encephalopathy (MNGIE) disease is a condition that affects several parts of the body, particularly the digestive system and nervous system. The major features of MNGIE disease can appear at any point from infancy to adulthood, but signs and symptoms most often begin by age twenty. MNGIE disease is also characterized by abnormalities of the nervous system, although these tend to be milder than the gastrointestinal problems. Affected individuals experience tingling, numbness, and weakness in their limbs (peripheral neuropathy), particularly in the hands and feet. Additional neurological signs and symptoms can include droopy eyelids (ptosis), weakness of the muscles that control eye movement (ophthalmoplegia), and hearing loss. Leukoencephalopathy, which is the deterioration of a type of brain tissue known as white matter, is a hallmark of MNGIE disease. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of mitochondrial neurogastrointestinal encephalopathy (MNGIE).

Neuropathy ataxia retinitis pigmentosa (NARP) syndrome is characterized by a variety of signs and symptoms that mainly affect the nervous system. Beginning in childhood or early adulthood, most people with NARP experience numbness, tingling, or pain in the arms and legs (sensory neuropathy), muscle weakness, and problems with balance and coordination (ataxia). Affected individuals may also have vision loss caused by a condition called retinitis pigmentosa. Mutations in the *MT-ATP6* gene cause NARP syndrome. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of neuropathy ataxia retinitis pigmentosa (NARP) syndrome.

Pearson syndrome affects many parts of the body, but especially the bone marrow and the pancreas. Pearson syndrome affects the cells in the bone marrow (hematopoietic stem cells) that produce red blood cells, white blood cells, and platelets. Pearson syndrome also affects the pancreas, which can cause frequent diarrhea and stomach pain, trouble gaining weight, and diabetes. Some children with Person syndrome may also have problems with their liver, kidneys, heart, eyes, ears, and/or brain. Pearson syndrome is caused by a mutation in the mitochondrial

DNA. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of Pearson syndrome.

POLG-related disorders comprise a continuum of overlapping phenotypes with onset from
5 infancy to late adulthood. Mutations in *POLG* can cause early childhood mitochondrial DNA (mtDNA) depletion syndromes or later-onset syndromes arising from mtDNA deletions. *POLG* mutations are the most common cause of inherited mitochondrial disorders, with as many as 2% of the population carrying these mutations. The six leading disorders caused by *POLG* mutations are Alpers-Huttenlocher syndrome, which is one of the most severe phenotypes; childhood
10 myocerebrohepatopathy spectrum, which presents within the first three years of life; myoclonic epilepsy myopathy sensory ataxia; ataxia neuropathy spectrum (which includes the phenotypes previously referred to as mitochondrial recessive ataxia syndrome (MIRAS) and sensory ataxia neuropathy dysarthria and ophthalmoplegia (SANDO)); autosomal recessive progressive external ophthalmoplegia; and, autosomal dominant progressive external ophthalmoplegia. In one
15 embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of a POLG-related disorder.

Pyruvate carboxylase deficiency is an inherited disorder that causes lactic acid and other potentially toxic compounds to accumulate in the blood. High levels of these substances can
20 damage the body's organs and tissues, particularly in the nervous system. There are at least three types of pyruvate carboxylase deficiency, types A, B, and C, which are distinguished by the severity of their signs and symptoms. This condition is caused by mutations in the *PC* gene and inherited in an autosomal recessive pattern. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including
25 CKLP1, is administered for the treatment of pyruvate carboxylase deficiency.

Pyruvate dehydrogenase complex (PDC) deficiency is a type of metabolic disease where the body is not able to efficiently break down nutrients in food to be used for energy. Symptoms of PDC deficiency include signs of metabolic dysfunction such as extreme tiredness (lethargy), poor feeding, and rapid breathing (tachypnea). Other symptoms may include signs of neurological
30 dysfunction such as developmental delay, periods of uncontrolled movements (ataxia), low muscle

tone (hypotonia), abnormal eye movements, and seizures. Symptoms usually begin in infancy, but signs can first appear at birth or later in childhood. The most common form of PDC deficiency is caused by genetic (mutations or pathogenic variants in the *PDHA1* gene. In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of pyruvate carboxylase deficiency.

TK2-Related mitochondrial DNA depletion syndrome, myopathic form (TK2-MDS) is an inherited condition that causes progressive myopathy. The signs and symptoms of TK2-MDS typically begin in early childhood. Development is usually normal early in life, but as muscle weakness progresses, people with TK2-MDS lose motor skills such as standing, walking, eating, and talking. Some affected individuals have increasing weakness in the muscles that control eye movement, leading to droopy eyelids (progressive external ophthalmoplegia). In one embodiment, an effective amount of a compound of Formula I-Formula III or its pharmaceutically acceptable salt thereof, including CKLP1, is administered for the treatment of TK2-related mitochondrial DNA depletion syndrome, myopathic form (TK2-MDS).

Selected Ocular Disorders

In additional aspects of the invention, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is used for the treatment of a selected ocular disorder, as described below.

Graves' ophthalmopathy or Graves' orbitopathy (or thyroid eye disease or thyroid-associated orbitopathy) are autoimmune inflammatory disorders of the orbit and periorbital tissues and typical signs of the diseases include upper eyelid retraction, lid lag, swelling, and bulging eyes. These disorders are orbital autoimmune disorders caused by an overactive thyroid. An effective amount of a CKLP1 prodrug of Formula I-III can be administered for the treatment of Graves' ophthalmopathy, Graves' orbitopathy, or thyroid-associated orbitopathy. The compound can be administered in any manner that achieves the desired effect, including as a topical drop taken as needed to reduce swelling and redness. In one embodiment, the prodrug of Formula I-III is taken in combination with a corticosteroid drug or an immune suppression medication (rituximab or mycophenolate).

Orbital tumors are benign or malignant space-occupying lesions of the orbit, often leading to dystopia of the eyeball, motility disturbances, diplopia, visual field defects, and sometimes a complete loss of vision. Often orbital tumors are removed via surgery and therefore a medication would be an advantageous therapeutic option. In one embodiment, an effective amount of a cromakalim prodrug of Formula I-Formula III or its pharmaceutically acceptable salt is administered for the treatment or reduction of orbital tumors. In one embodiment, the compound is administered topically one time, two times, three times, or more a day. In one embodiment, the compound is administered prior to or after surgery for the removal or reduction of orbital tumors.

Cavernous sinus thrombosis is the formation of a blood clot within the cavernous sinus, a cavity at the base of the brain which drains deoxygenated blood from the brain back to the heart. This is a rare disorder and can be of two types: septic cavernous thrombosis and aseptic cavernous thrombosis. The cause is often secondary to an infection in the nose, sinuses, ears, or teeth. A common disorder secondary to cavernous sinus pathology is superior ophthalmic vein thrombosis, an uncommon orbital pathology that can present with sudden onset proptosis, conjunctival injection, and visual disturbance.

In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered for the treatment of cavernous sinus thrombosis or superior ophthalmic vein thrombosis. In one embodiment, an effective amount is administered in combination or alternation with an antibiotic, heparin, or a steroid. In one aspect, the compound is administered orally and is given at least once, twice, three, or more times a day as needed.

Episcleral/orbital vein vasculitis is inflammation of the blood vessel wall. The clinical features of the eye vasculitis can vary from conjunctivitis, episcleritis, scleritis, peripheral ulcerative keratitis, proptosis, retinal vasculitis, orbititis to uveitis, depending on the site and distribution of the vessels involved. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered for the treatment of episcleral/orbital vein vasculitis. In one embodiment, the prodrug is administered as a topical drop.

Carotid-cavernous sinus fistula is an abnormal connection between an artery in the neck and the network of veins at the back of the eye. A fistula can raise the pressure in your cavernous

sinuses, which may compress the cranial nerves located around the cavernous sinuses. This compression may damage the nerve function, which is to control your eye movements. Carotid-cavernous sinus fistula can be direct or indirect. Direct carotid-cavernous sinus fistulas are often caused by accidents or injuries that tear the carotid artery wall, while indirect carotid-cavernous sinus fistulas often arise without warning and are associated with high blood pressure, hardened arteries, pregnancy, and connective tissue disorders. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered for the treatment of carotid-cavernous sinus fistula. In one embodiment, the prodrug is administered as an oral dosage form.

Dural cavernous sinus shunts are vascular communications in which blood flows through small meningeal branches of the carotid arteries to enter the venous circulation near the cavernous sinus. Often this disorder is congenital and the onset of clinical abnormalities may be associated with the occurrence of intracranial venous thrombosis. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered for the treatment of dural cavernous sinus shunts. In one embodiment, the prodrug is administered as an oral dosage form.

Orbital varices are a vascular hamartoma typified by a plexus of low pressure, low flow, thin walled and distensible vessels that intermingle with the normal orbital vessels. Most patients will experience positional proptosis with a head-down position, and intermittent proptosis that is exacerbated by coughing, straining, the Valsalva maneuver, or compression of the jugular veins. In one embodiment, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered for the treatment of orbital varices. In one embodiment, the prodrug is administered as an oral dosage form.

Sturge-Weber Syndrome is a condition that affects the development of certain blood vessels, causing abnormalities in the brain, skin, and eyes from birth. Sturge-Weber Syndrome has three major features: a red or pink birthmark called a port-wine birthmark, a brain abnormality called a leptomeningeal angioma, and increased IOP in the eye (glaucoma). In individuals with Sturge-Weber Syndrome, glaucoma typically develops either in infancy or early adulthood and can cause vision impairment. In some affected infants, the pressure can become so great that the eyeballs appear enlarged and bulging (buphthalmos). Individuals with Sturge-Weber Syndrome

can have tangles of abnormal blood vessels (hemangiomas) in various parts of the eye. When these abnormal blood vessels develop into a network of blood vessels at the back of the eye (choroid), it is called a diffuse choroidal hemangioma and occurs in about one-third of individuals with Sturge-Weber Syndrome. A diffuse choroidal hemangioma can cause vision loss. When present,
5 the eye abnormalities typically occur on the same side of the head as the port-wine birthmark.

In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered for the treatment of Sturge-Weber Syndrome. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered for the
10 treatment of Sturge-Weber Syndrome-induced glaucoma. In one embodiment, the compound is administered as an oral formulation once, twice, three, or more times a day. In one embodiment, the prodrug is administered as a topical ocular formulation and is administered once a day for long term therapy, as defined herein.

Central retinal vein occlusion, also known as CRVO, is a condition in which the main vein
15 that drains blood from the retina becomes blocked partially or completely. This can cause blurred vision and other problems with the eye. Risk factors for CRVO include diabetes, elevated IOP, and high blood pressure. The macula can swell from this fluid, affecting central vision. Eventually, without blood circulation, nerve cells in the eye can die and vision loss can occur. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt
20 of Formula I-III, including CKLP1, is administered for the treatment of central retinal vein occlusion. In one embodiment, the compound is administered as a topical drop that is given once, twice, or three times a day. In one embodiment, the prodrug is given in combination with an anti-VEGF inhibitor such as bevacizumab (Avastin®), ranibizumab (Lucentis®), and aflibercept (Eylea®).

Branch retinal vein occlusion (BRVO), is the blockage of branches of the retinal vein causing blood and fluid to spill into the retina. Risk factors for BRVO include diabetes, elevated IOP, and high blood pressure. The macula can swell from this fluid, affecting central vision. Eventually, without blood circulation, nerve cells in the eye can die and vision loss can occur. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable
25 salt of Formula I-III, including CKLP1, is administered for the treatment of branch retinal vein
30

occlusion (BRVO). In one embodiment, the prodrug is administered as a topical drop that is given once, twice, three, or more times a day.

Non-arteritic anterior ischemic optic neuropathy (NAION) refers to loss of blood flow to the optic nerve and is due to impaired circulation of blood at the optic nerve head. Non-arteritic
5 anterior ischemic optic neuropathy is associated with diabetes, high blood pressure, atherosclerosis, a small optic nerve, elevated IOP, and sleep apnea. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered for the treatment of non-arteritic anterior ischemic optic neuropathy. In one embodiment, the prodrug is administered as a topical drop that is given once,
10 twice, three, or more times a day.

In some embodiments, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is used as a secondary treatment to latanoprost for the treatment of an ocular disorder as described herein.

In some embodiments, it may be useful to administer a cromakalim prodrug or a
15 pharmaceutically acceptable salt of Formula I-III, including CKLP1, to a host in need thereof in combination with, for example,

(1) a prostaglandin analog, such as latanoprost (Xalatan), bimatoprost (Lumigan), travoprost (Travatan or Travatan Z), or Tafluprost (Zioptan);

(2) an α -2 adrenergic agonist, such as brimonidine (Alphagan®), epinephrine, dipivefrin
20 (Propine®) or apraclonidine (Lopidine®);

(3) a beta-blocker, such as timolol, levobunolol, metipranolol, or carteolol;

(4) a ROCK inhibitor, such as ripasudil, netarsudil (Rhopressa), fasudil, RKI-1447, GSK429286A, or Y-30141;

(5) a second potassium channel opener, such as minoxidil, diazoxide, nicorandil, or
25 pinacidil;

(6) a carbonic anhydrase inhibitor, such as dorzolamide (Trusopt®), brinzolamide (Azopt®), acetazolamide (Diamox®) or methazolamide (Neptazane®);

(7) a PI3K inhibitor, such as Wortmannin, demethoxyviridin, perifosine, idelalisib, Pictilisib, Palomid 529, ZSTK474, PWT33597, CUDC-907, and AEZS-136, duvelisib, GS-9820,
30 BKM120, GDC-0032 (Taselisib) (2-[4-[2-(2-Isopropyl-5-methyl-1,2,4-triazol-3-yl)-5,6-

dihydroimidazo[1,2-d][1,4]benzoxazepin-9-yl]pyrazol-1-yl]-2-methylpropanamide), MLN-1117
 ((2*R*)-1-Phenoxy-2-butanyl hydrogen (*S*)-methylphosphonate; or Methyl(oxo) {[(2*R*)-1-phenoxy-
 2-butanyl]oxy}phosphonium)), BYL-719 ((2*S*)-N1-[4-Methyl-5-[2-(2,2,2-trifluoro-1,1-
 dimethylethyl)-4-pyridinyl]-2-thiazolyl]-1,2-pyrrolidinedicarboxamide), GSK2126458 (2,4-
 5 Difluoro-*N*-{2-(methyloxy)-5-[4-(4-pyridazinyl)-6-quinolinyl]-3-pyridinyl}benzenesulfonamide)
 (omipalisib), TGX-221 ((±)-7-Methyl-2-(morpholin-4-yl)-9-(1-phenylaminoethyl)-pyrido[1,2-*a*]-
 pyrimidin-4-one), GSK2636771 (2-Methyl-1-(2-methyl-3-(trifluoromethyl)benzyl)-6-
 morpholino-1*H*-benzo[d]imidazole-4-carboxylic acid dihydrochloride), KIN-193 ((*R*)-2-((1-(7-
 methyl-2-morpholino-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-9-yl)ethyl)amino)benzoic acid), TGR-
 10 1202/RP5264, GS-9820 ((*S*)-1-(4-((2-(2-aminopyrimidin-5-yl)-7-methyl-4-mohydroxypropan-1-
 -one), GS-1101 (5-fluoro-3-phenyl-2-([*S*])-1-[9*H*-purin-6-ylamino]-propyl)-3*H*-quinazolin-4-
 one), AMG-319, GSK-2269557, SAR245409 (*N*-(4-(*N*-(3-((3,5-
 dimethoxyphenyl)amino)quinoxalin-2-yl)sulfamoyl)phenyl)-3-methoxy-4-methylbenzamide),
 BAY80-6946 (2-amino-*N*-(7-methoxy-8-(3-morpholinopropoxy)-2,3-dihydroimidazo[1,2-
 15 c]quinaz), AS 252424 (5-[1-[5-(4-Fluoro-2-hydroxy-phenyl)-furan-2-yl]-meth-(*Z*)-ylidene]-
 thiazolidine-2,4-dione), CZ 24832 (5-(2-amino-8-fluoro-[1,2,4]triazolo[1,5-*a*]pyridin-6-yl)-*N*-*tert*-
 butylpyridine-3-sulfonamide), Buparlisib (5-[2,6-Di(4-morpholinyl)-4-pyrimidinyl]-4-
 (trifluoromethyl)-2-pyridinamine), GDC-0941 (2-(1*H*-Indazol-4-yl)-6-[[4-(methylsulfonyl)-1-
 piperazinyl]methyl]-4-(4-morpholinyl)thieno[3,2-*d*]pyrimidine), GDC-0980 ((*S*)-1-(4-((2-(2-
 20 aminopyrimidin-5-yl)-7-methyl-4-morpholinothieno[3,2-*d*]pyrimidin-6-yl)methyl)piperazin-1-
 yl)-2-hydroxypropan-1-one (also known as RG7422)), SF1126 ((8*S*,14*S*,17*S*)-14-
 (carboxymethyl)-8-(3-guanidinopropyl)-17-(hydroxymethyl)-3,6,9,12,15-pentaoxo-1-(4-(4-oxo-
 8-phenyl-4*H*-chromen-2-yl)morpholino-4-ium)-2-oxa-7,10,13,16-tetrazaoctadecan-18-oate),
 PF-05212384 (*N*-[4-[[4-(Dimethylamino)-1-piperidinyl]carbonyl]phenyl]-*N'*-[4-(4,6-di-4-
 25 morpholinyl-1,3,5-triazin-2-yl)phenyl]urea) (gedatolisib), LY3023414, BEZ235 (2-Methyl-2-{4-
 [3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-
 yl]phenyl}propanenitrile) (dactolisib), XL-765 (*N*-(3-(*N*-(3-(3,5-
 dimethoxyphenylamino)quinoxalin-2-yl)sulfamoyl)phenyl)-3-methoxy-4-methylbenzamide), and
 GSK1059615 (5-[[4-(4-Pyridinyl)-6-quinolinyl]methylene]-2,4-thiazolidinedione), PX886
 30 [(3*aR*,6*E*,9*S*,9*aR*,10*R*,11*aS*)-6-[[bis(prop-2-enyl)amino]methylidene]-5-hydroxy-9-

(methoxymethyl)-9a,11a-dimethyl-1,4,7-trioxo-2,3,3a,9,10,11-hexahydroindeno[4,5h]isochromen-10-yl] acetate (also known as sonolisib)), LY294002, AZD8186, PF-4989216, pilaralisib, GNE-317, PI-3065, PI-103, NU7441 (KU-57788), HS 173, VS-5584 (SB2343), CZC24832, TG100-115, A66, YM201636, CAY10505, PIK-75, PIK-93, AS-605240, BGT226 (NVP-BGT226),
 5 AZD6482, voxtalisib, alpelisib, IC-87114, TGI100713, CH5132799, PKI-402, copanlisib (BAY 80-6946), XL 147, PIK-90, PIK-293, PIK-294, 3-MA (3-methyladenine), AS-252424, AS-604850, apitolisib (GDC-0980; RG7422);

(8) a BTK inhibitor, such as: ibrutinib (also known as PCI-32765)(Imbruvica™)(1-[(3R)-3-[4-amino-3-(4-phenoxy-phenyl)pyrazolo[3,4-d]pyrimidin-1-yl]piperidin-1-yl]prop-2-en-1-one), dianilinopyrimidine-based inhibitors such as AVL-101 and AVL-291/292 (N-(3-((5-fluoro-2-((4-(2-methoxyethoxy)phenyl)amino)pyrimidin-4-yl)amino)phenyl)acrylamide) (Avila Therapeutics) (US Patent publication No 2011/0117073, incorporated herein in its entirety), Dasatinib ([N-(2-chloro-6-methylphenyl)-2-(6-(4-(2-hydroxyethyl)piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide], LFM-A13 (alpha-cyano-beta-hydroxy-beta-methyl-N-(2,5-ibromophenyl) propenamide), GDC-0834 ([R-N-(3-(6-(4-(1,4-dimethyl-3-oxopiperazin-2-yl)phenylamino)-4-methyl-5-oxo-4,5-dihydropyrazin-2-yl)-2-methylphenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-2-carboxamide], CGI-560 4-(tert-butyl)-N-(3-(8-(phenylamino)imidazo[1,2-a]pyrazin-6-yl)phenyl)benzamide, CGI-1746 (4-(tert-butyl)-N-(2-methyl-3-(4-methyl-6-((4-(morpholine-4-carbonyl)phenyl)amino)-5-oxo-4,5-dihydropyrazin-2-yl)phenyl)benzamide), CNX-774 (4-(4-((4-((3-acrylamidophenyl)amino)-5-fluoropyrimidin-2-yl)amino)phenoxy)-N-methylpicolinamide), CTA056 (7-benzyl-1-(3-(piperidin-1-yl)propyl)-2-(4-(pyridin-4-yl)phenyl)-1H-imidazo[4,5-g]quinoxalin-6(5H)-one), GDC-0834 ((R)-N-(3-(6-((4-(1,4-dimethyl-3-oxopiperazin-2-yl)phenyl)amino)-4-methyl-5-oxo-4,5-dihydropyrazin-2-yl)-2-methylphenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-2-carboxamide), GDC-0837 ((R)-N-(3-(6-((4-(1,4-dimethyl-3-oxopiperazin-2-yl)phenyl)amino)-4-methyl-5-oxo-4,5-dihydropyrazin-2-yl)-2-methylphenyl)-4,5,6,7-tetrahydrobenzo[b]thiophene-2-carboxamide), HM-71224, ACP-196, ONO-4059 (Ono Pharmaceuticals), PRT062607 (4-((3-(2H-1,2,3-triazol-2-yl)phenyl)amino)-2-(((1R,2S)-2-aminocyclohexyl)amino)pyrimidine-5-carboxamide hydrochloride), QL-47 (1-(1-acryloylindolin-6-yl)-9-(1-methyl-1H-pyrazol-4-yl)benzo[h][1,6]naphthyridin-2(1H)-one), and

RN486 (6-cyclopropyl-8-fluoro-2-(2-hydroxymethyl-3-{1-methyl-5-[5-(4-methyl-piperazin-1-yl)-pyridin-2-ylamino]-6-oxo-1,6-dihydro-pyridin-3-yl}-phenyl)-2H-isoquinolin-1-one); or a

(9) a Syk inhibitor, such as Cerdulatinib (4-(cyclopropylamino)-2-((4-(4-(ethylsulfonyl)piperazin-1-yl)phenyl)amino)pyrimidine-5-carboxamide), entospletinib (6-(1H-indazol-6-yl)-N-(4-morpholinophenyl)imidazo[1,2-a]pyrazin-8-amine), fostamatinib ([6-({5-Fluoro-2-[(3,4,5-trimethoxyphenyl)amino]-4-pyrimidinyl}amino)-2,2-dimethyl-3-oxo-2,3-dihydro-4H-pyrido[3,2-b][1,4]oxazin-4-yl)methyl dihydrogen phosphate), fostamatinib disodium salt (sodium (6-((5-fluoro-2-((3,4,5-trimethoxyphenyl)amino)pyrimidin-4-yl)amino)-2,2-dimethyl-3-oxo-2H-pyrido[3,2-b][1,4]oxazin-4(3H)-yl)methyl phosphate), BAY 61-3606 (2-(7-(3,4-Dimethoxyphenyl)-imidazo[1,2-c]pyrimidin-5-ylamino)-nicotinamide HCl), RO9021 (6-[(1R,2S)-2-Amino-cyclohexylamino]-4-(5,6-dimethyl-pyridin-2-ylamino)-pyridazine-3-carboxylic acid amide), imatinib (Gleevec; 4-[(4-methylpiperazin-1-yl)methyl]-N-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)benzamide), staurosporine, GSK143 (2-(((3R,4R)-3-aminotetrahydro-2H-pyran-4-yl)amino)-4-(p-tolylamino)pyrimidine-5-carboxamide), PP2 (1-(tert-butyl)-3-(4-chlorophenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine), PRT-060318 (2-(((1R,2S)-2-aminocyclohexyl)amino)-4-(m-tolylamino)pyrimidine-5-carboxamide), PRT-062607 (4-((3-(2H-1,2,3-triazol-2-yl)phenyl)amino)-2-(((1R,2S)-2-aminocyclohexyl)amino)pyrimidine-5-carboxamide hydrochloride), R112 (3,3'-((5-fluoropyrimidine-2,4-diyl)bis(azanediyl))diphenol), R348 (3-Ethyl-4-methylpyridine), R406 (6-((5-fluoro-2-((3,4,5-trimethoxyphenyl)amino)pyrimidin-4-yl)amino)-2,2-dimethyl-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one), piceatannol (3-Hydroxyresveratol), YM193306, 7-azaindole, piceatannol, ER-27319, PRT060318, luteolin, apigenin, quercetin, fisetin, myricetin, morin.

In alternative embodiments, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered to a host in need thereof in combination with a nitric oxide donor, including, but not limited to, NCX-470, NCX-1728, NCX-4251, NCX-4016, NCX-434, NCX-667, Vyzulta (latanoprostene bunod ophthalmic solution), or sodium nitroprusside (SNP).

Ophthalmic Neuroprotection

Neuroprotection is a therapeutic strategy with the goal of maximizing the recovery of neural cells and minimizing neuronal cell death due to injury. The injury can be mechanical, ischemic, degenerative, or radiation. Many neurodegenerative disorders are associated with aging, which can be detrimental for the elderly population. For example, glaucoma is often characterized by the loss of retinal ganglion cells and is a major cause of vision loss and blindness in the elderly.

In one embodiment, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered to a host in need thereof for the treatment of an ocular-related neurodegenerative disorder. An ocular-related neurodegenerative disorder is any disorder that is associated with the dysfunction or degeneration of neurons or cells, including neural cells, such as retinal ganglion cells.

In one embodiment of the present invention, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered as a method for reducing neuronal or cellular damage in the eye of host in need thereof. In one embodiment, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered as a method for reducing neuronal or cellular damage in the eye of host in need thereof wherein the eye is glaucomatous.

In another embodiment, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, promotes the survival, growth, regeneration, and/or neurite outgrowth of retinal ganglion cells. In another embodiment, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, prevents the death of damaged neuronal cells.

Neuronal cell death can also be a result of retinal ischemia, and therefore in one embodiment, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered as a method of reducing neuronal or cellular damage in the eye following retinal ischemia in a host in need thereof.

Optic neuropathy, which is damage to the optic nerve often characterized by visual loss, results in the loss of retinal ganglion cells. There are many types of optic neuropathies, including ischemic optic neuropathy, optic neuritis, compressive optic neuropathy, infiltrative optic neuropathy, and traumatic optic neuropathy. Nutritional optic neuropathy can also result from

under nutrition and/or a vitamin B₁₂ deficiency. Toxic optic neuropathy can result from exposure to ethylene glycol, methanol, ethambutol, amiodarone, tobacco, or certain drugs, such as chloramphenicol or digitalis. Certain forms of optic neuropathy can be inherited, including Leber's hereditary optic neuropathy (LHON), dominant optic atrophy, Behr's syndrome, and Berk-
5 Tabatznik syndrome. In one embodiment, an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is administered as a method for reducing as a method of reducing neuronal or cellular damage in the eye of a host in need thereof with optic neuropathy.

Additional non-limiting examples of ocular-related neurodegenerative diseases include
10 lattice dystrophy, retinitis pigmentosa, age-related macular degeneration (wet or dry), photoreceptor degeneration associated with wet- or dry-age related macular degeneration, and optic nerve drusen.

Integrated or Adjunctive Therapy with Microinvasive Glaucoma Surgery (MIGS)

15 Minimally (or Micro) Invasive Glaucoma Surgery (MIGS) has become an innovative procedure in the evolution of glaucoma surgery. Since glaucoma is a disease in which the optic nerve gets damaged primarily due to elevated IOP, the goal of glaucoma surgery is to lower IOP to prevent or reduce damage to the optic nerve.

Standard glaucoma surgeries are still considered a major surgery and involve
20 trabeculectomy, ExPRESS shunts, or external tube-shunts such as the Ahmed, Molteno, and Baerveldt style valve implants. While such procedures have often been effective at lowering eye pressure and preventing progression of glaucoma, they have numerous potential complications such as double vision, devastating eye infections, exposure of a drainage implant, swelling of the cornea, and excessively low IOP.

25 According to Saheb and Ahmed, minimally (or micro) invasive glaucoma surgery refers to a group of procedures which share five preferable qualities:

1. an *ab interno* and/or *ab externo* approach through a clear corneal incision which may spare the conjunctiva of incision;
2. a minimally traumatic procedure to the target tissue;
- 30 3. an IOP lowering efficacy that justifies the approach;

4. a high safety profile avoiding serious complications compared to other glaucoma surgeries, and given lower likelihood of hypotony; and
5. a rapid recovery with minimal impact on the patient's quality of life.

5 The MIGS group of operations have been developed in recent years to reduce some of the complications of most standard glaucoma surgeries and therefore, in one embodiment, a prodrug of Formula I – Formula III is used as an additive in combination with a microinvasive glaucoma surgery (MIGS).

10 MIGS is intended to achieve lower IOP in patients with glaucoma with a less invasive surgical procedure, and ideally to achieve a medication sparing effect. MIGS procedures work by using microscopic-sized equipment and tiny incisions, enable controlled outflow and are often conducted at the time of cataract surgery. While they reduce the incidence of complications, some degree of effectiveness is traded for the increased safety. (Pillunat, L.E., *et al.*, *Clin Ophthalmol.* 2017; 11: 1583–1600)

The MIGS group of operations are divided into several categories:

- 15
1. Trabecular bypass operations (i.e., angle-based devices and or subconjunctival shunting devices);
 2. Microtrabeculectomies (miniaturized versions of trabeculectomy);
 3. Totally internal or suprachoroidal shunts; and,
 4. Milder, gentler versions of laser photocoagulation.

20 Trabecular Surgery (Trabeculotomy) involves the use of a special contact lens on the eye and cutting through the trabecular meshwork with a tiny device under high power microscopic control. This is done without damaging any other tissues in the ocular drainage pathway. The trabecular meshwork can either be destroyed (Trabectome or Trab360) or bypassed using a tiny snorkel-like device (the iStent) or using a plug-shaped stent device (iStent Inject). Both procedures are FDA-approved but generally do not reduce eye pressure low enough and are thus useful in
25 early to moderate stages of glaucoma. With these devices, the resistance of the trabecular meshwork is obviated, thus primarily leaving distal outflow facility and episcleral venous pressure as limits to further aqueous humor drainage. In certain embodiments, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is used as an additive in
30 combination with Trabectome or Trab360 and/or the iStent/iStent Inject for the treatment of

glaucoma by additively lowering IOP via increased distal outflow or reduced episcleral venous pressure prior to or after the procedure in an acute or chronic use setting.

Microtrabeculectomies work by inserting tiny, microscopic-sized tubes into the eye and draining the fluid from inside the eye to underneath the outer membrane of the eye (conjunctiva).

5 The Xen Gel Stent and PRESERFLO are two new devices that can make the trabeculectomy operation safer. Results have shown excellent pressure lowering with improved safety over trabeculectomy in studies done outside the United States. In certain embodiments, the compounds of the present invention are used as part of the protocols with Xen Gel Stent and/or Preserflo for the treatment of glaucoma by additively lowering IOP via increased distal outflow or reduced
10 episcleral venous pressure prior to or after the procedure in an acute or chronic use setting.

Suprachoroidal Shunts, including the Gold Micro-shunt, iStent Supra, Aquashunt, and STARflo, work by using tiny tubes with very small internal openings, the front of the eye is connected to the suprachoroidal space between the retina and the wall of the eye to augment the drainage of fluid from the eye. This operation has relatively few serious complications and lowers
15 pressures enough to be useful even in moderately severe glaucoma. In certain embodiments, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is used in combination with Suprachoroidal Shunts procedure for the treatment of glaucoma by additively lowering IOP via increased distal outflow or reduced episcleral venous pressure prior to or after the procedure in an acute or chronic use setting.

20 Trabecular bypass stents and shunts are investigational devices that work to dilate Schlemm's canal. These procedures facilitate the flow of aqueous into Schlemm's canal by shunting (Eyepass Glaucoma Implant; GMP Companies, Inc., Fort Lauderdale, FL) or by stenting the canal itself (iStent; Glaukos Corp., Laguna Hills, CA). Other devices such as the Solx Gold Micro-Shunt (OccuLogix, Inc., Mississauga, Ontario, Canada) divert aqueous into the
25 suprachoroidal space. In certain embodiments, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is used in combination with trabecular bypass stents or shunts procedure for the treatment of glaucoma by additively lowering IOP via increased distal outflow or reduced episcleral venous pressure prior to or after the procedure in an acute or chronic use setting.

Selective laser trabeculoplasty (SLT) is used any during the management to help lower IOP. Since the conduct of the LiGHT study, it has now been used more often as first line-treatment to help lower IOP, effectively working at the level of the trabecular meshwork to improve outflow. In certain embodiments, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is used alongside and/or in addition to SLT for the treatment of glaucoma by additively lowering IOP via increased distal outflow and/or reduced episcleral venous pressure prior to or after the procedure in an acute or chronic use setting.

Laser photocoagulation was previously reserved for advanced glaucoma that could not be controlled despite trabeculectomy or tube shunts. Endocyclophotocoagulation and micropulse Diode cyclophotocoagulation are two recent advances to the use of laser photocoagulation and have proven useful in cases where glaucoma has yet to become advanced. In certain embodiments, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is used in the endocyclophotocoagulation and micropulse cyclophotocoagulation protocol for the treatment of glaucoma by additively lowering IOP via increased distal outflow and/or reduced episcleral venous pressure prior to or after the procedure in an acute or chronic use setting.

Endocyclophotocoagulation in recent years has become a widely accepted and popular treatment of refractory glaucoma, pediatric glaucoma, and as an adjunct to cataract surgery in both medically controlled and uncontrolled glaucoma in conjunction with phacoemulsification with intraocular lens placement. Endocyclophotocoagulation is performed following lens removal and intraocular lens implantation by inserting an endolaser unit through the cataract incision, across the anterior segment, and into the posterior chamber on the nasal side of the eye. Laser energy is applied to the ciliary processes to destroy ciliary epithelial cells that produce aqueous humor. In certain embodiments, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is used in the endocyclophotocoagulation protocol for the treatment of glaucoma by additively lowering IOP via increased distal outflow and/or reduced episcleral venous pressure prior to or after the procedure in an acute or chronic use setting.

Micropulse cyclophotocoagulation delivers the laser in short bursts to allow the surgeon to target specific areas of the ciliary body while giving the tissue time to cool down between bursts, minimizing damage. MicroPulse P3 probe and the new Cyclo G6 glaucoma laser system (Iridex) have both been used successfully in retinal diseases, showing excellent safety and efficacy rates.

In certain embodiments, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is used in the Micropulse cyclophotocoagulation surgical protocol for the treatment of glaucoma by additively lowering IOP via increased distal outflow and/or reduced episcleral venous pressure prior to or after the procedure in an acute or chronic use setting.

5 Other devices include Gonioscopy-assisted transluminal trabeculotomy (GATT), Kahook Dual Blade, Ab interno canaloplasty and Hydrus Microstent, iStent Supra, Xen Glaucoma Treatment System and InnFocus MicroShunt. In certain embodiments, a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, is used in the surgical protocol of these devices for the treatment of glaucoma as described above.

10 Laser Trabeculoplasty, including Selective Laser Trabeculoplasty (SLT), Argon Laser Trabeculoplasty (ALT), Excimer Laser Trabeculostomy and Micropulse Laser Trabeculoplasty (MLT) are surgical laser procedures that help to reduce resistance at the trabecular meshwork by ablating cells of the trabecular meshwork and improving outflow in a manner similar to other forms of trabeculoplasty and certain MIGS devices. In certain embodiments, Excimer Laser
15 Trabeculostomy used as an additive in combination with Laser Trabeculoplasty for the treatment of glaucoma by additively lowering IOP via increased distal outflow or reduced episcleral venous pressure prior to or after the procedure in an acute or chronic use setting.

In one embodiment, a CKLP1 prodrug of Formula I-Formula III is used as a secondary therapy to a prostaglandin analog, such as latanoprost (Xalatan), bimatoprost (Lumigan),
20 travoprost (Travatan or Travatan Z), latanoprostene bunod (Vyzulta), or Tafluprost (Zioptan) and as an additive to a minimally (or micro) invasive glaucoma surgery (MIGS) as described herein. In a further embodiment, the MIGS is a trabeculotomy. In a further embodiment, the MIGS is a microtrabeculectomy. In a further embodiment, the MIGS is a suprachoroidal shunt. In a further embodiment, the MIGS is a trabecular bypass stent or shunt. In a further embodiment, the MIGS
25 is a selective laser trabeculoplasty (SLT). In a further embodiment, the MIGS is a laser photocoagulation. In a further embodiment, the MIGS is endocyclophotocoagulation. In a further embodiment, the MIGS is laser trabeculoplasty.

In one embodiment, a CKLP1 prodrug of Formula I-Formula III is used as a secondary therapy to latanoprost (Xalatan) and as an additive to a minimally (or micro) invasive glaucoma
30 surgery as described herein. In a further embodiment, the MIGS is a trabeculotomy. In a further

embodiment, the MIGS is a microtrabeculectomy. In a further embodiment, the MIGS is a suprachoroidal shunt. In a further embodiment, the MIGS is a trabecular bypass stent or shunt. In a further embodiment, the MIGS is a selective laser trabeculoplasty (SLT). In a further embodiment, the MIGS is a laser photocoagulation. In a further embodiment, the MIGS is endocyclophotocoagulation. In a further embodiment, the MIGS is laser trabeculoplasty.

In one embodiment, a CKLP1 prodrug of Formula I-Formula III is used as a secondary therapy to an α -2 adrenergic agonist, such as brimonidine (Alphagan®), epinephrine, dipivefrin (Propine®) or apraclonidine (Lopidine®) and as an additive to a minimally (or micro) invasive glaucoma surgery (MIGS) as described herein. In a further embodiment, the MIGS is a trabeculotomy. In a further embodiment, the MIGS is a microtrabeculectomy. In a further embodiment, the MIGS is a suprachoroidal shunt. In a further embodiment, the MIGS is a trabecular bypass stent or shunt. In a further embodiment, the MIGS is a selective laser trabeculoplasty (SLT). In a further embodiment, the MIGS is a laser photocoagulation. In a further embodiment, the MIGS is endocyclophotocoagulation. In a further embodiment, the MIGS is laser trabeculoplasty.

In one embodiment, a CKLP1 prodrug of Formula I-Formula III is used as a secondary therapy to a beta-blocker, such as timolol, levobunolol, metipranolol, or carteolol and as an additive to a minimally (or micro) invasive glaucoma surgery (MIGS) as described herein. In a further embodiment, the MIGS is a trabeculotomy. In a further embodiment, the MIGS is a microtrabeculectomy. In a further embodiment, the MIGS is a suprachoroidal shunt. In a further embodiment, the MIGS is a trabecular bypass stent or shunt. In a further embodiment, the MIGS is a selective laser trabeculoplasty (SLT). In a further embodiment, the MIGS is a laser photocoagulation. In a further embodiment, the MIGS is endocyclophotocoagulation. In a further embodiment, the MIGS is laser trabeculoplasty. In a further embodiment, the MIGS is a trabeculotomy. In a further embodiment, the MIGS is a microtrabeculectomy. In a further embodiment, the MIGS is a suprachoroidal shunt. In a further embodiment, the MIGS is a trabecular bypass stent or shunt. In a further embodiment, the MIGS is a selective laser trabeculoplasty (SLT). In a further embodiment, the MIGS is a laser photocoagulation. In a further embodiment, the MIGS is endocyclophotocoagulation. In a further embodiment, the MIGS is laser trabeculoplasty.

In one embodiment, a CKLP1 prodrug of Formula I-Formula III is used as a secondary therapy to a ROCK inhibitor, such as ripasudil, netarsudil (Rhopressa), fasudil, RKI-1447, GSK429286A, or Y-30141 and as an additive to a minimally (or micro) invasive glaucoma surgery (MIGS) as described herein. In a further embodiment, the MIGS is a trabeculotomy. In a further
5 embodiment, the MIGS is a microtrabeculectomy. In a further embodiment, the MIGS is a suprachoroidal shunt. In a further embodiment, the MIGS is a trabecular bypass stent or shunt. In a further embodiment, the MIGS is a selective laser trabeculoplasty (SLT). In a further embodiment, the MIGS is a laser photocoagulation. In a further embodiment, the MIGS is endocyclophotocoagulation. In a further embodiment, the MIGS is laser trabeculoplasty.

10 In one embodiment, a CKLP1 prodrug of Formula I-Formula III is used as a secondary therapy to a second potassium channel opener, such as minoxidil, diazoxide, nicorandil, or pinacidil and as an additive to a minimally (or micro) invasive glaucoma surgery (MIGS) as described herein. In a further embodiment, the MIGS is a trabeculotomy. In a further embodiment, the MIGS is a microtrabeculectomy. In a further embodiment, the MIGS is a suprachoroidal shunt.
15 In a further embodiment, the MIGS is a trabecular bypass stent or shunt. In a further embodiment, the MIGS is a selective laser trabeculoplasty (SLT). In a further embodiment, the MIGS is a laser photocoagulation. In a further embodiment, the MIGS is endocyclophotocoagulation. In a further embodiment, the MIGS is laser trabeculoplasty.

In one embodiment, a CKLP1 prodrug of Formula I-Formula III is used as a secondary
20 therapy to a carbonic anhydrase inhibitor, such as dorzolamide (Trusopt®), brinzolamide (Azopt®), acetazolamide (Diamox®) or methazolamide (Neptazane®) and as an additive to a minimally (or micro) invasive glaucoma surgery (MIGS) as described herein. In a further embodiment, the MIGS is a trabeculotomy. In a further embodiment, the MIGS is a microtrabeculectomy. In a further embodiment, the MIGS is a suprachoroidal shunt. In a further
25 embodiment, the MIGS is a trabecular bypass stent or shunt. In a further embodiment, the MIGS is a selective laser trabeculoplasty (SLT). In a further embodiment, the MIGS is a laser photocoagulation. In a further embodiment, the MIGS is endocyclophotocoagulation. In a further embodiment, the MIGS is laser trabeculoplasty.

III. Pharmaceutical Compositions and Dosage Forms

Formulas I, II or III, including CKLP1, or a pharmaceutically acceptable salt of the present invention described herein can be administered in an effective amount to a host, typically a human, in need thereof for any of the indications described herein. The compound or its salt can be provided as the neat chemical, but is more typically administered as a pharmaceutical composition that includes an effective amount for a host, typically a human, in need of such treatment of Formulas I, II or III, including CKLP1, or a pharmaceutically acceptable salt thereof. Thus, in one embodiment, the disclosure provides pharmaceutical compositions comprising an effective amount of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, with at least one pharmaceutically acceptable carrier for any of the uses described herein. The pharmaceutical composition may contain a compound or salt thereof as the only active agent, or, in an alternative embodiment, the compound or salt thereof and at least one additional active agent.

The exact amount of the active compound or pharmaceutical composition described herein to be delivered to the host, typically a human, in need thereof will be determined by the health care provider to achieve the desired clinical benefit.

The pharmaceutical compositions contemplated here optionally include a carrier, as described further below. Carriers must be of sufficiently high purity and sufficiently low toxicity to render them suitable for administration to the patient being treated. The carrier can be inert or it can possess pharmaceutical benefits of its own. The amount of carrier employed in conjunction with the compound is sufficient to provide a practical quantity of material for administration per unit dose of the compound. Representative carriers include solvents, diluents, pH modifying agents, preservatives, antioxidants, suspending agents, wetting agents, viscosity agents, tonicity agents, stabilizing agents, and combinations thereof. In some embodiments, the carrier is an aqueous carrier.

One or more viscosity agents may be added to the pharmaceutical composition to increase the viscosity of the composition as desired. Examples of useful viscosity agents include, but are not limited to, hyaluronic acid, sodium hyaluronate, carbomers, polyacrylic acid, cellulosic derivatives, polycarbophil, polyvinylpyrrolidone, gelatin, dextrin, polysaccharides,

polyacrylamide, polyvinyl alcohol (including partially hydrolyzed polyvinyl acetate), polyvinyl acetate, derivatives thereof and mixtures thereof.

Solutions, suspensions, or emulsions for administration may be buffered with an effective amount of buffer necessary to maintain a pH suitable for the selected administration. Suitable
5 buffers are well known by those skilled in the art. Some examples of useful buffers are acetate, borate, carbonate, citrate, and phosphate buffers.

Formulas I, II or III, including CKLP1, or its pharmaceutically acceptable salt of the present invention described herein can be provided in any dosage strength that achieves the desired results and also depends on the route of administration. In certain embodiments, the
10 pharmaceutical composition is in a dosage form that contains from about 0.1 mg to about 2000 mg, from about 10 mg to about 1000 mg, from about 100 mg to about 800 mg, or from about 200 mg to about 600 mg of the active compound and optionally from about 0.1 mg to about 2000 mg, from about 10 mg to about 1000 mg, from about 100 mg to about 800 mg, or from about 200 mg to about 600 mg of an additional active agent in a unit dosage form. Examples are dosage forms
15 with at least about 0.1, 0.2, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 10, 15, 20, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 900, 1000, 1100, 1200, 1250, 1300, 1400, 1500, or 1600 mg of active compound or its salt. In certain embodiments, the dosage form has at least about 0.1 mg, 0.25 mg, 0.5 mg, 0.75 mg, 1 mg, 5 mg, 10 mg, 25 mg, 50 mg, 75 mg, 100 mg, 200 mg, 400 mg, 500 mg, 600 mg, 1000mg, 1200 mg, or 1600 mg of active compound
20 or its salt. The amount of active compound in the dosage form is calculated without reference to the salt.

In alternative embodiments, the pharmaceutical composition is in a dosage form that contains from about 0.005 mg to about 5 mg, from about 0.003 mg to about 3 mg, from about 0.001 mg to about 1 mg, from about 0.05 mg to about 0.5 mg, from about 0.03 mg to about 0.3
25 mg, or from about 0.01 mg to about 0.1 mg, or from about 0.01 to about 0.05 mg of a compound of the cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1. In one embodiment, the dosage form has at least about 0.01 mg, 0.02 mg, 0.025 mg, or 0.05 mg of active compound or its salt.

As non-limiting embodiments, a therapeutically effective amount of the present
30 compounds in a pharmaceutical dosage form may range, for example, from about 0.001 mg/kg to

about 100 mg/kg per day or more. A compound of Formula I, Formula II, or Formula III or a pharmaceutically acceptable salt thereof, may for example in non-limiting embodiments, be administered in amounts ranging from about 0.1 mg/kg to about 35 mg/kg per day of the patient, depending upon the pharmacokinetics of the agent in the patient. In an alternative embodiment, a compound of Formula I, Formula II, or Formula III or a pharmaceutically acceptable salt thereof, may be administered in amounts ranging from about 0.01 mg/kg to about 3.5 mg/kg per day of the patient, depending upon the pharmacokinetics of the agent in the patient.

In certain embodiments, a compound of Formula I, Formula II, or Formula III or a pharmaceutically acceptable salt thereof, including CKLP1, is administered for at least about one day, two days, three days, four days, five days, six days, seven days, eight days, nine days, ten days, two weeks, three weeks, one month, at least two months, at least three months, at least four months, at least five months, at least six months or more, including indefinitely during therapy. In certain embodiments, a compound of Formula I, Formula II, or Formula III or a pharmaceutically acceptable salt thereof, including CKLP1, is administered once, twice, three, or more times a day.

Non-limiting examples of buffers, with or without additional excipients or other additives, that can be used as a pharmaceutically acceptable formulation for an appropriate indication as described herein include, for example (with illustrative, but not limiting concentrations and pHs), Acetate Buffer (0.1 M, pH 5.0); BES-Buffered Saline (2X) (0.05 M, pH 6.95); Bicine (1 M, pH 8.26); CAPS (1 M, pH 10.4); CHES (1 M, pH 9.5); Citrate Buffer (0.1 M, pH 6.0); Citrate-Phosphate Buffer (0.15 M, pH 5.0); Diethanolamine (1 M, pH 9.8); EBSS (magnesium, calcium, phenol red) (pH 7.0); Glycine-HCl Buffer (0.1 M, pH 3.0); Glycine-Sodium Hydroxide Buffer (0.08 M, pH 10); HBSS (Hank's Balanced Salt Solution); HEPPSO (1 M, pH 7.85); HHBS (Hank's Buffer with Hepes); Hydrochloric Acid-Potassium Chloride Buffer (0.1 M, pH 2.0); Imidazole-HCl Buffer (0.05 M, pH 7.0); MES (0.5 M, pH 6); MOPS Buffer (10X) (0.2 M, pH 7); PBS (Phosphate Buffered Saline) (1 X, pH 7.4); Sodium Borate Buffer (1 M, pH 8.5); TAE (1 M, pH 8.6); TAE Buffer (50X) (0.04 M, pH 8.5); TBS (1 M, pH 7.4); TE Buffer 10X; Tricine (1 M, pH 8.05); Tris Buffer (1 M, pH 7.2); Acetate Buffer (pH 3.6 to 5.6); Carbonate-Bicarbonate Buffer (pH 9.2 to 10.6); Citrate Buffer (pH 3.0 to 6.2); Phosphate Buffer (pH 5.8 to 8.0); Potassium Phosphate (pH 5.8 to 8.0); and, Trizma® Buffer (pH 7.0 to 9.2).

Formulations for ocular, topical, enteric and parenteral delivery are described in more detail below.

Ocular Delivery

When used for ocular treatment, an effective amount of a Formula I, II or III, including CKLP1, or its pharmaceutically acceptable salt of the present invention herein can be administered, for example, as a topical formulation, such as a solution, suspension, or emulsion. The topical formulation typically comprises a pharmaceutically acceptable carrier, which can be an aqueous or non-aqueous carrier.

Examples of aqueous carries include, but are not limited to, an aqueous solution or suspension, such as saline, plasma, bone marrow aspirate, buffers, such as Hank's Buffered Salt Solution (HBSS), HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), Ringers buffer, ProVisc®, diluted ProVisc®, Provisc® diluted with PBS, Krebs buffer, Dulbecco's PBS, normal PBS, sodium hyaluronate solution (HA, 5 mg/mL in PBS), simulated body fluids including simulated aqueous humor, tears, plasma platelet concentrate and tissue culture medium or an aqueous solution or suspension comprising an organic solvent. Pharmaceutical formulations for ocular administration are preferably in the form of a sterile aqueous solution. Acceptable solutions include, for example, water, Ringer's solution, phosphate buffered saline (PBS), citrate buffered saline, and isotonic sodium chloride solutions. The formulation may also be a sterile solution, suspension, or emulsion in a non-toxic diluent or solvent such as 1,3-butanediol. In one embodiment, the carrier is PBS. In one embodiment, the carrier is citrate-buffer, including citrate buffered saline. Further examples of buffers that can be used in a pharmaceutically acceptable ocular formulation for an appropriate indication are described above.

Suitable non-aqueous pharmaceutically acceptable carriers include but are not limited to oleoyl polyethyleneglycol glycerides, linoleoyl polyethyleneglycol glycerides, lauroyl polyethyleneglycol glycerides, hydrocarbon vehicles like liquid paraffin (Paraffinum liquidum, mineral oil), light liquid paraffin (low viscosity paraffin, Paraffinum perliquidum, light mineral oil), soft paraffin (vaseline), hard paraffin, vegetable fatty oils like castor oil, peanut oil or sesame oil, synthetic fatty oils like middle chain triglycerides (MCT, triglycerides with saturated fatty acids, preferably octanoic and decanoic acid), isopropyl myristate, caprylocaproyl macrogol-8

glyceride, caprylocaproyl polyoxyl-8 glycerides, wool alcohols like cetylstearylalcohols, wool fat, glycerol, propylene glycol, propylene glycol diesters of caprylic/capric acid, polyethyleneglycols (PEG), semifluorinated alkanes (e.g. as described in WO 2011/113855) or a mixture of thereof. Preferably non-aqueous pharmaceutically acceptable vehicles used for the solution are hydrophobic.

Pharmaceutically acceptable excipients used in the topical ophthalmological pharmaceutical composition according to the present invention include but are not limited to stabilizers, surfactants, polymer-based carriers like gelling agents, organic co-solvents, pH active components, osmotic active components and preservatives.

Surfactants used in the topical ophthalmological pharmaceutical composition according to the present invention include but are not limited to lipids such as phospholipids, phosphatidylcholines, lecithin, cardiolipins, fatty acids, phosphatidylethanolamines, phosphatides, tyloxapol, polyethyleneglycols and derivatives like PEG 400, PEG 1500, PEG 2000, poloxamer 407, poloxamer 188, polysorbate 80, polysorbate 20, sorbitan laurate, sorbitan stearate, sorbitan palmitate or a mixture thereof, preferably polysorbate 80. Suitable polymer base carriers like gelling agents used in the topical ophthalmological pharmaceutical composition according to the present invention include but are not limited to cellulose, hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose (HPC), carboxymethyl cellulose (CMC), methylcellulose (MC), hydroxyethylcellulose (HEC), amylase and derivatives, amylopectins and derivatives, dextran and derivatives, polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), and acrylic polymers such as derivatives of polyacrylic or polymethacrylic acid like HEMA, carbopol and derivatives of the before mentioned or a mixture thereof.

A suitable pH active component such as a buffering agent or pH-adjusting agent used in the pharmaceutical composition according to the invention include but are not limited to acetate, borate, carbonate, citrate, and phosphate buffers, including disodium phosphate, monosodium phosphate, boric acid, sodium borate, sodium citrate, hydrochloric acid, sodium hydroxide. The pH active components are chosen based on the target pH for the composition which generally ranges from pH 4 - 9. In certain embodiments, the formulation comprising a compound or pharmaceutically acceptable salt thereof of Formula I-III has a pH approximately between 5 and 8, between 5.5 and 7.4, between 6 and 7.5, or between 6.5 and 7. In one embodiment, the

formulation comprises a citrate buffer at a pH around 6.5 to 7. In another embodiment, the formulation comprises a phosphate buffer at a pH around 6.5 to 7. Suitable osmotic active components used in the pharmaceutical composition according to the invention include but are not limited to sodium chloride, mannitol and glycerol.

5 Organic co-solvents used in the pharmaceutical composition according to the invention include but are not limited to ethylene glycol, propylene glycol, N-methyl pyrrolidone, 2-pyrrolidone, 3- pyrrolidinol, 1,4-butanediol, dimethylglycol monomethylether, diethyleneglycol monomethylether, solketal, glycerol, polyethylene glycol, polypropylene glycol.

10 Preservatives used in the pharmaceutical composition according to the invention include but are not limited to benzalkonium chloride, alkyl dimethylbenzylammonium chloride, cetrimide, cetylpyridinium chloride, benzododecinium bromide, benzethonium chloride, thiomersal, chlorobutanol, benzyl alcohol, phenoxethanol, phenylethyl alcohol, sorbic acid, methyl and propyl parabens, chlorhexidine digluconate, EDTA or mixtures thereof.

15 Viscosity agents may be added to the pharmaceutical composition to increase the viscosity of the composition as desired. Examples of useful viscosity agents include, but are not limited to, hyaluronic acid, sodium hyaluronate, carbomers, polyacrylic acid, cellulosic derivatives, polycarbophil, polyvinylpyrrolidone, gelatin, dextrin, polysaccharides, polyacrylamide, polyvinyl alcohol (including partially hydrolyzed polyvinyl acetate), polyvinyl acetate, derivatives thereof and mixtures thereof. In one embodiment, the viscosity agent is hyaluronic acid and the hyaluronic acid is cross-linked. In one embodiment, the viscosity agent is hyaluronic acid and hyaluronic acid is linear.

20 The topical dosage form can be administered, for example, once a day (q.d.), twice a day (b.i.d.), three times a day (t.i.d.), four times a day (q.i.d.), once every other day (Q2d), once every third day (Q3d), as needed, or any dosage schedule that provides treatment of a disorder described herein.

25 In certain nonlimiting embodiments, the pharmaceutical composition is in an ocular dosage form that contains from about 0.005 mg to about 5 mg, from about 0.003 mg to about 3 mg, from about 0.001 mg to about 1 mg, from about 0.05 mg to about 0.5 mg, from about 0.03 mg to about 0.3 mg, or from about 0.01 mg to about 0.1 mg, or from about 0.01 to about 0.05 mg of a compound

of the cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1.

In certain embodiments, the ocular solution comprises approximately 0.1% to 5.0% of a compound of Formula I-III or a pharmaceutically acceptable salt thereof as measured in mg/mL.

5 In certain embodiments, the ocular solution comprises approximately 5% to 30% of a compound of Formula I-III as measured in mg/mL. In certain embodiments, the solution comprises approximately 0.2% to 4.5%, 0.3% to 3.0%, 0.4% to 2.0%, or 0.5% to 1.5% of a compound of Formula I-III as measured in mg/mL. In certain embodiments, the solution comprises at least 10%, at least 8%, at least 5%, at least 4%, at least 3%, at least 2 %, at least 1%, at least 0.9%, at least
10 0.7%, at least 0.5%, at least 0.3%, or at least 0.1% of a compound of Formula I-III. In other embodiments, the solution comprises at least 30%, at least 25%, at least 20%, or at least 15% of a compound of Formula I-III. In certain embodiments, the solution comprises approximately 0.2%, 0.4%, or 0.8% of a compound of Formula I-III or salts thereof. In certain embodiments, the solution comprises approximately 0.5%, 1%, or 2% of a compound of Formula I-III or salts thereof.

15 In alternative embodiments, the ocular solution comprises approximately 0.01% to 5.0% of a compound of Formula I-III or a pharmaceutically acceptable salt thereof, including CKLP1, as measured in mg/mL. In certain embodiments, the solution comprises approximately 0.01% to 3%, 0.01% to 1.0%, 0.01% to 0.5%, 0.01% to 0.1%, 0.01% to 0.08%, or 0.01% to 0.05% of a compound of Formula I-III as measured in mg/mL.

20 In other embodiments, the solution has a concentration of a compound of Formula I-III or a pharmaceutically acceptable salt thereof, including CKLP1, ranging from about 2.5 mM to 500 mM. In certain embodiments, the concentration is not greater than about 550 mM, 500 mM, 450 mM, 400 mM, 350 mM, 300 mM, 250 mM, 200 mM, 150 mM, 100 mM, 50 mM, 45 mM, 40 mM, 35 mM, 30 mM, 25 mM, 20 mM, 15 mM, 10 mM, 8 mM, 6 mM, 5 mM, 4 mM, 3 mM, 2.5 mM,
25 2.0 mM, 1.5 mM, or 1.0 mM.

In alternative embodiments, the solution has a concentration of a compound of Formula I-III or a pharmaceutically acceptable salt thereof, including CKLP1, ranging from about 0.1 mM to 2.5 mM. In certain embodiments, the concentration is not greater than about 1.0 mM, 0.9 mM, 0.8 mM, 0.7 mM, 0.6 mM, 0.5 mM, 0.4 mM, 0.3 mM, 0.2 mM, or 0.1 mM.

In certain embodiments, the concentration of a compound of Formula I-III or a pharmaceutically acceptable salt thereof, including CKLP1, is in the range of approximately 0.2% - 2% (equivalent to a 5mM to 52mM solution). In certain embodiments, the concentration is at least 0.2% (equivalent to 5M), at least 0.4% (equivalent to 10 mM), at least 0.5% (equivalent to 12.5 mM), at least 0.8% (equivalent to 20 mM), at least 1% (equivalent to approximately 25 mM), or at least 2% (equivalent to approximately 50 mM).

In alternative embodiments, the concentration of a compound of Formula I-III or a pharmaceutically acceptable salt thereof, including CKLP1, is in the range of approximately 0.02% - 0.2%. In one embodiment, the concentration is at least 0.02%, at least 0.04%, at least 0.05%, at least 0.08%, at least 0.1%, or at least 0.2%.

A cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, can also be used for ocular therapy using an alternative route: intravitreal, intrastromal, intracameral, sub-tenon, sub-retinal, retro-bulbar, peribulbar, suprachoroidal, subchoroidal, choroidal, conjunctival, subconjunctival, episcleral, periocular, transscleral, posterior juxtасcleral, circumcorneal, or tear duct injections, or through a mucus, mucin, or a mucosal barrier, in an immediate or controlled release fashion or via an ocular device, or injection. In one embodiment, the ocular device is a contact lens that releases the cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1.

In one embodiment, a compound of a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, is administered via suprachoroidal injection. Suprachoroidal delivery is described in U.S. Patent Nos. 9,636,332; 9,539,139; 10,188,550; 9,956,114; 8,197,435; 7,918,814 and PCT Applications WO 2012/051575; WO 2015/095772; WO 2018/031913; WO 2017/192565; WO 2017/190142; WO 2017/120601; and WO 2017/120600.

A device for minimally invasive delivery of drugs to the suprachoroidal space may comprise a needle for injection of drugs or drug containing materials directly to the suprachoroidal space. The device may also comprise elements to advance the needle through the conjunctiva and sclera tissues to or just adjacent to the suprachoroidal space without perforation or trauma to the inner choroid layer. The position of the leading tip of the delivery device may be confirmed by non-invasive imaging such as ultrasound or optical coherence tomography, external depth markers

or stops on the tissue-contacting portion of the device, depth or location sensors incorporated into the device or a combination of such sensors. For example, the delivery device may incorporate a sensor at the leading tip such as a light pipe or ultrasound sensor to determine depth and the location of the choroid or a pressure transducer to determine a change in local fluid pressure from entering the suprachoroidal space. In certain embodiments, the suprachoroidal injection is conducted with a thin- or regular-walled needle of 26-, 27-, 28-, 29- or 30-gauge. In alternative embodiments, the suprachoroidal injection is conducted with a thin- or regular-walled needle of 31, 32, or 33-gauge. In further alternative embodiments, the suprachoroidal injection is conducted with a thin- or regular-walled needle of 34-gauge or smaller gauge.

Additional non-limiting examples of how to deliver the active compounds are provided in WO/2015/085251 titled "Intracameral Implant for Treatment of an Ocular Condition" (Envisia Therapeutics, Inc.); WO/2011/008737 titled "Engineered Aerosol Particles, and Associated Methods", WO/2013/082111 titled "Geometrically Engineered Particles and Methods for Modulating Macrophage or Immune Responses", WO/2009/132265 titled "Degradable compounds and methods of use thereof, particularly with particle replication in non-wetting templates", WO/2010/099321 titled "Interventional drug delivery system and associated methods", WO/2008/100304 titled "Polymer particle composite having high fidelity order, size, and shape particles", WO/2007/024323 titled "Nanoparticle fabrication methods, systems, and materials" (Liquidia Technologies, Inc. and the University of North Carolina at Chapel Hill); WO/2010/009087 titled "Iontophoretic Delivery of a Controlled-Release Formulation in the Eye", (Liquidia Technologies, Inc. and Eyegate Pharmaceuticals, Inc.) and WO/2009/132206 titled "Compositions and Methods for Intracellular Delivery and Release of Cargo", WO/2007/133808 titled "Nano-particles for cosmetic applications", WO/2007/056561 titled "Medical device, materials, and methods", WO/2010/065748 titled "Method for producing patterned materials", WO/2007/081876 titled "Nanostructured surfaces for biomedical/biomaterial applications and processes thereof" (Liquidia Technologies, Inc.).

In one embodiment, a cromakalim prodrug of Formula I-Formula III is stored as a depot in tissues and then slowly released over time where it is converted to lev cromakalim to induce an IOP-lowering effect. In one embodiment, a cromakalim prodrug of Formula I-Formula III is stored in the trabecular meshwork and then slowly released to the proximal distal outflow pathway. In

one embodiment, the return to baseline IOP following a dosage form of a cromakalim prodrug of Formula I-Formula III in a host in need thereof, including a human, is at least about 12 hours, at least about 24 hours, at least about 36 hours, at least about 48 hours, at least about 60 hours, or at least about 72 hours.

5

Topical Skin or Transdermal Delivery

Administration of a cromakalim prodrug or a pharmaceutically acceptable salt of Formula I-III, including CKLP1, may also include topical or transdermal administration. Pharmaceutical compositions suitable for topical application to the skin may take the form of a gel, ointment, cream, lotion, paste, spray, aerosol, or oil, and may optionally include petroleum jelly, lanoline, polyethylene glycol, alcohol, or a combination thereof.

Pharmaceutical compositions suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Pharmaceutical compositions suitable for transdermal administration may also be delivered by iontophoresis (see, for example, Pharmaceutical Research 3 (6):318 (1986)) and typically take the form of an optionally buffered aqueous solution of the active compound. In one embodiment, microneedle patches or devices are provided for delivery of drugs across or into biological tissue, particularly the skin. The microneedle patches or devices permit drug delivery at clinically relevant rates across or into skin or other tissue barriers, with minimal or no damage, pain, or irritation to the tissue.

A wide variety of skin care active and inactive ingredients may be advantageously combined with the present compounds in accordance with the present invention, including, but not limited to, conditioning agents, skin protectants, other antioxidants, UV absorbing agents, sunscreen actives, cleansing agents, viscosity modifying agents, film formers, emollients, surfactants, solubilizing agents, preservatives, fragrance, chelating agents, foaming or antifoaming agents, opacifying agents, stabilizing agents, pH adjustors, absorbents, anti-caking agents, slip modifiers, various solvents, solubilizing agents, denaturants, abrasives, bulking agents, emulsion stabilizing agents, suspending agents, colorants, binders, conditioning agent-emollients, surfactant emulsifying agents, biological products, anti-acne actives, anti-wrinkle and anti-skin atrophy actives, skin barrier repair aids, cosmetic soothing aids, topical anesthetics, artificial tanning agents

and accelerators, skin lightening actives, antimicrobial and antifungal actives, sebum stimulators, sebum inhibitors, humectants, and/or combinations thereof.

Conditioning agents may generally be used to improve the appearance and/or feel of the skin upon and after topical application via moisturization, hydration, plasticization, lubrication, and occlusion, or a combination thereof. Non-limiting examples of the conditioning component include, but are not limited to, mineral oil, petrolatum, C₇-C₄₀ branched chain hydrocarbons, C₁-C₃₀ alcohol esters of C₁-C₃₀ carboxylic acids, C₁-C₃₀ alcohol esters of C₂-C₃₀ dicarboxylic acids, monoglycerides of C₁-C₃₀ carboxylic acids, diglycerides of C₁-C₃₀ carboxylic acids, triglycerides of C₁-C₃₀ carboxylic acids, ethylene glycol monoesters of C₁-C₃₀ carboxylic acids, ethylene glycol diesters of C₁-C₃₀ carboxylic acids, propylene glycol monoesters of C₁-C₃₀ carboxylic acids, propylene glycol diesters of C₁-C₃₀ carboxylic acids, C₁-C₃₀ carboxylic acid monoesters and polyesters of sugars, polydialkylsiloxanes, polydiarylsiloxanes, polyalkarylsiloxanes, cyclomethicones having 3 to 9 silicon atoms, vegetable oils, hydrogenated vegetable oils, polypropylene glycol C₄-C₂₀ alkyl ethers, di C₈-C₃₀ alkyl ethers, and mixtures thereof. Non-limiting examples of straight and branched chain hydrocarbons having from about 7 to about 40 carbon atoms include, but are not limited to, dodecane, isododecane, squalane, cholesterol, hydrogenated oleyl isobutylene, docosane hexadecane, isohexadecane, C₇-C₄₀ isoparaffins, monoglycerides of C₁-C₃₀ carboxylic acids, diglycerides of C₁-C₃₀ carboxylic acids, triglycerides of C₁-C₃₀ carboxylic acids, ethylene glycol monoesters of C₁-C₃₀ carboxylic acids, ethylene glycol diesters of C₁-C₃₀ carboxylic acids, propylene glycol monoesters of C₁-C₃₀ carboxylic acids, and propylene glycol diesters of C₁-C₃₀ carboxylic acids, including straight chain, branched chain and aryl carboxylic acids, and propoxylated and ethoxylated derivatives of these materials.

Non-limiting examples of sugars include sucrose, mannitol, trehalose, glucose, arabinose, fucose, mannose, rhamnose, xylose, D-xylose, glucose, fructose, ribose, D-ribose, galactose, dextrose, dextran, lactose, maltodextrin, maltose, glycerol, erythritol, threitol, arabitol, xylitol, ribitol, sorbitol, galactitol, fucitol, iditol, inositol, volemitol, isomalt, maltitol, lactitol, maltotriitol, maltotetraitol, polyglycitol, aspartame, saccharin, stevia, sucralose, acesulfame potassium, advantame, alitame, neotame, and sucralose.

Non-limiting examples of sunscreens which are useful in the compositions include 4-N,N-(2-ethylhexyl)methylaminobenzoic acid ester of 2,4-dihydroxybenzophenone, 4-N,N-(2-

ethylhexyl)methylaminobenzoic acid ester with 4-hydroxydibenzoylmethane, 4-N,N-(2-ethylhexyl)-methylaminobenzoic acid ester of 2-hydroxy-4-(2-hydroxyethoxy)benzophenone, 4-N,N-(2-ethylhexyl)-methylaminobenzoic acid ester of 4-(2-hydroxyethoxy)dibenzoylmethane, 2-ethylhexyl p-methoxycinnamate, 2-ethylhexyl N,N-dimethyl-p-aminobenzoate, p-aminobenzoic acid, 2-phenylbenzimidazole-5-sulfonic acid, octocrylene, oxybenzone, homomenthyl salicylate, octyl salicylate, 4,4'-methoxy-t-butyl dibenzoylmethane, 4-isopropyl dibenzoylmethane, 3-benzylidene camphor, 3-(4-methylbenzylidene) camphor, titanium dioxide, zinc oxide, silica, iron oxide, and mixtures thereof. Other useful sunscreens include 4-aminobenzoic acid (PABA), benzylidene camphor, butyl methoxy dibenzoyl methane, diethanolamine p-methoxycinnamate, 5-dioxybenzone, ethyl dihydroxypropyl PABA, glyceryl aminobenzoate, homomenthyl salicylate, isopropyl dibenzoyl methane, lawsone and dihydroxyacetone, menthyl anthranilate, methyl anthranilate, methyl benzylidene camphor, octocrylene, octyl dimethyl PABA, octyl methoxycinnamate, oxybenzone, 2-phenylbenzimidazole-5-sulfonic acid, red petrolatum, sulisobenzene, titanium dioxide, triethanolamine salicylate, zinc oxide, and mixtures thereof.

Exact amounts of sunscreens which can be employed will vary depending upon the sunscreen chosen and the desired Sun Protection Factor (SPF) to be achieved.

Viscosity agents may be added to the topical formulation to increase the viscosity of the composition as desired. Examples of useful viscosity agents include, but are not limited to, water-soluble polyacrylic and hydrophobically modified polyacrylic resins such as Carbopol and Pemulen; starches such as corn starch, potato starch, and tapioca; gums such as guar gum and gum arabic; and, cellulose ethers such as hydroxypropyl cellulose, hydroxyethyl cellulose, carboxymethyl cellulose, and the like.

A wide variety of emulsifiers are also useful and include, but are not limited to, sorbitan esters, glyceryl esters, poly glyceryl esters, methyl glucose esters, sucrose esters, ethoxylated fatty alcohols, hydrogenated castor oil ethoxylates, sorbitan ester ethoxylates, polymeric emulsifiers, silicone emulsifiers, glyceryl monoesters, preferably glyceryl monoesters of C₁₆-C₂₂ saturated, unsaturated and branched chain fatty acids such as glyceryl oleate, glyceryl monostearate, glyceryl monopalmitate, glyceryl monobehenate, and mixtures thereof; polyglyceryl esters of C₁₆-C₂₂ saturated, unsaturated and branched chain fatty acids, such as polyglyceryl-4 isostearate, polyglyceryl-3 oleate, diglycerol monooleate, tetraglycerol monooleate and mixtures thereof;

5 methyl glucose esters, preferably methyl glucose esters of C₁₆-C₂₂ saturated, unsaturated and branched chain fatty acids such as methyl glucose dioleate, methyl glucose sesquihydrostearate, and mixtures thereof; sucrose fatty acid esters, preferably sucrose esters of C₁₂-C₂₂ saturated, unsaturated and branched chain fatty acids such as sucrose stearate, sucrose laurate, sucrose distearate (e.g., CRODESTA.RTM. F10), and mixtures thereof, C₁₂-C₂₂ ethoxylated fatty alcohols such as oleyl-2, oleyl-3, stearyl-2, and mixtures thereof; hydrogenated castor oil ethoxylates such as PEG-7 hydrogenated castor oil; sorbitan ester ethoxylates such as PEG-40 sorbitan monooleate, Polysorbate-80, and mixtures thereof; polymeric emulsifiers such as ethoxylated dodecyl glycol copolymer; and silicone emulsifiers such as lauryldimethicone copolyol, cetyldimethicone, dimethicone copolyol, and mixtures thereof.

Systemic Delivery

15 In another embodiment, a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, is administered in an effective amount via any systemic route that achieves the desired effect. Examples are enteral or parenteral administration, including via oral, buccal, sublingual, intravenous, subcutaneous, intramuscular, intrathecal, or intranasal delivery, including a solution, a suspension, emulsion, or a lyophilized powder. In some instances, the composition is distributed or packaged in a liquid form. Alternatively, formulations can be packaged as a solid, obtained, for example by lyophilization of a suitable liquid formulation. The solid can be reconstituted with an appropriate carrier or diluent prior to administration. In one embodiment, the compound is administered vaginally via a suppository, a cream, a gel, a lotion, or an ointment.

25 Other forms of administration include oral, rectal, sublingual, sublabial, or buccal and typical dosage forms for these routes include a pill, a tablet, a capsule, a solution, a suspension, an emulsion, or a suppository.

In one embodiment, a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, is administered via the inhaled pulmonary route. Dosage forms for pulmonary drug delivery include propellants, non-aqueous inhalers, dry powder inhalers, and jet or ultrasonic nebulizers.

Oral Delivery

In one aspect, a cromakalim prodrug or a pharmaceutically acceptable salt thereof of Formula I-III, including CKLP1, is administered orally. The cromakalim prodrug can be formulated using any desired techniques including formulating the prodrug as a neat chemical (for example a powder, morphic form, amorphous form, or oil), or mixing the prodrug with a pharmaceutically acceptable excipient. The resulting pharmaceutically acceptable composition for oral delivery contains an effective amount of the prodrug or a pharmaceutically acceptable salt thereof and one or more pharmaceutically acceptable excipients.

Excipients

Pharmaceutically acceptable excipients should be of sufficiently high purity and sufficiently low toxicity to render them suitable for administration to the patient being treated. The excipient can be inert or it can possess pharmaceutical benefits of its own. The amount of excipient employed in conjunction with the compound is sufficient to provide a practical quantity of material for administration per unit dose of the compound. Classes of excipients include, but are not limited to binders, buffering agents, coloring agents, diluents, disintegrants, emulsifiers, fillers, flavorants, glidants, lubricants, pH modifiers, preservatives, stabilizers, surfactants, solubilizers, tableting agents, and wetting agents. Exemplary pharmaceutically acceptable excipients include sugars, starches, celluloses, powdered tragacanth, malt, gelatin, talc, and vegetable oils. Examples of other matrix materials, fillers, or diluents include lactose, mannitol, xylitol, microcrystalline cellulose, calcium diphosphate, and starch. Examples of surface-active agents include sodium lauryl sulfate and polysorbate 80. Examples of drug complexing agents or solubilizers include the polyethylene glycols, caffeine, xanthene, gentisic acid and cyclodextrins. Examples of disintegrants include sodium starch glycolate, sodium alginate, carboxymethyl cellulose sodium, methyl cellulose, colloidal silicon dioxide, and croscarmellose sodium. Examples of binders include methyl cellulose, microcrystalline cellulose, starch, gums, and tragacanth. Examples of lubricants include magnesium stearate and calcium stearate. Examples of pH modifiers include acids such as citric acid, acetic acid, ascorbic acid, lactic acid, aspartic acid, succinic acid, phosphoric acid, and the like; bases such as sodium acetate, potassium acetate, calcium oxide, magnesium oxide, trisodium phosphate, sodium hydroxide, calcium hydroxide, aluminum hydroxide, and the like, and buffers

generally comprising mixtures of acids and the salts of said acids. Optionally, other active agents may be included in a pharmaceutical composition, so long as they do not substantially interfere with the activity of the compound of the present invention.

In certain embodiments the excipient is selected from phosphoglyceride; phosphatidylcholine; dipalmitoyl phosphatidylcholine (DPPC); dioleoylphosphatidyl ethanolamine (DOPE); dioleyloxypropyltriethylammonium (DOTMA); dioleoylphosphatidylcholine; cholesterol; cholesterol ester; diacylglycerol; diacylglycerolsuccinate; diphosphatidyl glycerol (DPPG); hexanedecanol; fatty alcohol, polyethylene glycol (PEG); polyoxyethylene-9-lauryl ether; a surface active fatty acid, such as palmitic acid or oleic acid; fatty acid; fatty acid monoglyceride; fatty acid diglyceride; fatty acid amide; sorbitan trioleate (Span®85) glycocholate; sorbitan monolaurate (Span®20); polysorbate 20 (Tween®20); polysorbate 60 (Tween®60); polysorbate 65 (Tween®65); polysorbate 80 (Tween®80); polysorbate 85 (Tween®85); polyoxyethylene monostearate; surfactin; a poloxomer; a sorbitan fatty acid ester such as sorbitan trioleate; lecithin; lysolecithin; phosphatidylserine; phosphatidylinositol; sphingomyelin; phosphatidylethanolamine (cephalin); cardiolipin; phosphatidic acid; cerebroside; dicetylphosphate; dipalmitoylphosphatidylglycerol; stearylamine; dodecylamine; hexadecylamine; acetyl palmitate; glycerol ricinoleate; hexadecyl stearate; isopropyl myristate; tyloxapol; poly(ethylene glycol)5000-phosphatidylethanolamine; poly(ethylene glycol)400-monostearate; phospholipid; synthetic and/or natural detergent having high surfactant properties; deoxycholate; cyclodextrin; chaotropic salt; ion pairing agent; glucose, fructose, galactose, ribose, lactose, sucrose, maltose, trehalose, cellbiose, mannose, xylose, arabinose, glucuronic acid, galacturonic acid, mannuronic acid, glucosamine, galactosamine, and neuramic acid; pullulan, cellulose, microcrystalline cellulose, hydroxypropyl methylcellulose (HPMC), hydroxycellulose (HC), methylcellulose (MC), dextran, cyclodextrin, glycogen, hydroxyethylstarch, carageenan, glycon, amylose, chitosan, N,O-carboxymethylchitosan, algin and alginic acid, starch, chitin, inulin, konjac, glucomannan, pustulan, heparin, hyaluronic acid, curdlan, and xanthan, mannitol, sorbitol, xylitol, erythritol, maltitol, and lactitol, a pluronic polymer, polyethylene, polycarbonate (e.g. poly(1,3-dioxan-2-one)), polyanhydride (e.g. poly(sebacic anhydride)), polypropylfumerate, polyamide (e.g. polycaprolactam), polyacetal, polyether, polyester (e.g., polylactide, polyglycolide, polylactide-co-glycolide, polycaprolactone, polyhydroxyacid (e.g. poly(β-

hydroxyalkanoate)), poly(orthoester), polycyanoacrylate, polyvinyl alcohol, polyurethane, polyphosphazene, polyacrylate, polymethacrylate, polyurea, polystyrene, and polyamine, polylysine, polylysine-PEG copolymer, and poly(ethyleneimine), poly(ethylene imine)-PEG copolymer, glycerol monocaprylocaprate, propylene glycol, Vitamin E TPGS (also known as d- α -Tocopheryl polyethylene glycol 1000 succinate), gelatin, titanium dioxide, polyvinylpyrrolidone (PVP), hydroxypropyl methyl cellulose (HPMC), hydroxypropyl cellulose (HPC), methyl cellulose (MC), block copolymers of ethylene oxide and propylene oxide (PEO/PPO), polyethyleneglycol (PEG), sodium carboxymethylcellulose (NaCMC), or hydroxypropylmethyl cellulose acetate succinate (HPMCAS).

Oral Dosage Forms

Typical dosage forms for oral administration includes a pill, a tablet, a capsule, a gel cap, a solution, a suspension, or an emulsion. The dosage form may also feature compartmentalization. For example, when the dosage form is a pill, tablet, or capsule, it may have different layers of material which have different excipients or different concentrations of excipients. For example, an enteric coated oral tablet may be used to enhance bioavailability of the compounds for an oral route of administration. The enteric coating will be a layer of excipient that allows the tablet to survive stomach acid. The most effective dosage form will depend upon the bioavailability/pharmacokinetic of the particular agent chosen as well as the severity of disease in the patient. Oral dosage forms are particularly preferred, because of ease of administration and prospective favorable patient compliance.

In certain embodiments the oral dosage form contains one or more additional active agents as described herein. In certain embodiments the second active agent is administered separately from the compound of the present invention.

In another embodiment one dosage form may be converted to another to favorably improve the properties. For example, when making a solid pharmaceutically acceptable composition a suitable liquid formulation can be lyophilization. The solid can be reconstituted with an appropriate carrier or diluent prior to administration.

Oral pharmaceutical compositions can contain any amount of active compound that achieves the desired result, for example between 0.1 and 99 weight % (wt.%) of the compound

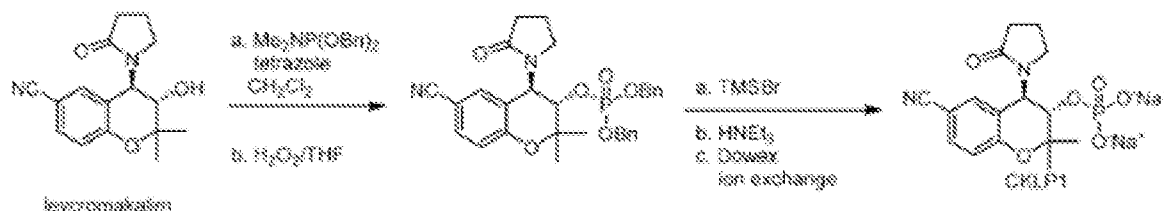
and usually at least about 5 wt.% of the compound. Some embodiments contain at least about 10%, 15%, 20%, 25 wt.% to about 50 wt.% or from about 5 wt.% to about 75 wt.% of the compound.

The oral dosage form can be administered, for example, once a day (q.d.), twice a day (b.i.d.), three times a day (t.i.d.), four times a day (q.i.d.), once every other day (Q2d), once every third day (Q3d), as needed, or any dosage schedule that provides treatment of a disorder described herein.

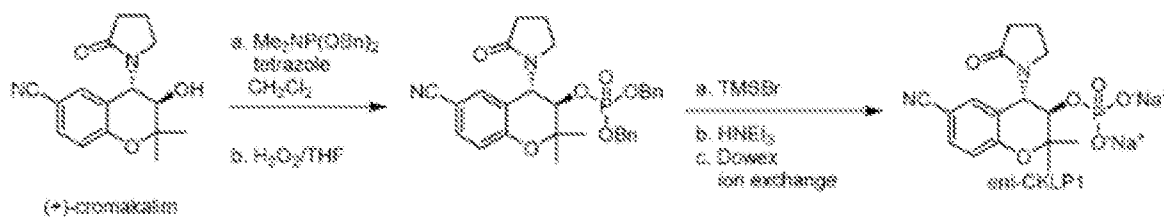
General Synthesis of Compounds of the Present Invention and Pharmaceutically Acceptable Salts Thereof.

The described pharmaceutically acceptable salts of the present invention can be prepared according to known methods. For example, the skilled artisan can prepare the sodium salt of CKLP1 or its enantiomer (ent-CKLP1) via Scheme 1 and Scheme 2 below. Various modifications can be made to these synthetic sequences to prepare other pharmaceutically acceptable salts. This process is described in more detail below.

General Scheme 1:



General Scheme 2:



These schemes and other methods of synthesis for CKLP1 and ent-CKLP1 are described in more detail in Roy Chowdhury *et al.*, *J. Med. Chem.*, 2016, 59(13), 6221-6231 and WO2015/117024 application.

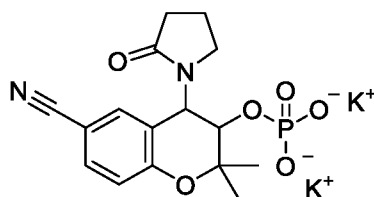
Synthesis of CKLP1 and ent-CKLP1 from dibenzyl ((3S,4R)-6-cyano-2,2-dimethyl-4-(2-oxopyrrolidin-1-yl)chroman-3-yl) phosphate

To a solution of dibenzyl ((3S,4R)-6-cyano-2,2-dimethyl-4-(2-oxopyrrolidin-1-yl)chroman-3-yl) phosphate (65.5 mg, 0.120 mmol) in dry CH₂Cl₂ (3 mL) was added TMSBr (53
 5 μL, 0.40 mmol) by syringe. After stirring for 6 hours, the reaction mixture was concentrated under reduced pressure. The resulting residue was purified by chromatography (0% acetonitrile/20 mM triethylammonium acetate buffer to 100% acetonitrile, Cis column) to yield 53.5 mg white solid after lyophilization. To prepare the sodium salt, a 1 cm wide column was filled with 12 cm of
 10 DOWEX 50W2 (50-100 mesh) ion exchange resin. The column was prepared by sequentially washing with 1:1 acetonitrile/water, 1M aqueous NaHCO₃, water, and then finally 1:1 acetonitrile/water. The reaction product was dissolved in 1:1 acetonitrile/water and loaded onto the column, which was eluted with 1:1 acetonitrile/water. The product containing fractions were lyophilized to furnish as a white solid (40.9 mg, 83% yield).

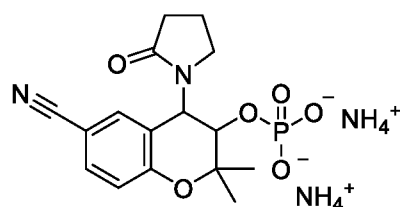
Ion Exchange Chromatography

In certain embodiments, the phosphate ester salts described herein can be formed via ion exchange as described in Scheme 1 and Scheme 2. When using ion exchange chromatography, the resulting cation is the cation that was present in the ion exchange wash solution. For example, in Scheme 1 and Scheme 2 the sodium cation of CKLP1 and ent-CKLP1 is the sodium in NaHCO₃.
 20 Thus, the skilled artisan could instead wash the ion exchange column with a different salt instead to prepare different pharmaceutically acceptable salts of the present invention.

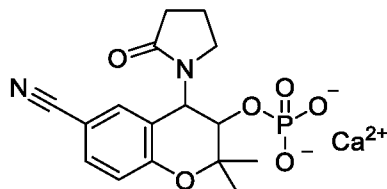
For example, the potassium salt can be generated by substituting 1M NaHCO₃ for 1M K₂CO₃, KHCO₃ or KOH, to afford the compound



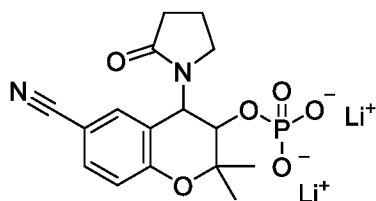
For example, the ammonium salt can be generated by substituting 1M NaHCO₃ for 1M (NH₄)₂CO₃ or NH₄OH, to afford the compound



For example, the calcium salt can be generated by substituting 1M NaHCO₃ for 1M CaCO₃ or Ca(OH)₂, to afford the compound



5 For example, the calcium salt can be generated by substituting 1M NaHCO₃ for 1M Li₂CO₃ or LiOH, to afford the compound



Other column material, salt washes, and concentrations can be utilized as desired.

10 *Synthetic Salt Formation*

In certain embodiments, the phosphate esters described herein can be formed by direct chemical reaction as an alternative to ion exchange. For example, to generate a sodium salt of the compounds described herein, the acid version of the compound can be reacted with an aqueous solution or base solution such as NaOH, NaHCO₃, Na₂CO₃, or sodium acetate in a reaction vessel.

15 In certain embodiments, other aqueous solutions may be used. For example, potassium hydride, lithium hydride, calcium hydride, acetate salts, sulfate salts, phosphate salts, and the like.

In certain embodiments, the chemical reaction can occur wherein the equivalence ratio is the same, for example a 1:1 ratio or wherein the equivalence ratio is different, for example, a ratio of 1:10; 1:5; 1:3; 1:2; or 1:1.5 CKLP1 to cation source. Concentrations of salt in solution can also
20 be varied. For example, the chemical reaction can occur wherein the sample is washed with 1M aqueous NaHCO₃; however, the chemical reaction can also occur where the sample is washed with

<1M or >1M aqueous NaHCO_3 as desired. This variance in equivalency is also applicable for chemical reactions involving other salts or base solutions as desired to afford a salt of the present invention.

Alternately, other salts may be prepared from the following: (a) metal hydroxides, for example any alkali metal hydroxides (e.g., NaOH and KOH), divalent metals (such as magnesium, calcium, and the like), and (b) organic hydroxides, for example organic compounds which include at least one tertiary amine, ammonium group, or at least one quaternary ammonium ion (e.g., diethylaminoethanol, triethylamine, hydroxyethylpyrrolidine, choline and hexamethylhexamethylenediammonium, and the like).

Salts of the compounds described herein, may be prepared by reacting the compound with an alkali metal hydroxide or alkali metal alkoxide, such as for example, NaOH , KOH or NaOCH_3 , in a variety of solvents which may be selected for example from low molecular weight ketones (e.g., acetone, methyl ethyl ketone, and the like), tetrahydrofuran (THF), dimethylformamide (DMF), and n-methylpyrrolidinone, and the like. In one embodiment the solvent is water. In another embodiment the solvent is THF.

The compounds described herein, may also form salts with organic cations that include at least one tertiary amine or ammonium cation. Organic cation compounds can have +1, +2, +3, or +4 charge per molecule by inclusion of one, two, three or four tertiary amine or ammonium ions within the compound, respectively. When a multicharged compound is used, the tertiary amine or quaternary ammonium moieties are preferably separated by a chain of at least 4 atoms, more preferably by a chain of at least 6 atoms, such as for example, hexamethyl hexamethylene diammonium dihydroxide, wherein the quaternary ammonium moieties are separated by — $(\text{CH}_2)_6$ —.

Salts of the compounds described herein, may be prepared by reacting the compound with compounds that include at least one tertiary amine or quaternary ammonium ion (e.g., choline hydroxide, hexamethylhexamethylene diammonium dihydroxide) in a solvent selected from low molecular weight ketones (e.g., acetone, methyl ethyl ketone), tetrahydrofuran, dimethylformamide, and n-methyl pyrrolidinone. As with the preparation of salts from alkali metal hydroxides, amine and ammonium containing compounds typically do not form salts when the solvent is an alcohol.

Typically, basic addition of salts of the compounds described herein, may include those containing hexamethyl hexamethylene diammonium, choline, sodium, potassium, methyldiethyl amine, triethylamine, diethylamino-ethanol, hydroxyethyl pyrrolidine, tetrapropylammonium and tetrabutylphosphonium ions.

5 Typically, basic addition of salts of the compounds described herein, may be prepared using any suitable reagent, for example, hexamethyl hexamethylene diammonium dihydroxide, choline hydroxide, sodium hydroxide, sodium methoxide, potassium hydroxide, potassium methoxide, ammonium hydroxide, tetrapropylammonium hydroxide, or tetrabutylphosphonium hydroxide. The basic addition of salts can be separated into inorganic salts (e.g., sodium, potassium
10 and the like) and organic salts (e.g., choline, hexamethyl hexamethylene diammonium hydroxide, and the like).

Salts of the compounds described herein may include organic or inorganic counter ions, including but not limited to, calcium, dimeglumine, dipotassium, disodium, meglumine, polistirex, or tromethamine. Suitable, organic cations include compounds having tertiary amines or
15 quaternary ammonium groups.

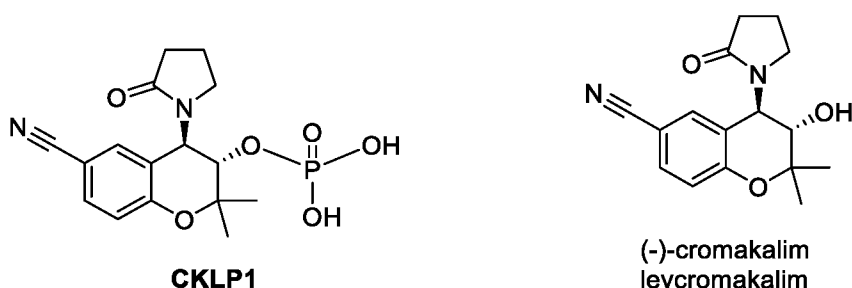
Pharmaceutically acceptable salts of the compounds described herein may also include basic addition of salts such as those containing chloroprocaine, procaine, aluminum, calcium, lithium, magnesium, potassium, sodium, ammonium, and alkylamine. For example, see Remington's Pharmaceutical Sciences, 19th ed., Mack Publishing Co., Easton, Pa., Vol. 2, p. 1457,
20 1995.

Salts of the compounds described herein, may be prepared, for example, by dissolving the free-base form of a compound in a suitable solvent, such as an aqueous or aqueous-alcohol in solution containing the appropriate acid and then isolated by evaporating the solution. In another example, a salt is prepared by reacting the free base and acid in an organic solvent.

25 Solvents useful in the preparation of pharmaceutically acceptable salts of the compounds described herein include organic solvents, such as for example, acetonitrile, acetone, alcohols (e.g., methanol, ethanol and isopropanol), tetrahydrofuran, methyl ethyl ketone (MEK), ethers (e.g., diethyl ether), benzene, toluene, xylenes, dimethylformamide (DMF), and N-methylpyrrolidinone (NMP), and the like. In one embodiment the solvents are selected from acetonitrile and MEK.

Example 1. Levcromakalim modulates the Human ATP-sensitive Potassium Channel

The ability of CKLP1 and the active moiety of CKLP1, levcromakalim, to pharmacologically modulate the function of the human ATP-sensitive potassium channel comprised of Kir6.2 (encoded by the human gene KCNJ11) coupled to the sulfonylurea receptor SUR2B (encoded by the human gene ABCC9) was studied. KATP channel modulatory activity of both compounds was determined using fluorescence-based changes in cellular membrane potential and the activity was compared to known reference KATP channel activators (pinacidil and cromakalim).



Compounds were dissolved up to 10 mM stock solution in DMSO. Ten-point concentration response curves were generated at 100x concentration in 100% DMSO. Compound source plates were made by serially diluting 10 mM compound stocks in DMSO to generate a progressive semilog dilution schema. Dose response stock plate (10 μ L) were then transferred into assay plates containing 90 μ L of assay buffer, generating 10x working concentrations. Final assay test concentration ranges of 100 μ M to 0.003 μ M with a final DMSO concentration of 1.0%.

Human embryonic kidney (HEK) cells stably expressing human Kir6.2/SUR2B KATP channel subunits were plated onto 96 well, black poly-d-lysine (PDL)-coated microplates and maintained in growth media the day prior to use for experiments. Media was removed from the plates and 90 μ L of 1X stock of the membrane potential sensitive fluorescent dye FMP-Blue resuspended in assay buffer (EBSS – in mM: NaCl 145, KCl 2, Glucose 5, CaCl₂ 1.8, MgCl₂ 0.8, HEPES 10, pH 7.4 with NaOH, 290-300 mOsm) was added to the cells. Cells were incubated at room temperature, protected from light, for 45-60 minutes. After the incubation period, cell, test compound and glibenclamide plates were loaded onto the fluorescence plate reader (FLIPR-TETRA™) and the scanning initiated. The FLIPR measured a 10 second baseline and then added

10 μ L of 10x the final desired concentration of test agent. Changes in fluorescence were monitored for an additional 5 minutes. After the 5-minute compound incubation, 10 μ L of the receptor inhibitor glibenclamide was added to the cell plate (10 μ M final glibenclamide concentration). Changes in fluorescence was then monitored for an additional 5 minutes.

- 5 Compound modulation of Kir6.2/SUR2B KATP channel mediated changes in cellular membrane potential was determined as follows. After the administration of compound, FMP fluorescence was monitored for a 5-minute period. The following parameters were recorded and exported from the FLIPR: the average relative fluorescence response (RFU) of 5 images taken at the 5-minute point in the assay with the average background EBSS buffer response subtracted.
- 10 Data was then normalized against the control response to 100 μ M pinacidil. Test agent effect was calculated as % activation using the following formula:

$$\frac{\% \text{ activation} = (\text{RFU test agent} - \text{Plate Ave RFU Buffer Control})}{(\text{Plate Ave. RFU } 100 \mu\text{M pinacidil Control} - \text{Plate Ave RFU Buffer Control}) \times 100}$$

15

- Activation of potassium efflux through human Kir6.2 post binding of levcromakalim to the SUR2B receptor resulted in a concentration-dependent hyperpolarization of the cell membrane potential in a glibenclamide sensitive manner. FIG. 1A illustrates the time course of the average FLIPR fluorescence response seen for levcromakalim across three test concentrations (30 μ M, 3 μ M, and 0.3 μ M). FIG. 1B shows the fitted concentration response curve used to determine the EC₅₀ for levcromakalim. Curve fits were performed in GraphPad Prism graphing software using a 4-parameter, variable slope fit equation.
- 20 Application of CKLP1 did not result in any significant hyperpolarization up to the maximum tested concentration of 100 μ M. FIG. 2A illustrates the average FLIPR time course response observed for CKLP1 for top tested concentration (100 μ M). FIG. 2B shows the fitted concentration response curve used to determine the EC₅₀ of CKLP1. Curve fits were performed in GraphPad Prism using a 4-parameter, variable slope fit equation. The fitted EC₅₀ for all test agents are summarized in Table 1. FIG. 3 shows the fitted concentration response curve used to determine the EC₅₀ of reference compounds pinacidil and cromakalim.

25

Table 1: Ability of Test Compounds to Modulate KATP Channel

Test Compound	EC ₅₀ (μM) (mean ± SEM)	# replicates
Levcromakalim	0.534 ± 0.05	6 (across 2 experiment days)
CKLP1	>100 ± NA	6 (across 2 experiment days)
Pinacidil	5.49 ± 0.99	6 (across 2 experiment days)
Cromakalim	1.35 ± 0.12	6 (across 2 experiment days)

Levcromakalim produced a concentration dependent hyperpolarization of HEK-Kir6.2/SUR2B cells with an EC₅₀ of 0.53 μM. By comparison, CKLP1 failed to produce any significant activation of human Kir6.2/SUR2B channels up to the top concentration tested in the assay (100 μM). Reference KATP channel activators pinacidil and cromakalim both produced concentration dependent hyperpolarization of HEK-Kir6.2/SUR2B cells with EC₅₀ values of 5.5 μM and 1.4 μM respectively. Hyperpolarization of HEK-Kir6.2/SUR2B cells by levcromakalim and the reference KATP activators pinacidil and cromakalim were observed to be reversed by coadministration of the agents with the established KATP sulfonylurea inhibitor glibenclamide (10 μM) confirming that levcromakalim mediated hyperpolarization was mediated by activation of Kir6.2/SUR2B KATP channels. In contrast, prodrug CKLP1 lacked any clear activation of Kir6.2/SUR2B KATP channels when present at up to 100 μM. The maximal response seen at the top concentration tested of 100 μM was 9.4% activation +/- 3.6% (standard deviation).

Example 2. *In Vitro* Conversion of CKLP1 to Levcromakalim

Conversion of CKLP1 (200 μM-5.0 mM) was examined by LC/MS-MS following incubation at pH 7.4 with either human alkaline phosphatase (ALP), acid phosphatase, or 5'-nucleotidase (2.01 nM-1.0 μM) for up to 2 hours. Human ALP, but not acid phosphatase or 5'-nucleotidase, converted CKLP1 to levcromakalim *in vitro*, with Michaelis constant (K_m) and the rate constants for the catalytic conversion of substrate into product (k_{cat}) values of 630 μM and 15 min⁻¹, respectively.

In the two separate studies described below, dose- and time-dependent analysis of CKLP1 (0.01-40.0 mM) conversion to levcromakalim was determined following incubation with human ALP (0.0002-0.2 U/μl) for up to 72 hours. To activate CKLP1, the phosphate is hydrolyzed from

phosphatases to produce levcromakalim, the active moiety that can open (activate) ATP-sensitive potassium channels. As discussed below, CKLP1 is converted by human alkaline phosphatase to levcromakalim in a concentration-dependent manner.

5 ***Fixed Concentration of CKLP1 With Varying Concentrations of Human Alkaline Phosphatase***

In the first experiment, a fixed concentration of CKLP1 solution was incubated with varying concentrations of placenta-derived human alkaline phosphatase. To accomplish this, placenta-derived human alkaline phosphatase (0.0002 U) in a 100- μ L volume was added to each
10 of thirteen 1.5-mL tubes. To each tube, CKLP1 was then added at a fixed concentration [10 mM (0.4%)]. Tubes were inverted twice and incubated in a water bath at 37°C. At each of 13 different time points (0, 1, 2.5, 5, 15, and 30 minutes and at 1, 2, 4, 8, 24, 48, and 72 hours), a single tube was removed, and the reaction was stopped by the addition of 2 volumes (200 μ L) of acetonitrile. Samples were stored at -80°C. The experiment was repeated with 10 mM CKLP1 (0.4%) in the
15 presence of placenta-derived human alkaline phosphatase at concentrations of 0.002 U, 0.02 U, and 0.2 U. All assays were performed at pH 10.

At a fixed concentration of CKLP1 in solution [10 mM (0.4%)], up to approximately 21% of CKLP1 was converted to levcromakalim in a human alkaline phosphatase concentration-dependent manner (FIG. 4A and FIG. 4B). For example, the conversion rates of CKLP1 (fixed at
20 a concentration of 10 mM [0.4%]) to levcromakalim at 24 hours were 0.4%, 1.3%, 4.8%, and 13.7% in the presence of human alkaline phosphatase in concentrations of 0.0002 U/100 μ L, 0.002 U/100 μ L, 0.02 U/100 μ L, and 0.2 U/100 μ L, respectively.

Fixed Concentration of Human Alkaline Phosphatase with Varying Concentrations of CKLP1

25 In a second experiment, a fixed concentration of placenta-derived human alkaline phosphatase was incubated with varying concentrations of CKLP1. To accomplish this, a fixed concentration of 0.02 U of placenta-derived human alkaline phosphatase in a 100- μ L volume was added to each of thirteen 1.5-mL tubes. To each tube, CKLP1 was then added [0.01 mM (0.0004%)]. Tubes were inverted twice and incubated in a water bath at 37°C. At each of 13
30 different time points (0, 1, 2.5, 5, 15, and 30 minutes and at 1, 2, 4, 8, 24, 48, and 72 hours), a

single tube was removed and the reaction was stopped by the addition of 2 volumes (200 μ L) of acetonitrile. Samples were stored at -80°C . The experiment was repeated with the same concentration of placenta-derived human alkaline phosphatase (0.02 U) and various concentrations of CKLP1 [0.1 mM (0.004%), 1 mM (0.04%), 10 mM (0.4%), 20 mM (0.8%), and 40 mM (1.6%)].

5 All assays were performed at pH 10.

Human alkaline phosphatase, present at a fixed concentration [0.02 U/100 μ L], converted CKLP1 to levcromakalim in a CKLP1 inverse-concentration-dependent manner (FIG. 5A and FIG. 5B). For example, in the presence of the fixed concentration of placenta-derived human alkaline phosphatase (0.02 U/100 μ L), the conversion rates of CKLP1 to levcromakalim at 24 hours were
10 26.9%, 20.0%, 5.2%, 4.9%, 3.2%, and 1.7% when CKLP1 was present in concentrations of 0.01 mM, 0.1 mM, 1 mM, 10 mM, 20 mM, and 40 mM, respectively. The maximum rate of reaction (V_{max}) was 1.35×10^{-4} mM/min and the Michaelis constant (K_m) was 0.399 mM.

Example 3. *In Vitro* Conversion of CKLP1 to Levcromakalim in Human Ocular Tissues

15 A study was conducted to determine if CKLP1 is converted to levcromakalim in ocular tissue and fluid. To assess conversion of CKLP1 to levcromakalim in human ocular tissues and fluids, human donor eyes from a 70-year old female were obtained so that human ocular tissues could be dissected and used for conversion studies.

Aqueous humor was collected from each eye and combined in a single 1.5-mL tube. The
20 eyes were then bisected at the equator, and vitreous humor was collected from both eyes, combined, and placed in a 15-mL conical tube and centrifuged at 1500 rpm for 10 minutes. Following centrifugation, the upper supernatant phase (less viscous region) was isolated and placed in a 1.5-mL tube. Tubes containing aqueous and vitreous humor were stored on ice. The following tissues were dissected from the eyes: cornea, retina, optic nerve, sclera, iris, ciliary body,
25 and trabecular meshwork. The tissue samples were placed in 1.5-mL tubes containing approximately 200 μ L of 50 mM Tris buffer pH 7.1. Each sample, except for the optic nerve sample, contained tissue from both eyes. Samples were stored on ice. Tissues were independently homogenized with a Polytron PT 1200 (setting 8) and placed on ice. The trabecular meshwork was lysed using a small pestle. In between samples, the homogenizer was cleaned and washed
30 thoroughly with a minimum of 200 mL of distilled water.

Remaining tissue and debris were pelleted in an Eppendorf 5415C centrifuge at 13,000 rpm for 2 minutes. Supernatants were isolated and placed in clean 1.5-mL tubes. A Bradford protein assay was performed with 5 μ L of supernatant of each sample. At the completion of the assay, 200- μ L aliquots of each sample were placed in a 96-well plate, and protein concentrations were read using a TECAN Infinite® M200 plate reader at 595 nm. The final concentrations for all samples was 150 μ g of protein in a solution of 10 μ M CKLP1, except for vitreous humor, which only contained 100 μ g of protein in 10 μ M CKLP1 due to low protein concentration. There were 18 samples in total. Samples were mixed, briefly centrifuged and then, for each sample, a 100- μ L portion was removed and placed in a new tube so that both tubes contained 100 μ L (75 μ g of protein each except vitreous humor, which contained 50 μ g each). All pairs of samples were placed in incubation at 37°C. For each pair of tubes, one tube was incubated for 4 hours and one tube for 24 hours. At the completion of the incubation period, 200 μ L of acetonitrile was added to each tube and mixed. Tubes were briefly centrifuged and placed at -80°C.

Ocular tissue samples were analyzed for CKLP1 and levromakalim using high pressure liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). CKLP1 was converted to levromakalim over the course of 24 hours in the ciliary body (2.6%), optic nerve (0.9%), iris (3.9%), sclera (1.6%), retina (0.7%), cornea (0.8%), and trabecular meshwork (1.6%), but not in aqueous humor and vitreous humor. The iris, ciliary body, sclera, and trabecular meshwork showed the most efficient conversion.

Example 4. Pharmacological Profile and Ocular Hypotensive Effects of CKLP1 in Normotensive Hound Dogs and Non-human Primates

Pharmacokinetic parameters of CKLP1 were measured in hound dogs and ocular hypotensive effects of CKLP1 were measured in hound dogs and African green monkeys. As discussed below, CKLP1 was shown to significantly lower IOP over extended periods of time with no effects on systemic blood pressure in both models. Pharmacokinetic analysis indicated that CKLP1 is cleaved into levromakalim at sufficient amounts that result in significant lowering of IOP in normotensive animal eyes. Further, a detailed histologic analysis of ocular tissues and fluids along with systemic organ and blood from CKLP1-treated hound dogs did not reveal any significant pathology.

Determination of optimal dose of CKLP1 for lowering IOP in hound dogs

In order to determine the optimal topical ocular dose of CKLP1 for subsequent pharmacokinetic studies, hound dogs (n=3) were treated with four different concentrations of CKLP1 (5 mM, 10 mM, 15 mM and 20 mM) once daily for 5 consecutive days. All CKLP1 concentrations showed a significant IOP lowering ($p \leq 0.01$) with greatest effect observed with 10 mM (2.3 ± 0.5 mmHg) and 15 mM (2.5 ± 0.4 mmHg) (FIG. 6). No difference in IOP reduction was noted between the 10 and 15 mM doses ($p=0.57$). To utilize the lowest dose concentration with effective IOP reduction, a 50 μ L topical ocular administration of 10 mM CKLP1 was selected as the optimal dose for all subsequent experiments.

IOP was measured three times each day at times that corresponded to 1 hour, 4 hours and 23 hours post treatment. The average of the measurements at three time points on any given day was recorded as the daily IOP.

For the dose response study, baseline IOP measurements were obtained and recorded (three consecutive days prior to treatment). One eye of each dog was treated with a 50 μ L topical ocular administration of 5 mM CKLP1 and the contralateral eye was treated with 10 mM CKLP1 once daily for 5 consecutive days. After 5 days, the eyes that received 5 mM CKLP1 was treated with 15 mM CKLP1 while the eye with 10 mM CKLP1 received 20 mM CKLP1. IOPs were measured every day at times corresponding to 1, 4, and 23 hours post treatment. For all experiments, the right eye was used as control while the left eye was selected as the treatment eye.

Effect of CKLP1 on IOP and systemic blood pressure in hound dogs

To evaluate the effect of IOP lowering long-term, hound dogs (n=5) were treated with 10 mM CKLP1 in one eye and vehicle (PBS) in the contralateral eye once daily for 61 consecutive days. As shown in FIG. 7A, over the course of the experiment, the average IOP in the vehicle-treated eye was 16.0 ± 2.4 mmHg, while the treated eye was significantly lower (12.9 ± 2.0 mmHg, $p < 0.001$). On average, IOP was reduced by $18.9 \pm 1.3\%$ (reduction of 3.0 ± 0.5 mmHg; $p < 0.001$) in all five hound dogs over the entire treatment period. Additionally, no significant change in systolic (baseline, 141.0 ± 6.7 ; treatment, 138.9 ± 9.5 ; $p=0.56$) or diastolic (baseline, 80.1 ± 8.9 ; treatment, 78.1 ± 5.9 ; $p=0.76$) blood pressure was observed during the treatment period (FIG. 7B).

Hound dogs were also evaluated for eye redness, swelling of the eye or eyelids, unusual discharge from eye and overall food intake. No notable findings in these parameters were identified.

For the extended dose study, baseline IOPs were measured three times daily for 5 consecutive days. The average of the three measurements was recorded as the daily IOP and averaged over the 5 days for the final pre-treatment value. Following baseline IOP measurements, dogs (n=5) were treated with 10 mM CKLP1 in one eye, while the contralateral eye received vehicle (PBS). IOP was measured at least three times every week at times corresponding to 1, 4, and 23 hours post treatment. Blood pressure was measured three times each week at the 4 hour post treatment time point.

Effect of CKLP1 on IOP and systemic blood pressure in African green monkeys

To further validate the IOP lowering effects of CKLP1 in large animal models, one eye of five African green monkeys was treated with topical ocular administration of 10 mM CKLP1, while the contralateral eye received vehicle (PBS). Baseline IOP in control and treated eyes were 20.1 ± 1.8 mmHg and 21.9 ± 2.5 mmHg, respectively. Following treatment, the eye that received CKLP1 had an IOP reduction of 3.8 ± 1.8 mmHg compared to baseline (p=0.01), which corresponded to a 16.7 ± 6.7% change in IOP. In contrast and as shown in FIG. 8A, the vehicle treated eyes showed an increase in IOP of 0.1 ± 1.0 mmHg, which was not statistically different from baseline (p=0.80). Similar to the hound dogs, topical ocular instillation of CLKP1 did not have any effect on systemic blood pressure when compared to baseline. Average baseline systolic pressure was 118.7 ± 12.0 mmHg, which slightly increased to 121.1 ± 7.3 mmHg after treatment (p=0.6). Likewise, as shown in FIG. 8B, there was no significant change in diastolic pressure following CKLP1 treatment (76.0 ± 8.2 mmHg) compared to baseline (68.1 ± 6.0 mmHg; p=0.13).

For treatment days, 10 mM CKLP1 (dissolved in PBS) was added to one eye of each monkey in a 50 µl topical ocular administration, once daily, for 7 consecutive days, while the contralateral eye received a 50 µl ocular administration of vehicle (PBS).

In summary, CKLP1 lowered IOP by approximately 19% and 17% in hound dogs and African green monkeys, respectively. It has previously been reported that CKLP1 lowers IOP by approximately 17% in mice and 16% in Dutch-belted pigmented rabbits (Roy Chowdhury, U. *et al. J. Med. Chem.* 2016, 59, 6221; Roy Chowdhury, U. *et al. Invest. Ophthalmol. Vis. Sci.* 2017,

58, 5731). The trend of seeing 15-20% IOP reduction in normotensive animals is consistent between small and large animals (Roy Chowdhury, U. *et al. PLoS One*, 2015, 10, e0141783; Roy Chowdhury, U. *et al. Exp. Eye Res.* 2017, 158, 85; Roy Chowdhury, U. *et al. J. Med. Chem.* 2016, 59, 6221; Roy Chowdhury, U. *et al. Invest. Ophthalmol. Vis. Sci.* 2017, 58, 5731).

5 In both hound dogs and African green monkeys, CKLP1 had no significant effect on either systolic or diastolic pressure. While the treatment in African green monkeys was for only 7 days, hound dogs showed no effect on blood pressure after 61 consecutive days of once daily CKLP1 treatment. This may be due to the low concentrations of levcromakalim found in plasma (1 ng/ml), which is much lower than the reported threshold of the drug needed to elicit a systemic effect on
10 blood pressure (Hamilton TC, *et al. Gen. Pharmacol.* 1989; 20, 1; Hamilton TC, *et al. Levcromakalim. Cardiovascular Drug Reviews.* 1993; 11, 199; Wilson C, *et al. Eur. J. Pharmacol.* 1988; 152:331-339). However, this low level of levcromakalim is still enough to exert a localized IOP lowering effect, potentially through dilation of the vessels in the distal outflow pathway. The topical ocular application converts enough CKLP1 to levcromakalim to induce IOP reduction, but
15 is not enough to have an effect on blood pressure.

Analysis of pharmacokinetic parameters of CKLP1 and levcromakalim in hound dogs

To assess the pharmacokinetic parameters, hound dogs (n=3) were treated with 50 µL topical ocular administration of 10 mM CKLP1 or vehicle (PBS) (n=2) in both eyes, once daily
20 for eight days. Blood (approximately 3 mL) was collected in heparin blood collection tubes at eight different time points (5 minutes, 15 minutes, 30 minutes, 60 minutes, 2 hours, 4 hours, 8 hours and 24 hours) following treatment on days 1, 4 and 8. Plasma was separated from the blood by centrifugation at 2000 rpm for 5 minutes.

Pharmacokinetic analysis of these samples showed characteristic distribution, absorption
25 and elimination profiles of CKLP1 and levcromakalim (FIG. 9A, FIG. 9B, and FIG. 9C). Maximum concentration of CKLP1 (10.5 ± 1.7 ng/ml) in plasma was obtained generally within 60 minutes following topical dose. Maximum concentration of levcromakalim (1.2 ± 0.2 ng/ml) occurred around 120 minutes. The half-lives of CKLP1 were 180.5 minutes, 451.8 minutes, and 253.7 minutes on days 1, 4 and 8. Half-lives of the parent compound levcromakalim on those same
30 days were 74.3 min, 87.8 minutes and 126.4 minutes. Average area under the concentration versus

time curve (AUC) for CKLP1 (5261.4 ± 918.9 ng*min/ml) was 22.4-fold greater than levcromakalim (233.0 ± 102.8 ng/ml x minutes), indicating a possible slow release of CLKP1 from an internal tissue source in the animals. This is further indicated by a longer T_{last} (time when drug was last detected in plasma) of CKLP1 on days 4 and 8 compared to day 1 (Table 2A and Table 2B).

CKLP1 treatment of hound dogs showed conversion to its parent compound levcromakalim as evidenced by the longer T_{max} of levcromakalim (approximately 120 minutes) compared to CKLP1 (approximately 60 minutes). The 10% conversion value reported is an estimate based on the comparison of levcromakalim to CKLP1 concentrations in blood. The optimum concentration of CKLP1 for lowering pressure, based on the dose response studies in hound dogs, was the same used in previous studies performed in Dutch-belted pigmented rabbits (Roy Chowdhury, U. *et al. Invest. Ophthalmol. Vis. Sci.* 2017, 58, 5731; Roy Chowdhury, U. *et al. PLoS One*, 2020, 15, e0231841).

Table 2A. PK Parameters of CKLP1 following Topical Dosing in Hound Dogs

	Half-life (min)	T_{max} (min)	C_{max} (ng/mL)	T_{last} (h)	C_{last} (ng/mL)	AUC_{last} (ng/mL)
Day 1	180.5	60.0	10.3	480	2.6	4373.8
Day 4	451.8	120.0	12.2	1441	1.4	6208.6
Day 8	253.7	60.0	8.9	1450	0.3	5201.9

Table 2B. PK Parameters of Levcromakalim following Topical Dosing in Hound Dogs

	Half-life (min)	T_{max} (min)	C_{max} (ng/mL)	T_{last} (h)	C_{last} (ng/mL)	AUC_{last} (ng/mL)
Day 1	74.3	120.0	1.3	480	0.1	305.6
Day 4	87.8	120.0	1.4	480	0.1	377.8
Day 8	126.4	120.0	1.0	240	0.5	160.3

Concentration of CKLP1 and levcromakalim in select ocular and systemic tissues

After blood collection for pharmacokinetic studies, bilateral ocular treatment with CKLP1 (10 mM) continued in the hound dogs for 4-5 additional days. At 23 hours following the last treatment, animals were euthanized and select ocular and systemic tissue samples were collected and analyzed for the presence of CKLP1 and levcromakalim by LC-MS/MS.

For ocular tissue collection, eyes were enucleated and aqueous humor, vitreous humor, trabecular meshwork, optic nerve, ciliary body, iris, retina and cornea were isolated and stored at -80°C. While collecting tissues during necropsy, portions of heart, kidney, lung, brain, liver and skeletal muscle were immediately frozen for pharmacodynamics analysis, while the remaining tissue samples were immediately fixed in 10% neutral buffered formalin.

CKLP1 and levcromakalim concentrations in the biological samples (fluids and tissues) were determined by an established LC-MS/MS based assay. Immediately prior to analysis, tissues were thawed and their weight was measured. PBS was added at double the tissue volume homogenized in a rotor stator homogenizer for 30 seconds. Briefly, CKLP1, levcromakalim and flavopiridol (internal standard) were separated on a Waters Acquity UPLCBEH C18 column (1.7 µm, 2.1 x 50 mm) coupled with an Agilent EC-C18 pre-column (2.7 µm, 2.1 x 5mm). Detection was accomplished using positive electrospray ionization with multiple-reaction monitoring (MRM). The MRM precursor and product ions were monitored at m/z 367>86, 287>86 and 402>341 for CKLP1, levcromakalim and flavopiridol (internal standard) respectively. Data were acquired and analyzed using Waters MassLynx v4.1 software.

Values are expressed as mean ± standard deviations. Group means within the same animal were compared using paired t-tests. Means for more than two groups (dose response studies) were compared using one-way ANOVA followed by pairwise t-tests. Statistical tests were performed using JMP software.

Two of the hound dogs showed significant levels of CKLP1 and levcromakalim in their tissues, while the levels in the third hound dog were below quantitative levels (BQL). As shown in FIG. 10, using data from the two animals, high concentration of CKLP1 was found in optic nerve (63.8 ± 63.1 ng/g), trabecular meshwork (169.5 ± 21.6 ng/g), cornea (31.3 ± 10.8 ng/g) and vitreous humor (24.4 ± 2.4 ng/g) with lower levels found in ciliary body (10.3 ± 8.1 ng/g), iris (4.8 ± 1.1 ng/g), retina (6.2 ± 3.8 ng/g) and aqueous humor (10.2 ± 14.4 ng/g). Levcromakalim was also

present in these samples but at lower concentrations. The highest concentration of levromakalim was found in the trabecular meshwork (2.0 ± 0.5 ng/g) followed by cornea (1.4 ± 0.3 ng/g) and aqueous humor (1.1 ± 1.5 ng/ml). Optic nerve, ciliary body, iris, retina and vitreous humor also showed levromakalim albeit at <1 ng/g. A high concentration of both CKLP1 (88.0 ± 134.9 ng/ml) and levromakalim (3.7 ± 4.5 ng/ml) was noted in urine of the treated animals indicating this to be an important route of drug excretion from the body. Among systemic organs, both CKLP1 and levromakalim were either absent or found at low concentrations in heart (3.7 ± 0.5 ng/g CKLP1; 0.9 ± 0.8 ng/g levromakalim), kidney (2.7 ± 2.9 ng/g CKLP1; 0.8 ± 1.2 ng/g levromakalim), and lung (CKLP1 was undetected; 0.3 ± 0.4 ng/g levromakalim).

To evaluate local and systemic side effects of bilateral topical administration of CKLP1 to the eyes, additional tissue samples were harvested for histological examination. Tissues collected and fixed during necropsy were processed into paraffin blocks, sectioned, and stained with hematoxylin and eosin.

Of the 40 different tissues evaluated from each hound dog, none of the analyzed tissues showed any significant pathology beyond incidental findings. Absence of significant pathological changes suggest that treatment with CKLP1 was absent of any observable toxicity. FIG. 11A, FIG. 11B, FIG. 11C, and FIG. 11D show representative images from select tissues (trabecular meshwork, retina, kidney, liver) treated with CKLP1.

Typical blood chemistry was within normal range for hound dogs compared to the historical range except for albumin, which was found to be at a slightly lower concentration in both treated and control animals. Additionally, no changes were observed in food intake or behavior of the hound dogs during the treatment period. Likewise, the weight of the dogs pre- and post-experiment did not show any significant changes ($p>0.36$ for both treated and control groups).

Together, these results suggest that bilateral eye treatment with CKLP1 was well tolerated and did not result in any observed ocular or systemic toxicity.

The low concentrations of levromakalim found in blood may also be due to tissues acting as reservoirs for CKLP1, first by storing and then slowly releasing the drug. Values of AUC, which indicates the amount of available drug, is 22.4-fold higher for CKLP1 than levromakalim, indicating that CKLP1 may be stored and then slowly released over time. Additionally, several ocular tissues show high concentrations of CKLP1 and levromakalim. One such tissue appears to

be the trabecular meshwork, which contains the most CKLP1 and levromakalim among the analyzed ocular tissues. High concentration of CKLP1 identified in the trabecular meshwork may be acting as a reservoir for slow release of levromakalim in clinically relevant concentrations. This may also partially explain the delay in IOP returning to baseline following cessation of treatment, which has been identified in small animal models (Roy Chowdhury, U. *et al. PLoS One*, 2015, 10, e0141783; Roy Chowdhury, U. *et al. Invest. Ophthalmol. Vis. Sci.* 2017, 58, 5731; Roy Chowdhury, U. *et al. PLoS One*, 2020, 15, e0231841) and also reported above. Because the trabecular meshwork is immediately proximal to the distal outflow region, it is an advantageous location for CKLP1 to levromakalim conversion to induce an effect on the distal outflow pathway.

Example 5. Intravenous CKLP1 induces Peripheral Vasodilation in Dogs

Two beagles (one male and one female) were intravenously injected with escalating doses of CKLP1 (0.05 mg/kg, 0.5 mg/kg, 1.5 mg/kg, 3 mg/kg, and 5 mg/kg) to assess the toxicity of CKLP1. The injection was done through the cephalic vein and CKLP1 was administered in a phosphate buffered saline solution ((0.096% sodium phosphate dibasic, 0.089% sodium dihydrogen orthophosphate monohydrate, 0.83% sodium chloride) pH 6.5 ± 0.1 in sterile water for injection USP). The dosing schedule is shown below in Table 3.

Table 3. Dosing Schedule for Toxicity Study

Escalating Phase No.	Dose Level (mg/kg/day)	Dose Volume ^a (mL/kg)	Dose Concentration ^b (mg/mL)	Day of Dosing
1	0.05	1	0.05	1
2	0.5	1	0.5	3
3	1.5	1	1.5	8
4	3	1	3	10
5	5	1	5	14

^aBased on the most recent body weight measurement.

^bPrepared from stock solution at 5 mg/mL; diluted with the vehicle on each dosing occasion

The following parameters and endpoints were evaluated: mortality, clinical observations, body and organ weights, and food consumption. Bioanalytical samples for toxicokinetic

parameters (for both CKLP1 and levcromakalim) were collected after each dose level (days 1, 3, 8, 10, and 14) at predose, 1, 3, 6, 8, and 24 hours postdose. Animals were released from the study after completion of the bioanalytical sample collection schedule.

Toxicokinetics (TK) were determined based on the individual exposures for each animal on each sampled study day (days 1, 3, 8, 10, and 14). Toxicokinetic analysis was not performed on levcromakalim for the female at 0.05 mg/kg on day 1 because there was insufficient plasma concentration data available to perform the assessment with only 2 of the collected time points quantified in the profile. The levcromakalim AUC_{T_{last}} at 0.05 mg/kg for the female dog was estimated using the AUC_{T_{last}} from the next lowest dose level (0.5 mg/kg) normalized by the dose ratio (10-fold) in order to estimate the R_{AUC} values at the higher doses for levcromakalim in the female dog. Predose samples had no quantifiable exposure in any animal on any day, with the exception of the female dog on day 10. The predose concentration for the female dog on day 10 was excluded from the TK analysis in order to allow for estimation of the IV C₀. Male and female summary of TK parameters for CKLP1 and levcromakalim following IV bolus dosing of CKLP1 are presented below. The parameters for CKLP1 are shown in Table 4A and the parameters for levcromakalim are shown in FIG. 4B.

Table 4A. CKLP1 Toxicokinetic Parameters Following Administration of CKLP1

Study Day	1	3	8	10	14
Dose (mg/kg)	0.05	0.5	1.5	3	5
Male Dog					
T _{max} (h)	1.00	1.00	1.00	1.00	1.00
C _{max} (ng/mL)	245	2600	7690	14400	22400
C _{max} /Dose (g/L)	4900	5200	5130	4800	4480
C ₀ (ng/mL)	301	3580	10400	18500	25900
C ₀ /Dose (g/L)	6030	7160	6910	6160	5170
AUC _{T_{last}} (ng*h/mL)	1120	14300	43200	85100	159000
AUC _{T_{last}} /Dose (g*h/mL)	22400	28500	28800	28400	31700
R _{AUC}	1.00	12.8	38.6	76.0	142
T _{last} (h)	8.00	24.0	24.0	24.0	24.0
T _{1/2} (h)	2.23	2.84	2.91	6.22	3.47
λ _Z range (h)	3-8	3-24	1-24	6-24	1-24
λ _Z R ²	1.00	1.00	0.998	0.990	0.998

AUC _(0-inf) (ng*h/mL)	1230	14300	43300	89000	160000
Cl (mL/h/kg)	40.7	35.0	34.7	33.7	31.3
V _Z (mL/kg)	131	143	146	303	156
Female Dog					
T _{max} (h)	1.00	1.00	1.00	1.00	1.00
C _{max} (ng/mL)	356	2990	9050	18400	29300
C _{max} /Dose (g/L)	7120	5980	6030	6130	5860
C ₀ (ng/mL)	461	3670	11000	22700	32200
C ₀ /Dose (g/L)	9230	7350	7300	7560	6430
AUC _{last} (ng*h/mL)	2100	20900	61200	128000	311000
AUC _{last} /Dose (g*h/mL)	41900	41900	40800	42800	62300
R _{AUC}	1.00	10.0	29.1	61.0	148
T _{last} (h)	24.0	24.0	24.0	24.0	24.0
T _{1/2} (h)	3.26	4.11	4.68	2.94	5.98
λ _Z range (h)	6-24	6-24	6-24	6-24	3-24
λ _Z R ²	1.00	0.999	1.00	1.00	0.999
AUC _(0-inf) (ng*h/mL)	2110	21200	62500	129000	330000
Cl (mL/h/kg)	23.7	23.5	24.0	23.3	15.2
V _Z (mL/kg)	112	139	162	99.0	131

Table 4B. Levchromakalim Toxicokinetic Parameters Following Administration of CKLP1

Study Day	1	3	8	10	14
Dose (mg/kg)	0.05	0.5	1.5	3	5
Male Dog					
T _{max} (h)	1.00	1.00	1.00	1.00	3.00
C _{max} (ng/mL)	0.826	7.30	18.0	42.7	65.8
AUC _{last} (ng*h/mL)	5.04	45.5	183	476	741
R _{AUC}	1.00	9.03	36.3	94.4	147
T _{last} (h)	8.00	8.00	24.0	24.0	24.0
T _{1/2} (h)	9.66	6.89	5.27	6.48	7.67
λ _Z range (h)	1-8	3-8	3-24	6-24	6-24
λ _Z R ²	0.999	1.00	0.992	0.996	0.991
AUC _(0-inf) (ng*h/mL)	12	87.5	192	518	837
Female Dog					
T _{max} (h)	NR	3.00	3.00	6.00	3.00
C _{max} (ng/mL)	NR	5.13	15.6	28.3	82.7
AUC _{last} (ng*h/mL)	NR	81.3	241	386	1040
R _{AUC}	NR	10.0	29.6	47.5	128
T _{last} (h)	NR	24.0	24.0	24.0	24.0
T _{1/2} (h)	NR	10.3	7.58	5.07	7.82
λ _Z range (h)	NR	6-24	6-24	6-24	6-24
λ _Z R ²	NR	0.999	0.978	1.00	0.992
AUC _(0-inf) (ng*h/mL)	NR	103	272	404	1190

In general, CKLP1 exposure based on the theoretical concentration at time zero after intravenous bolus dosing only (C₀), the maximum observed plasma concentration (C_{max}) and the area under the concentration versus time curve (AUC) was approximately dose proportional in both dogs. No consistent gender differences were noted as differences in AUC values following each dose were less than 2-fold.

Levchromakalim exposure in both dogs appeared to be proportional to the CKLP1 dose administered. There were no consistent, obvious differences in levchromakalim TK parameters between the male and female dogs. Plasma concentrations of CKLP1 were more than 300-fold higher than levchromakalim at the early time points. Differences in C_{max} were 300- to 400-fold higher in males and 350- to 650-fold higher in females. Levchromakalim appeared to have a somewhat longer T_{1/2} than CKLP1, so the relative difference decreased over the time after dosing.

CKLP1 area under the concentration versus time curve from time zero extrapolated to infinity ($AUC_{(0-\infty)}$) was 100- to 200-fold higher than levromakalim in males and 200- to 300-fold higher in females.

In dogs administered single escalating IV doses, the maximum tolerated dose (MTD) was determined to be 3 mg/kg. The MTD corresponded to sex-combined C_{max} and AUC_{last} values of 16.4 $\mu\text{g/mL}$ and 106.55 $\mu\text{g}\cdot\text{h/mL}$ for CKLP1, and 35.5 ng/mL and 431 $\text{ng}\cdot\text{h/mL}$, for levromakalim. No mortality, change in body weight or food consumption were noted. Histological examination showed no systemic toxicity as a result of CKLP1 treatment. There was no mortality during this study, and no CKLP1-related effect on food consumption or body weight.

Surprisingly, as shown in Table 5 below, CKLP1-related clinical signs included inconsistent observations of red discoloration of the skin (pinnae, gums, generalized area, and/or left forelimb [female only]) in the male at ≥ 0.05 mg/kg and in the female at ≥ 0.5 mg/kg. The no-observed adverse effect level (NOAEL) was 3 mg/kg. At 5 mg/kg, CKLP1-related adverse clinical signs of increased heart rate, warm to touch, and/or partly closed eyes (female only) were observed.

Table 5. Clinical Signs of male and female dogs dosed with intravenous CKLP1

Dose	Clinical Signs
0.5 mg/kg	red discoloration of the skin (pinnae, gums, generalized area, and/or left forelimb ^a)
1.5 mg/kg	red discoloration of the skin (pinnae, gums, generalized area, and/or left forelimb ^a)
3 mg/kg	red discoloration of the skin (pinnae, gums, generalized area, and/or left forelimb ^a)
5 mg/kg	red discoloration of the skin (pinnae, gums, generalized area, and/or left forelimb ^a)

^aFemale only

This study confirms that CKLP1 induces peripheral vasodilation following intravenous administration, which is beneficial for blood vessel disorders, including Raynaud's disease.

Example 6. Plasma Pharmacokinetics Following Ophthalmic Dosing in Beagle Dogs for 28 Days

The plasma pharmacokinetics of CKLP1 and levromakalim following topical ophthalmic dosing in beagle dogs for 28 days was evaluated. In the study, dogs (3 males and 3 females) received bilateral daily topical administration of 40 μL /eye of 2.0%, 4.0%, or 8.0% as measured

in mg/mL (equivalent to 0.8, 1.6, or 3.2 mg CKLP1 per eye) of CKLP1 in phosphate buffered saline. Based on an average male dog weight of 7.9 kg at the start of the study, these doses were equivalent to 0.20 mg/kg, 0.41 mg/kg, or 0.81 mg/kg and based on an average female dog weight of 6.0 kg at the start of the study, these doses were equivalent to 0.27 mg/kg, 0.53 mg/kg, or 1.07 mg/kg. Blood was collected for toxicokinetic analysis pre-dose and at 1, 2, 4, 8, 12, and 24 hours post dosing on days 1 and 28. In addition, 2 animals of each gender were allowed 336 hours (14 days) of recovery time following the final dose, at which point final blood samples were obtained. Samples were analyzed for CKLP1 and levromakalim by a validated LC-MS/MS method (MET244v1).

When dosed topically, the no-observed-adverse-effect level (NOAEL) was determined to be 8.0% as measured in mg/mL. At this dose, the mean C_{max} and AUC T_{last} values on day 28 were 147 ng/mL and 1.26 ug*h/ml, respectively, for males, with similar results in females. CKLP1 C_{max} and AUC T_{last} levels at NOAEL were 31 ng/ml and 166 ng*h/ml in males, with similar results in females.

Non-adverse, ocular effects were observed at 3.2 mg/eye/dose (8% as measured in mg/mL) primarily consisting of slight to moderate redness (congestion) that increased in incidence and severity and a minor reduction in red cell mass. CKLP1-related microscopic findings were limited to non-adverse mild increased mitoses in the corneal epithelium of males at ≥ 1.6 mg/eye/dose (4%) and 1 female at 3.2 mg/eye/dose (8%), and mild lacrimal gland acinar atrophy, in 2 females at 3.2 mg/eye/dose (8%), of unknown toxicological significance.

In several animals across treated groups including, 1 control animal, a reduction in thymus weight and size was observed macroscopically with minimal to mild decreased lymphoid cellularity microscopically. These changes were considered to be non-adverse and secondary to a combination of physiological involution and stress.

Following a 14-day recovery period, all ocular findings were fully recovered and non-adverse changes in hematology parameters (at 3.2 mg/eye/dose (8%)) and thymus were partially reversed.

Low exposure occurred in one male and one female at the mid dose on day 28, and in one high dose female on days 1 and 28. This was considered to have impacted the comparison between

genders for CKLP1 at both doses and the levchromakalim plasma concentrations in females at the high dose.

As seen in Table 6, there were no consistent differences in CKLP1 exposures between day 1 and day 28, but levchromakalim exposures and the percentage conversion of CKLP1 to levchromakalim tended to be lower on day 28 than on day 1. Exposure to CKLP1 and levchromakalim increased with dose, but the increases were not strictly proportional to dose.

Females at the low and mid doses exhibited higher CKLP1 levels than males even after accounting for the higher effective mg/kg doses in the females. After adjusting for the higher dose received by females, exposures to CKLP1 and levchromakalim in females at the high dose appeared slightly lower than males. No gender difference was observed with respect to levchromakalim at the low and mid doses.

Toxicokinetic analyses indicated that exposure to CKLP1 exceeded that of levchromakalim across all doses and time points. Mean levchromakalim C_{max} and AUC_{last} values were 18.1% to 69% and 10.2% to 43.2% those of CKLP1 on day 1, and 9.86% to 27% and 6.01 % to 18.5 % those of CKLP1 on day 28, respectively.

The maximum concentration (C_{max}) of CKLP1 occurred at 1 or 2 hours in both genders on days 1 and 28. The mean $T_{1/2}$ of CKLP1 ranged between 3.32 and 6.18 hours. Higher exposure to CKLP1 ($AUC > 2$ -fold) was observed in females compared to males at 0.8 (2%) and 1.6 mg/eye/dose (4%) on days 1 and 28, although similar exposure between genders was observed at 3.2 mg/eye/dose (8%). Dose proportionality in the context of exposure was variable in males and females on both days 1 and 28. Generally, there was no accumulation of CKLP1 (mean values) on day 28 relative to day 1 at all dose levels, though some individual animals showed an increased exposure on day 28.

The C_{max} of levchromakalim was observed between 1 and 4 hours in both genders on day 1, and between 1 and 8 hours in males and at 2 or 4 hours in females on day 28. Mean $T_{1/2}$ was between 2.06 and 4.90 hours. Sex differences in exposure (AUC) at all doses and time points were less than 2-fold. Dose proportionality in the context of exposure to levchromakalim was variable on both days 1 and 28. Generally, systemic exposure to levchromakalim decreased on day 28 relative to day 1 at all dose levels in males, and at 3.2 mg/eye/dose (8%) in females and was approximately similar between days 1 and 28 at 0.8 (2%) and 1.6 mg/eye/dose (4%) in females.

Terminal elimination half-lives for CKLP1 ranged between approximately 3 and 6 hours. Those for levromakalim were slightly lower, ranging between 2 and 5 hours post dose.

Plasma samples collected pre-dose on day 28 showed detectable levels of CKLP1 in one female at the low dose, all females at the mid dose, and all males and females at the high dose.

- 5 Predose levels of CKLP1 were between 16 and 25 times lower than peak plasma concentrations after dosing. Predose levromakalim levels on day 28 were either below the lower limit of quantitation (0.499 ng/mL) or marginally above it. Plasma samples collected at the end of the recovery period were below the lower limits of quantitation for both CKLP1 (1.999 ng/mL) and levromakalim (0.499 ng/mL) in both male and female dogs.

Table 6. 28-Day Ophthalmic Daily Dosing Plasma Pharmacokinetics in Dogs

Pharmacokinetic Parameters		2.0% ^a CKLP1		4.0% ^a CKLP1		8.0% ^a CKLP1	
		Male	Female	Male	Female	Male	Female
CKLP1							
C _{max} (ng/mL)	Day 1	36.5	55.5	73.7	129	166	156
	Day 28	47.0	80.2	57.3	201	147	139
AUC _{0-last} (ng·h/mL)	Day 1	231	496	520	1220	1520	1480
	Day 28	272	545	381	1750	1260	1200
AUC _{0-inf} (ng·h/mL)	Day 1	299	660	571	1300	1580	1860
	Day 28	NA	NA	NA	NA	NA	NA
T _{max} (h)	Day 1	1	2	1	2	2	2
	Day 28	1	2	1	2	2	2
t _{1/2} (h)	Day 1	3.69	4.12	4.26	6.18	4.82	4.92
	Day 28	3.44	3.68	3.32	4.95	5.08	5.15
Levcromakalim							
C _{max} (ng/mL)	Day 1	25.3	25.1	24.4	23.2	76.0	72.2
	Day 28	10.6	18.4	15.5	19.9	31.0	32.9
AUC _{0-last} (ng·h/mL)	Day 1	99.8	92.7	133	125	361	294
	Day 28	49.1	80.1	70.7	105	166	154
AUC _{0-inf} (ng·h/mL)	Day 1	111	96.8	185	131	371	304
	Day 28	NA	NA	NA	NA	NA	NA
T _{max} (h)	Day 1	2	2	4	2	2	2
	Day 28	2	2	2	2	2	2
t _{1/2} (h)	Day 1	3.64	2.65	3.24	3.61	3.44	2.73

Pharmacokinetic Parameters		2.0% ^a CKLP1		4.0% ^a CKLP1		8.0% ^a CKLP1	
		Male	Female	Male	Female	Male	Female
	Day 28	2.35	2.51	3.12	2.06	3.38	4.90
Ratio AUC _{0-last} (%) Levcromakalim: CKLP1 (μM*h)	Day 1	55.3	23.9	32.8	13.1	30.4	25.5
	Day 28	23.2	18.8	23.8	7.7	16.9	16.4

^a as measured in mg/mL

N/A = not applicable

Example 7. Intravenous CKLP1 induces Peripheral Vasodilation in Rats

5 Three groups of rats were intravenously administered different doses of CKLP1. The study details are provided in Table 7. The study was 28 days long with a 14-day recovery period.

Table 7. Study Design for Intravenous Administration of CKLP1

Group No.	Compd.	Dose Level (mg/kg/day)	Dose Volume (mg/mL)	Dose Conc. (mg/mL)	Number of Animals					
					Main Study		Recovery Study		Toxicokinetic Study	
					M	F	M	F	M	F
1	Control	0	0.5	0	10	10	5	5	3	3
2	CKLP1	0.15	0.5	0.3	10	10	-	-	9	9
3	CKLP1	1.5	0.5	3	10	10	-	-	9	9
4	CKLP1	15	0.5	30	10	10	5	5	9	9

10 At the end of the study period, there was no difference between the CKLP1 groups and the control group with regard to food consumption, body weight, or body weight gains. As shown in Table 8 below, clinical signs of the male and female rats administered ≥ 0.15 mg/kg/day of CKLP1 included red skin forelimbs and hindlimbs, red pinnae, and red scrotums (male only). Red skin forepaws were observed in males and females administered ≥ 1.5 mg/kg/day and red muzzles in

15 males and females administered 1.5 mg/kg/day CKLP1 were observed between days 8 and 14. Abnormal eye color in males given the 15 mg/kg dose was also observed. This study further

confirms that CKLP1 induces peripheral vasodilation following intravenous administration, which is beneficial for blood vessel disorders, including Raynaud's disease.

Table 8. Clinical Signs of Male and Female Rats Dosed with Intravenous CKLP1

Dose	Clinical Signs
0.15 mg/kg/day	Red skin forelimbs and hindlimbs; red pinnae; red scrotum (males only)
1.5 mg/kg/day	Red skin forelimbs and hindlimbs; red skin forepaws; red pinnae; red muzzle (between days 8 and 14); red scrotum (males only)
15 mg/kg/day	Red skin forelimbs and hindlimbs; red skin forepaws; red pinnae; red scrotum (males only)

5

Example 8. Use of Levcromakalim in a 3D-Glaucomatous Human Trabecular Meshwork/Schlemm's Canal Tissue Model

Simulating glaucoma pathology and medication-induced changes to the anatomy and physiology of the conventional outflow pathway presents a unique challenge. In the study described below, a 3D-bioengineered glaucomatous conventional outflow model was used to investigate the ability of levcromakalim to modulate outflow *in vitro*. The effects of levcromakalim on fibrotic and endothelial junctional markers in human trabecular meshwork/Schlemm's canal co-cultures are also described below.

Bioengineered 3D conventional outflow tract constructs using 4 donors (ages 47-91) were treated with TGF- β 2 (5 ng/mL) for 6 days. Constructs were then treated with levcromakalim (1, 10 or 100 μ M), or Y-27632 (10 μ M), a Rho kinase inhibitor. The effect of levcromakalim (1 μ M) on outflow facility (hydraulic conductivity) was assessed by perfusion studies where pressure was constantly recorded at various perfusion rates. Protein expression of α -smooth muscle actin (α -SMA), CD31, endothelin-I, fibronectin, VE-cadherin, phospho-eNOS and total eNOS was analyzed via western blot. Cellular expression of α -SMA, fibronectin, phospho-eNOS and total eNOS was determined by immunocytochemistry and confocal microscopy. Statistical significance was determined by one-way ANOVA with a Tukey's multiple comparisons test, or by two-way ANOVA.

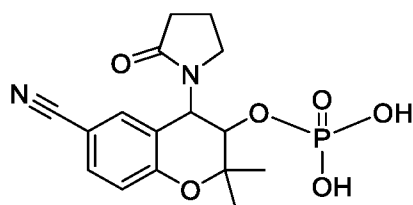
Levcromakalim significantly increased outflow facility across all donors as compared to TGF- β 2 or Y-27632 treated donors ($P < 0.0001$ and $P < 0.05$, respectively). Levcromakalim did not significantly affect expression of the cell adhesion proteins CD31 and VE-Cadherin, while Y-27632 significantly decreased their content ($P < 0.01$). Neither compound significantly altered
5 protein expression or distribution of endothelin, fibronectin, α -SMA, or phospho-eNOS or total eNOS.

Levcromakalim significantly improved outflow facility in glaucomatous constructs without impacting protein expression of fibrotic or endothelial junctional markers. In contrast, Y27632 decreased expression of endothelial junctional markers. These results indicate that
10 levcromakalim is a treatment that may lower elevated IOP without altering vessel integrity, and therefore without inducing significant hyperemia.

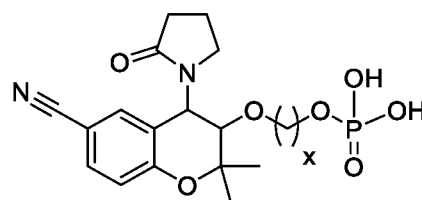
This specification has been described with reference to embodiments of the invention. Given the teaching herein, one of ordinary skill in the art will be able to modify the invention for a desired purpose and such variations are considered within the scope of the invention.

WE CLAIM:

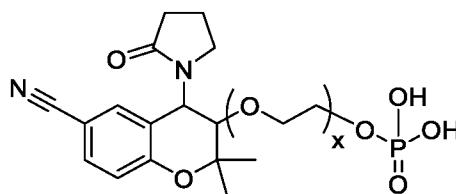
1. A method to treat an ocular disorder selected from Graves' ophthalmopathy, cavernous sinus thrombosis, orbital vein vasculitis, carotid-cavernous sinus fistula, orbital varices, central retinal vein occlusion, branch retinal vein occlusion, and non-arteritic anterior ischemic optic neuropathy in a host in need thereof comprising administering an effective amount of a compound of Formula I, Formula II, or Formula III:



CKLP1
Formula I



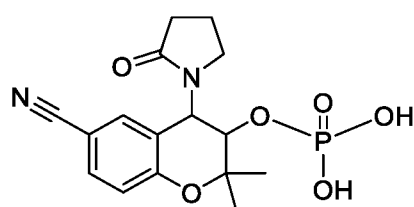
Formula II



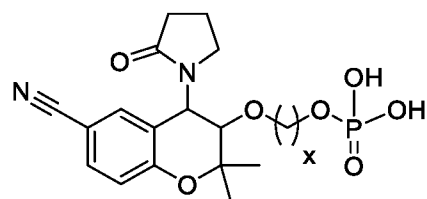
Formula III

or a pharmaceutically acceptable salt, optionally in a pharmaceutically acceptable carrier, wherein x is an integer selected from 1, 2, 3, 4, and 5.

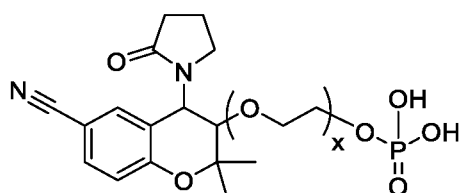
2. The method of claim 1, wherein the ocular disorder is non-arteritic anterior ischemic optic neuropathy.
3. A method to treat a blood vessel disorder selected from Raynaud's disease, peripheral artery disease, chronic limb ischemia, thrombophlebitis, pulmonary arterial hypertension, and chronic venous insufficiency in a host in need thereof comprising administering an effective amount of a compound of Formula I, Formula II, or Formula III:



CKLP1
Formula I



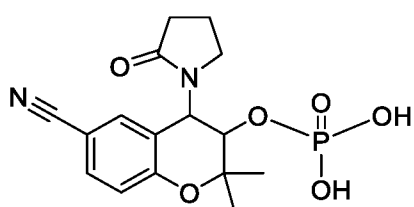
Formula II



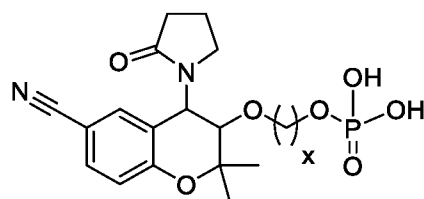
Formula III

or a pharmaceutically acceptable salt, optionally in a pharmaceutically acceptable carrier, wherein x is an integer selected from 1, 2, 3, 4, and 5.

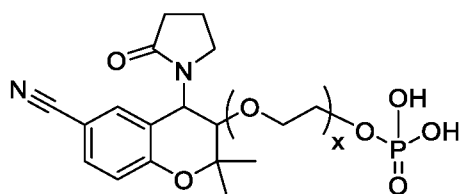
4. The method of claim 3, wherein the blood vessel disorder is Raynaud's disease.
5. The method of claim 3, wherein the blood vessel disorder is pulmonary arterial hypertension.
6. A method to treat a cardiovascular disease selected from chronic or acute myocardial ischemia, microvascular dysfunction, coronary artery disease, arrhythmia, high blood pressure, endothelial dysfunction, and a heart attack in a host in need thereof comprising administering an effective amount of a compound of Formula I, Formula II, or Formula III:



CKLP1
Formula I



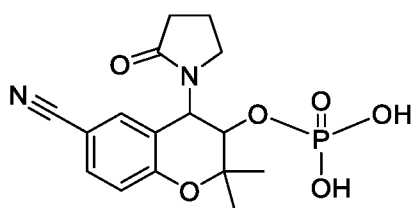
Formula II



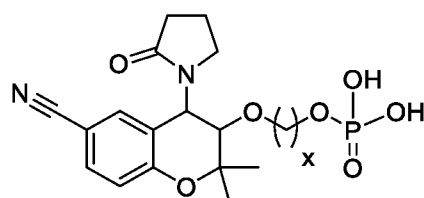
Formula III

or a pharmaceutically acceptable salt, optionally in a pharmaceutically acceptable carrier, wherein x is an integer selected from 1, 2, 3, 4, and 5.

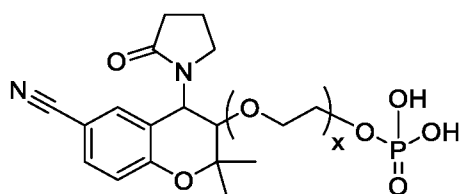
7. A method to treat erectile dysfunction or female sexual arousal disorder in a host in need thereof comprising administering an effective amount of a compound of Formula I, Formula II, or Formula III:



CKLP1
Formula I

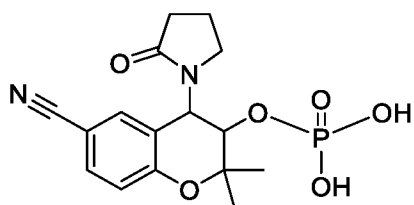


Formula II

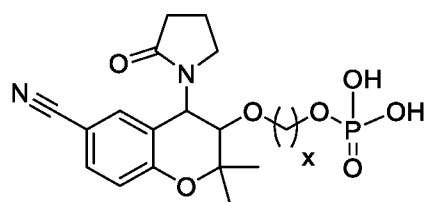
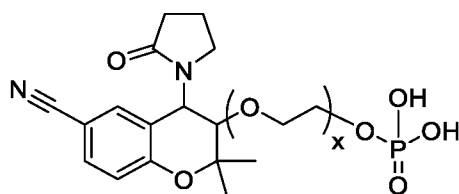
**Formula III**

or a pharmaceutically acceptable salt, optionally in a pharmaceutically acceptable carrier, wherein x is an integer selected from 1, 2, 3, 4, and 5.

8. The method of claim 7, to treat erectile dysfunction.
9. A method to treat a lymphatic disease selected from lymphadenopathy, lymphangitis, lymphangiectasia, lymphadenitis, and lymphangiomatosis in a host in need thereof comprising administering an effective amount of a compound of Formula I, Formula II, or Formula III:

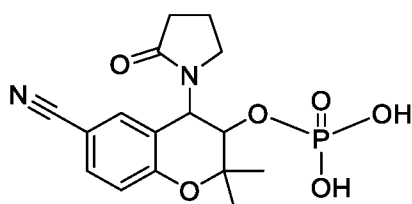


CKLP1
Formula I

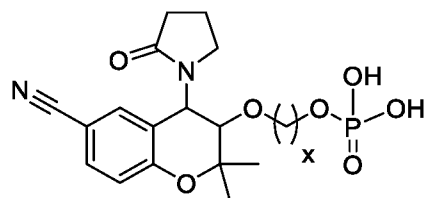
**Formula II****Formula III**

or a pharmaceutically acceptable salt, optionally in a pharmaceutically acceptable carrier, wherein x is an integer selected from 1, 2, 3, 4, and 5.

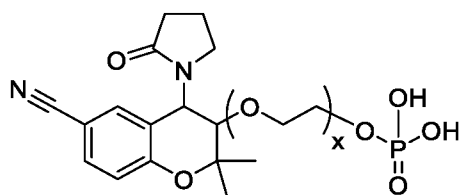
10. A method to treat an ocular lymphatic disorder selected from conjunctival myxoma, dry eye, conjunctival lymphangiectasia, chemosis, mustard gas keratitis, corneal inflammation, orbital cellulitis, chalazion, dermatochalasis, and blepharochalasis in a host in need thereof comprising administering an effective amount of a compound of Formula I, Formula II, or Formula III:



CKLP1
Formula I



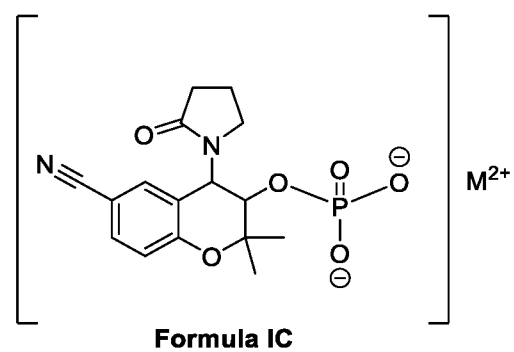
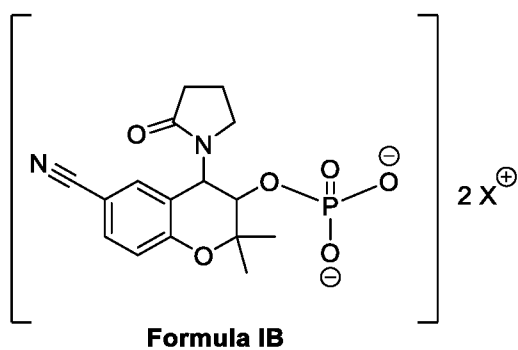
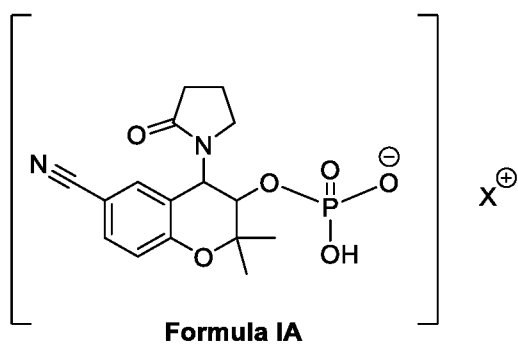
Formula II



Formula III

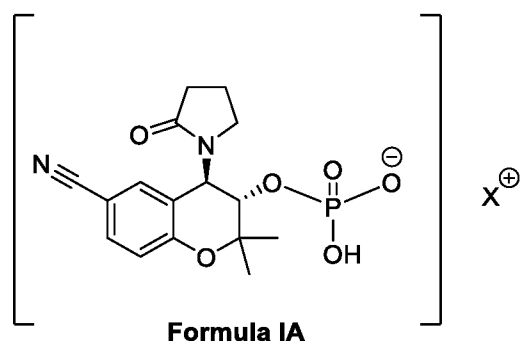
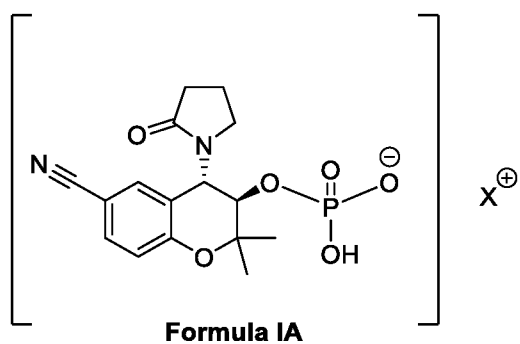
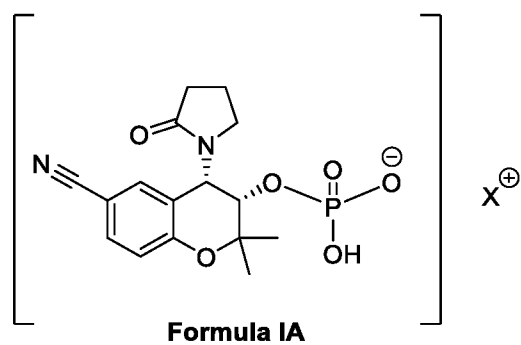
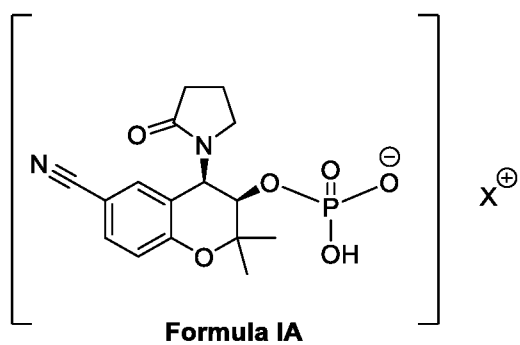
or a pharmaceutically acceptable salt, optionally in a pharmaceutically acceptable carrier, wherein x is an integer selected from 1, 2, 3, 4, and 5.

11. The method of claim 1 or 10, wherein the effective amount of a compound of Formula I, Formula II, or Formula III does not cause significant hyperemia.
12. The method of any one of claim 1-11, wherein the compound is a pharmaceutically acceptable salt of Formula I selected from Formula IA, Formula IB, or Formula IC:

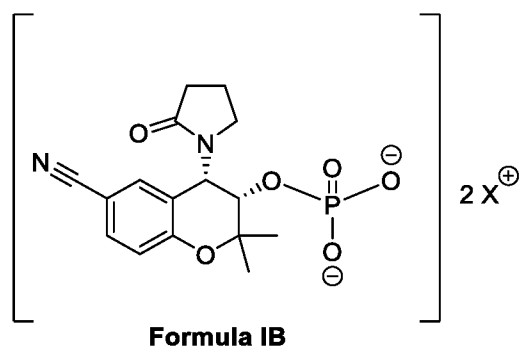
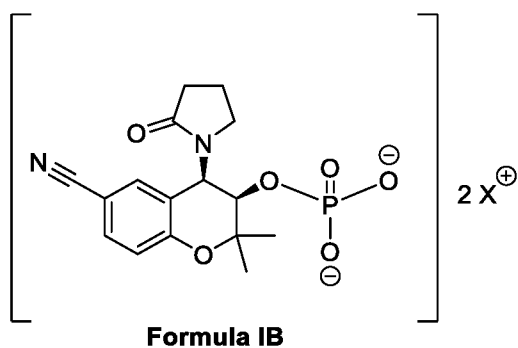


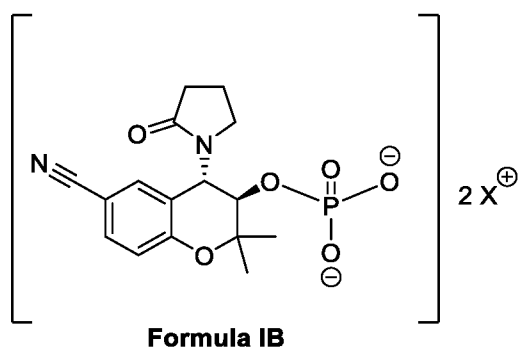
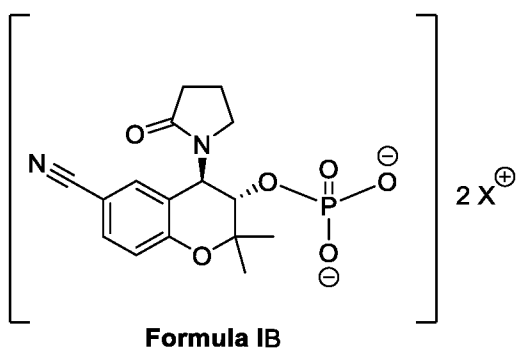
wherein X^+ and M^{2+} are a pharmaceutically acceptable cation.

13. The method of claim 12, wherein the compound of Formula IA is selected from:

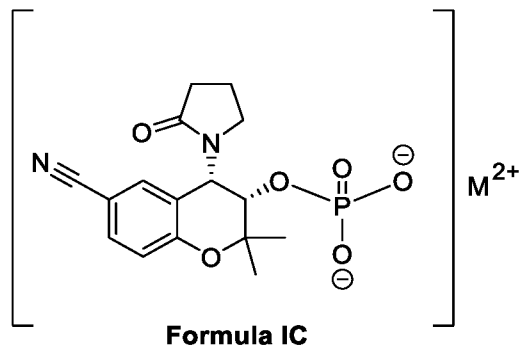
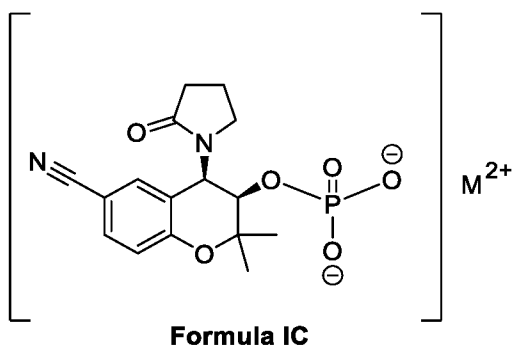
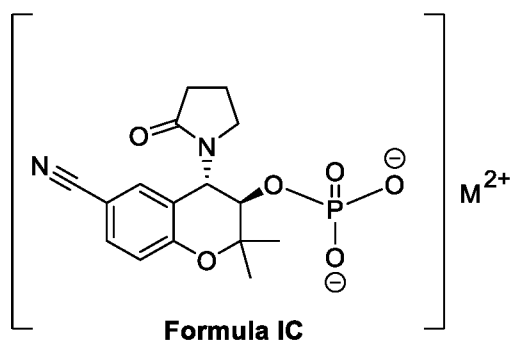
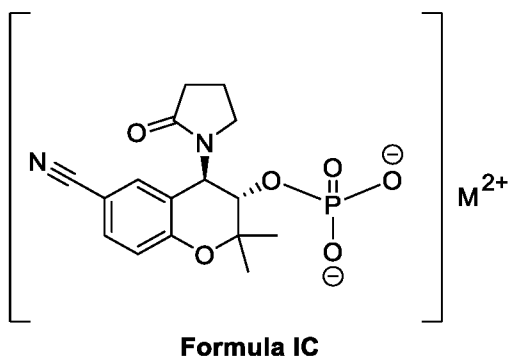


14. The method of claim 12, wherein the compound of Formula IB is selected from:

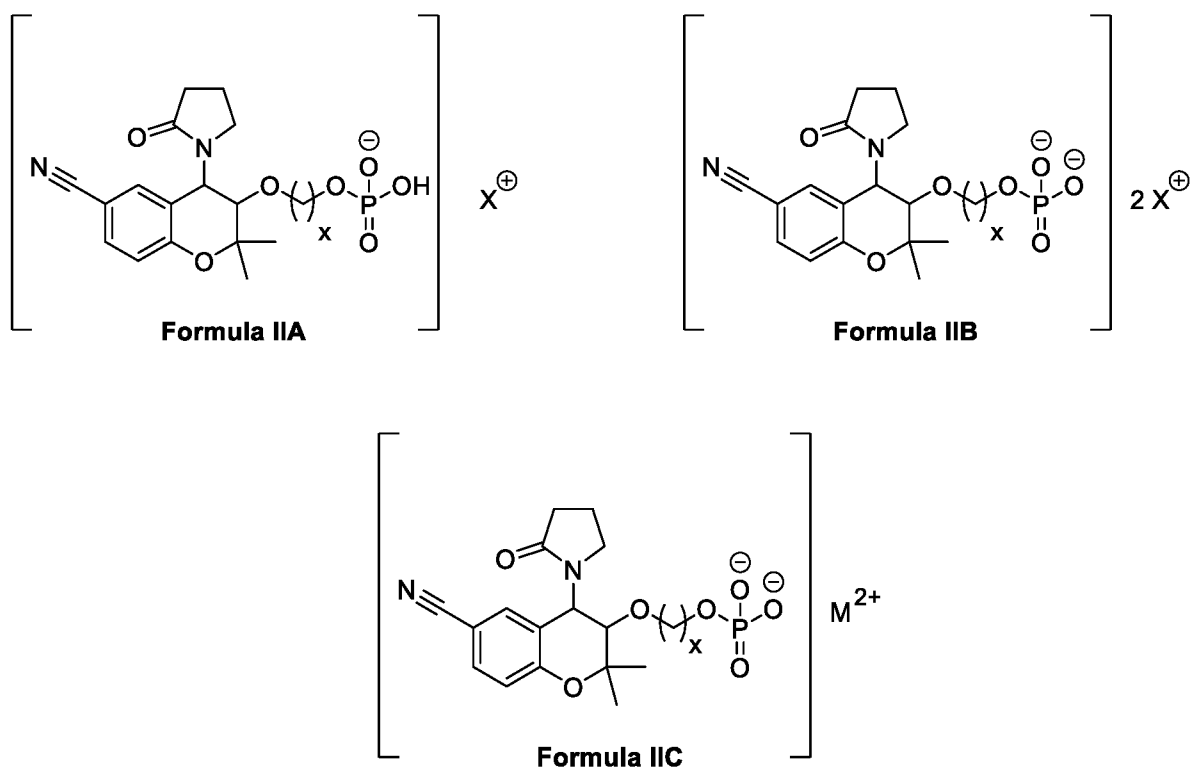




15. The method of claim 12, wherein the compound of Formula IC is selected from:

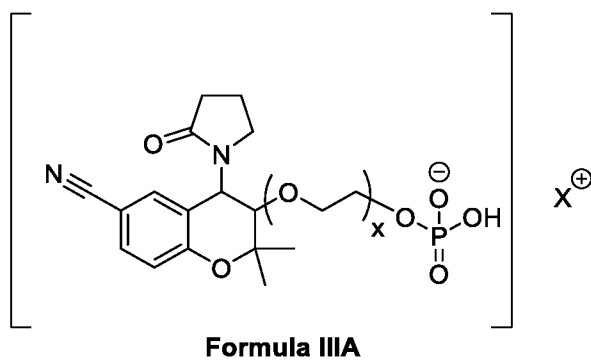


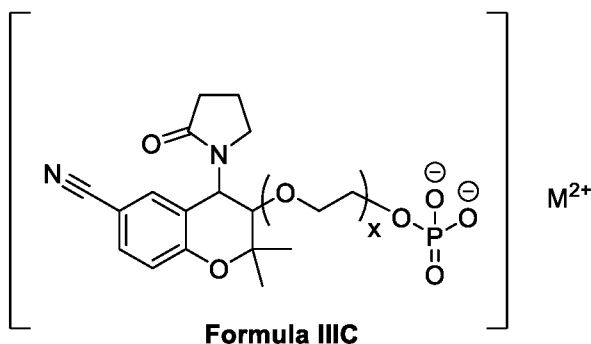
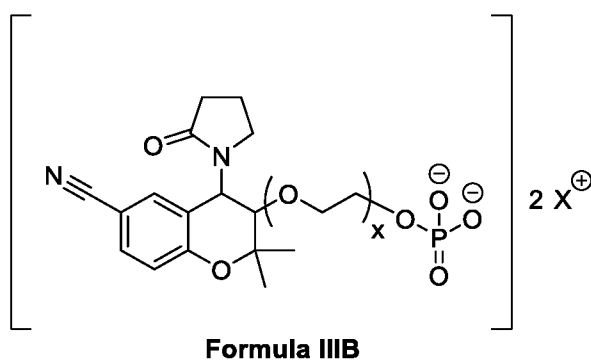
16. The method of any one of claim 1-11, wherein the compound is a pharmaceutically acceptable salt of Formula II selected from Formula IIA, Formula IIB, or Formula IIC:



wherein X^+ and M^{2+} are a pharmaceutically acceptable cation; and
 x is an integer selected from 1, 2, 3, 4, or 5.

17. The method of any one of claim 1-11, wherein the compound is a pharmaceutically acceptable salt of Formula III selected from Formula IIIA, Formula IIIB, or Formula IIIC:

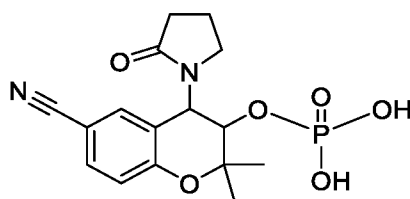




wherein X^+ and M^{2+} are a pharmaceutically acceptable cation; and
 x is an integer selected from 1, 2, 3, 4, and 5.

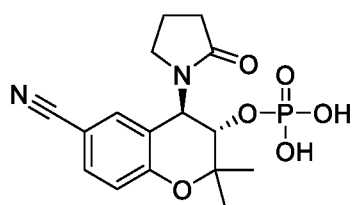
18. The method of any one of claim 12-14 and 16-17, wherein X^+ is selected from Na^+ , K^+ , Li^+ , Cs^+ , or an ammonium ion with a net positive charge of one.
19. The method of any one of claim 12 and 15-17, wherein M^{2+} is selected from Mg^{2+} , Ca^{2+} , Sr^{2+} , Zn^{2+} , Fe^{2+} , or an ammonium ion with a net positive charge of two.
20. The method of any one of claims 1-11 and 16-19, wherein x is 1.
21. The method of any one of claims 1-11 and 16-19, wherein x is 1, 2, or 3.
22. The method of any one of claims 1-11 and 16-19, wherein x is 4, or 5.

23. The method of any one of claims 1 and 10-22, wherein the pharmaceutically acceptable carrier is a dosage form suitable for ocular topical delivery.
24. The method of any one of claims 1-22, wherein the pharmaceutically acceptable carrier is a dosage form suitable for oral administration.
25. The method of claim 24, wherein the dosage form is a solid dosage form.
26. The method of claim 25, wherein the dosage form is a pill, capsule, or gel-cap.
27. The method of claim 24, wherein the dosage form is a liquid dosage form.
28. The method of claim 27, wherein the dosage form is a suspension or solution.
29. The method of any one of claims 1-22, wherein the pharmaceutically acceptable carrier is a dosage form suitable for topical administration.
30. The method of any one of claims 1-22, wherein the pharmaceutically acceptable carrier is a dosage form suitable for intravenous administration.
31. The method of any one of claims 1-22, wherein the pharmaceutically acceptable carrier is a dosage form suitable for parental administration.
32. The method of any one of claims 1-11 and 23-31, wherein the compound is CKLP1



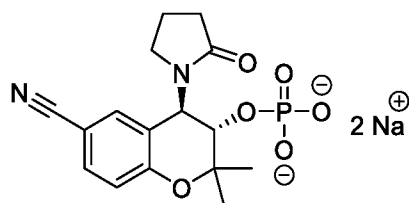
or a pharmaceutically acceptable salt thereof.

33. The method of claim 32, wherein the compound is of the formula:



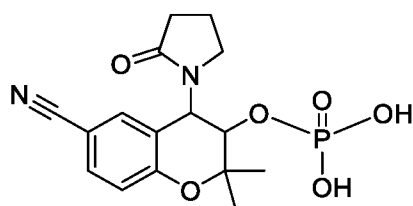
or a pharmaceutically acceptable salt thereof.

34. The method of claim 33, wherein the compound is of the formula:

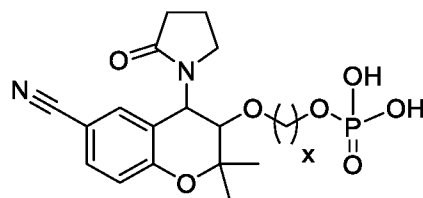


35. The method of any one of claims 1-34, wherein the host is a human.

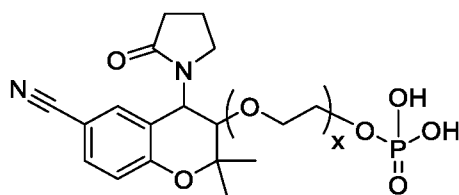
36. An effective amount of a compound of Formula I, Formula II, or Formula III,



CKLP1
Formula I



Formula II



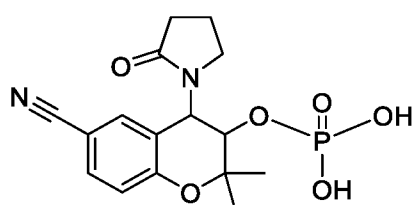
Formula III

or a pharmaceutically acceptable salt, optionally in a pharmaceutically acceptable carrier,
for use to treat an ocular disorder selected from Graves' ophthalmopathy, cavernous sinus

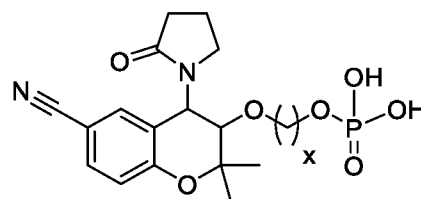
thrombosis, orbital vein vasculitis, carotid-cavernous sinus fistula, orbital varices, central retinal vein occlusion, branch retinal vein occlusion, and non-arteritic anterior ischemic optic neuropathy in a host in need thereof wherein x is an integer selected from 1, 2, 3, 4, and 5.

37. The compound of claim 36, wherein the ocular disorder is non-arteritic anterior ischemic optic neuropathy.

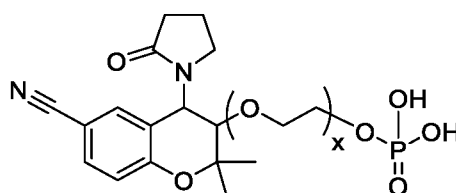
38. An effective amount of a compound of Formula I, Formula II, or Formula III:



CKLP1
Formula I



Formula II



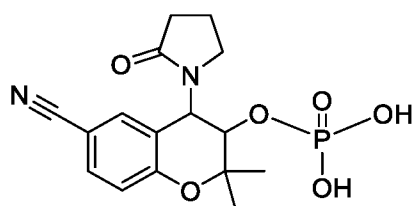
Formula III

or a pharmaceutically acceptable salt, optionally in a pharmaceutically acceptable carrier, for use to treat a blood vessel disorder selected from Raynaud's disease, peripheral artery disease, chronic limb ischemia, thrombophlebitis, pulmonary arterial hypertension, and chronic venous insufficiency in a host in need thereof wherein x is an integer selected from 1, 2, 3, 4, and 5.

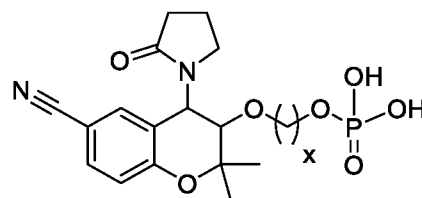
39. The compound of claim 38, wherein the blood vessel disorder is Raynaud's disease.

40. The compound of claim 38, wherein the blood vessel disorder is pulmonary arterial hypertension.

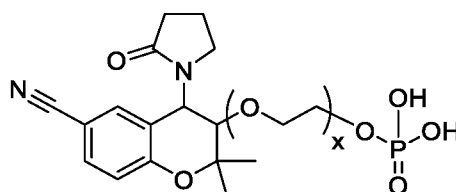
41. An effective amount of a compound of Formula I, Formula II, or Formula III:



CKLP1
Formula I



Formula II

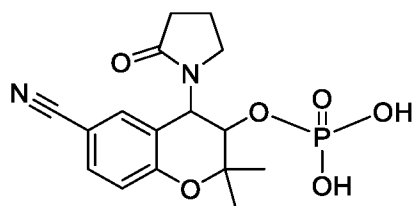


Formula III

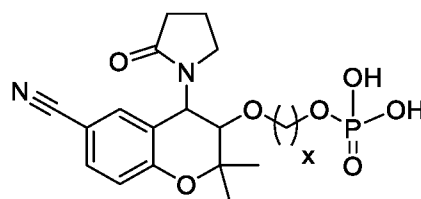
or a pharmaceutically acceptable salt, optionally in a pharmaceutically acceptable carrier, for use to treat a disorder or disease described herein in a host in need thereof wherein x is an integer selected from 1, 2, 3, 4, and 5.

42. An effective amount of the compounds of claim 41 for any of the method of treatment claims 1-35.

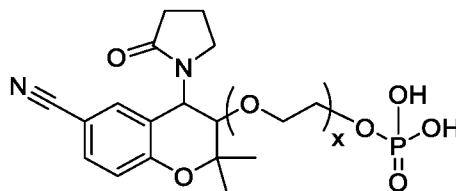
43. The use of an effective amount of a compound of Formula I, Formula II, or Formula III,



CKLP1
Formula I



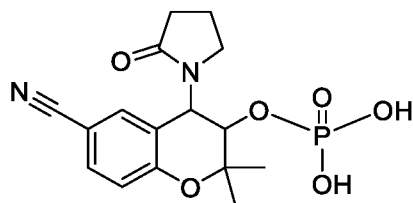
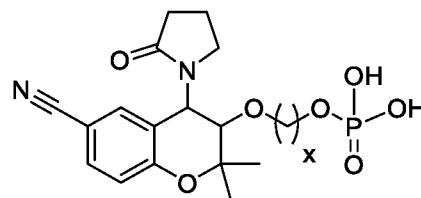
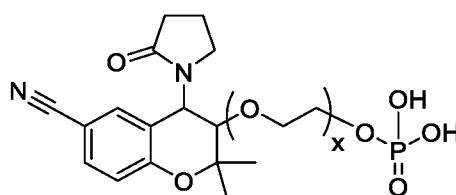
Formula II

**Formula III**

or a pharmaceutically acceptable salt, optionally in a pharmaceutically acceptable carrier, in the manufacture of a medicament for the treatment of an ocular disorder selected from Graves' ophthalmopathy, cavernous sinus thrombosis, orbital vein vasculitis, carotid-cavernous sinus fistula, orbital varices, central retinal vein occlusion, branch retinal vein occlusion, and non-arteritic anterior ischemic optic neuropathy in a host in need thereof wherein x is an integer selected from 1, 2, 3, 4, and 5.

44. The use of claim 43, wherein the ocular disorder is non-arteritic anterior ischemic optic neuropathy.

45. The use of an effective amount of a compound of Formula I, Formula II, or Formula III:

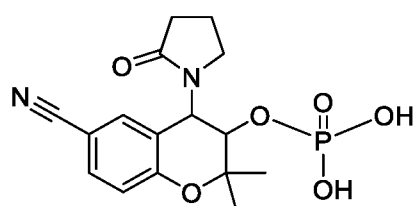
**CKLP1**
Formula I**Formula II****Formula III**

or a pharmaceutically acceptable salt, optionally in a pharmaceutically acceptable carrier, in the manufacture of medicament for the treatment of a blood vessel disorder selected from Raynaud's disease, peripheral artery disease, chronic limb ischemia, thrombophlebitis, pulmonary arterial hypertension, and chronic venous insufficiency in a host in need thereof wherein x is an integer selected from 1, 2, 3, 4, and 5.

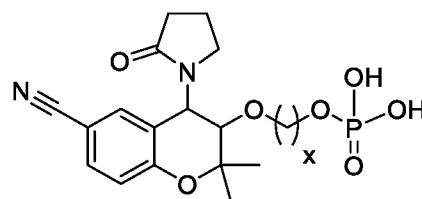
46. The use of claim 45, wherein the blood vessel disorder is Raynaud's disease.

47. The use of claim 45, wherein the blood vessel disorder is pulmonary arterial hypertension.

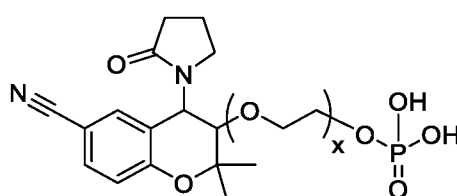
48. The use of an effective amount of a compound of Formula I, Formula II, or Formula III:



CKLP1
Formula I



Formula II

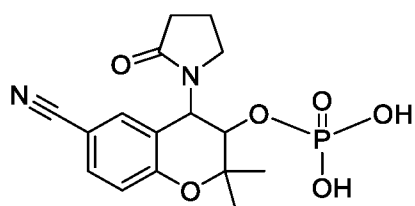


Formula III

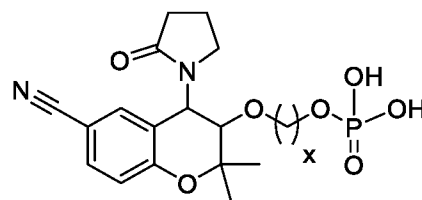
or a pharmaceutically acceptable salt, optionally in a pharmaceutically acceptable carrier, in the manufacture of a medicament for the treatment of a disorder or disease described herein in a host in need thereof wherein x is an integer selected from 1, 2, 3, 4, and 5.

49. The use of an effective amount of the compounds of claim 48 for any of the method of treatment claims 1-35.

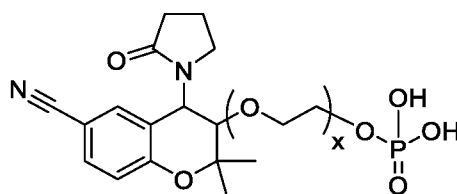
50. A pharmaceutical composition that includes an effective amount of a compound of Formula I, Formula II, or Formula III



CKLP1
Formula I



Formula II



Formula III

or a pharmaceutically acceptable salt thereof for any of the method of treatment claims 1-35.

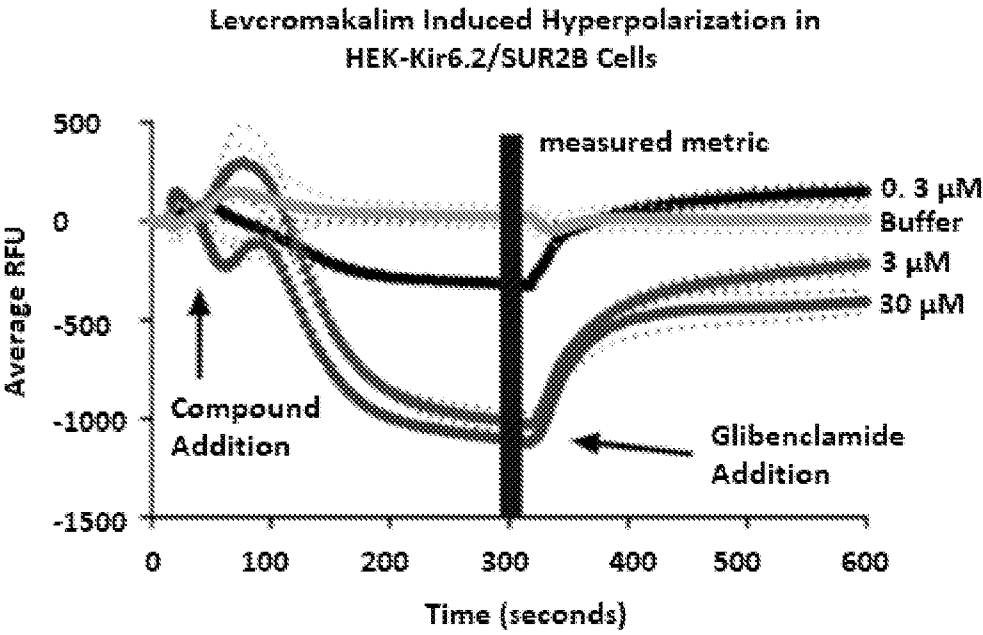


FIG. 1A

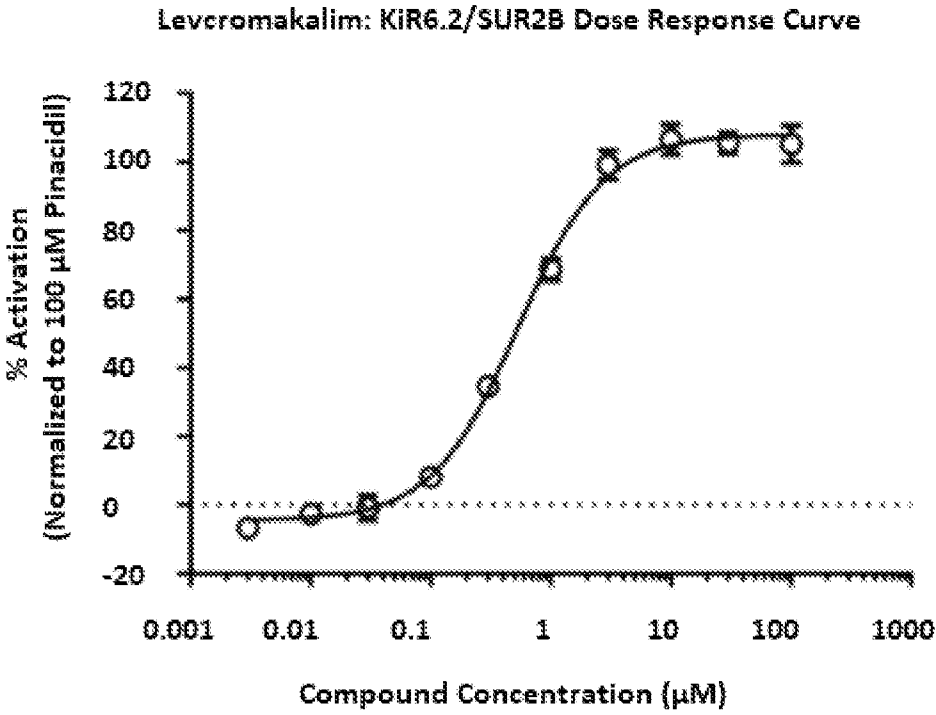


FIG. 1B

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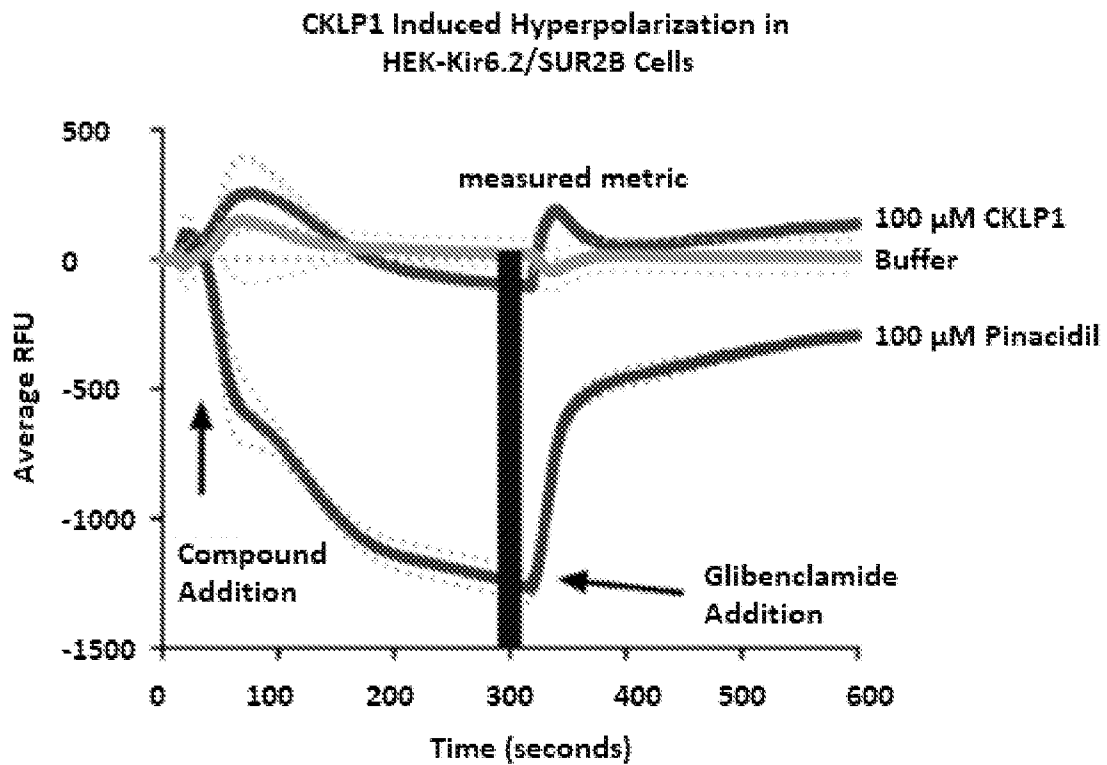


FIG. 2A

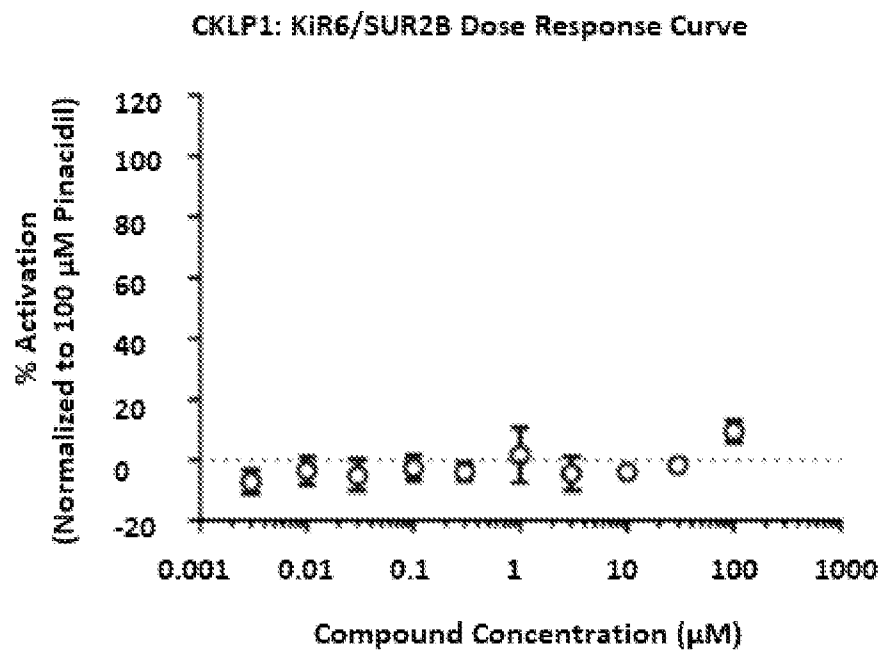


FIG. 2B

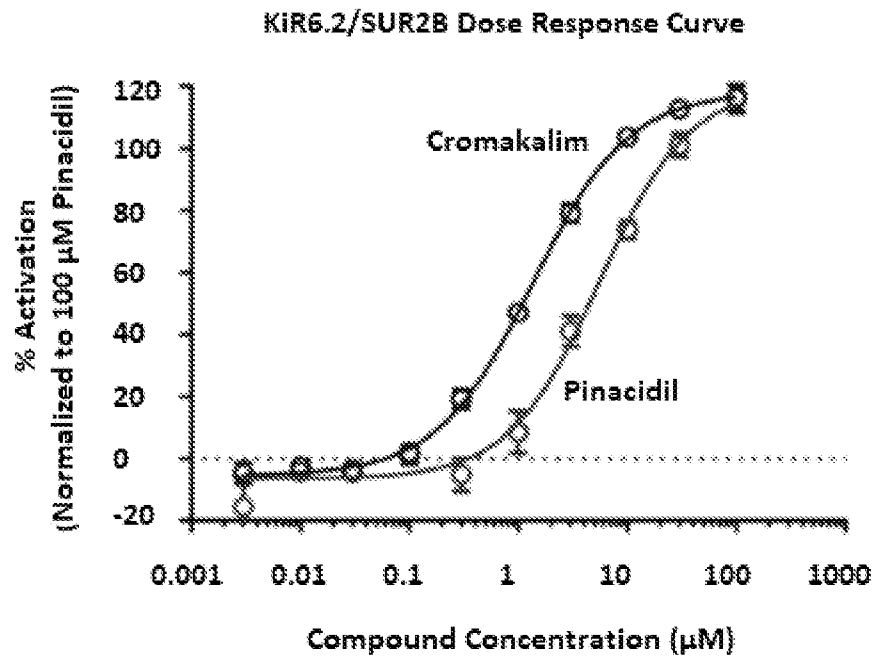


FIG. 3

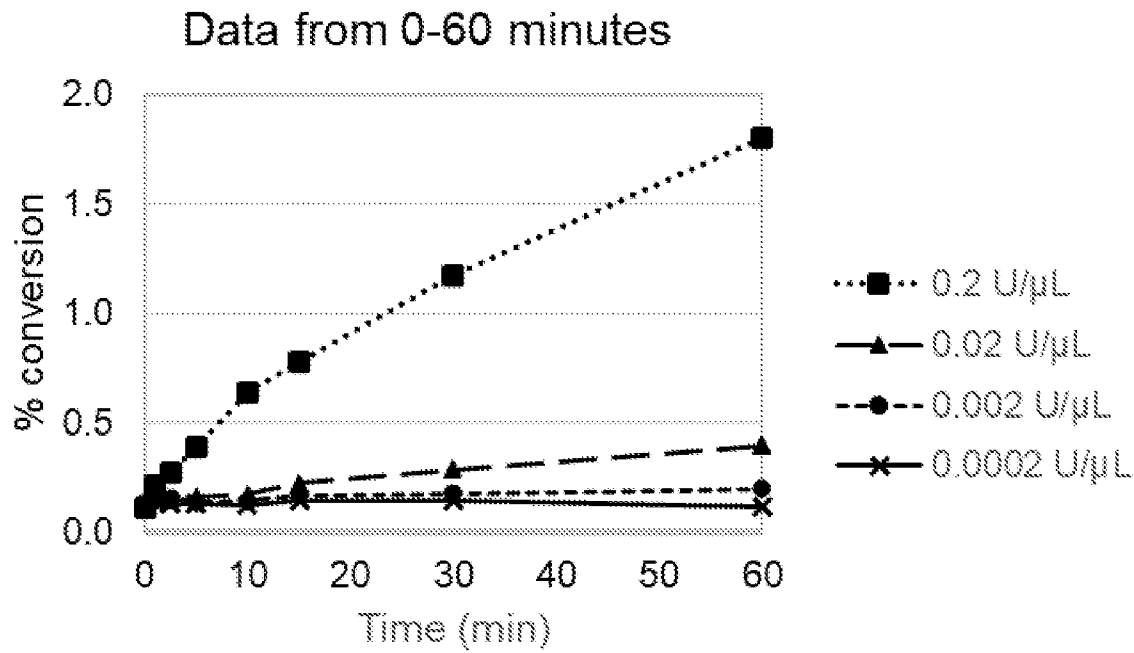


FIG. 4A

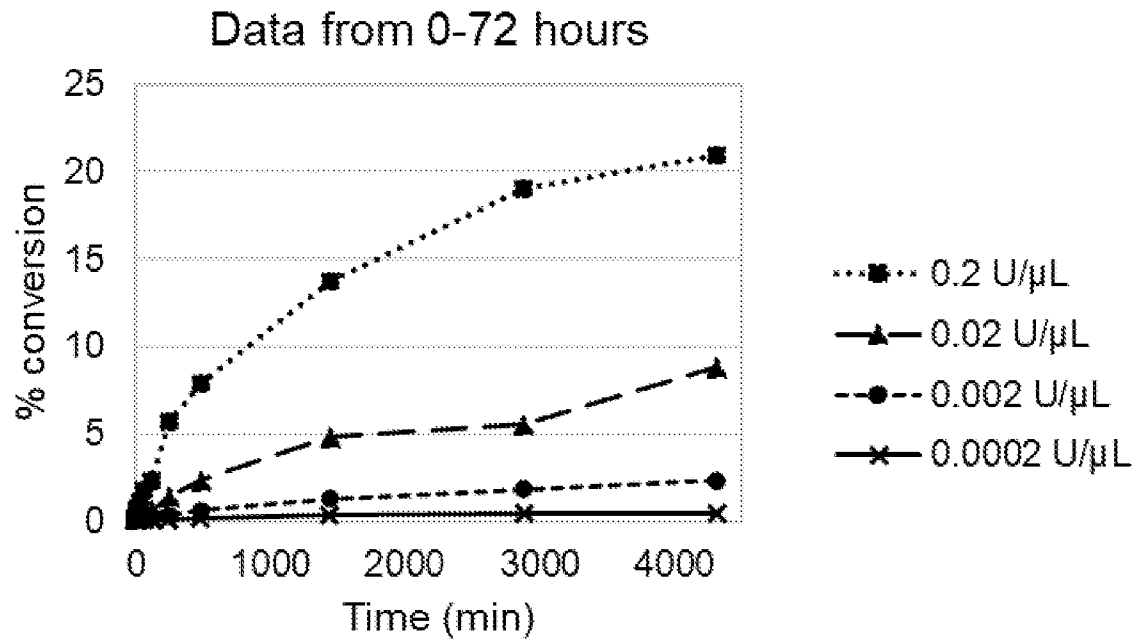


FIG. 4B

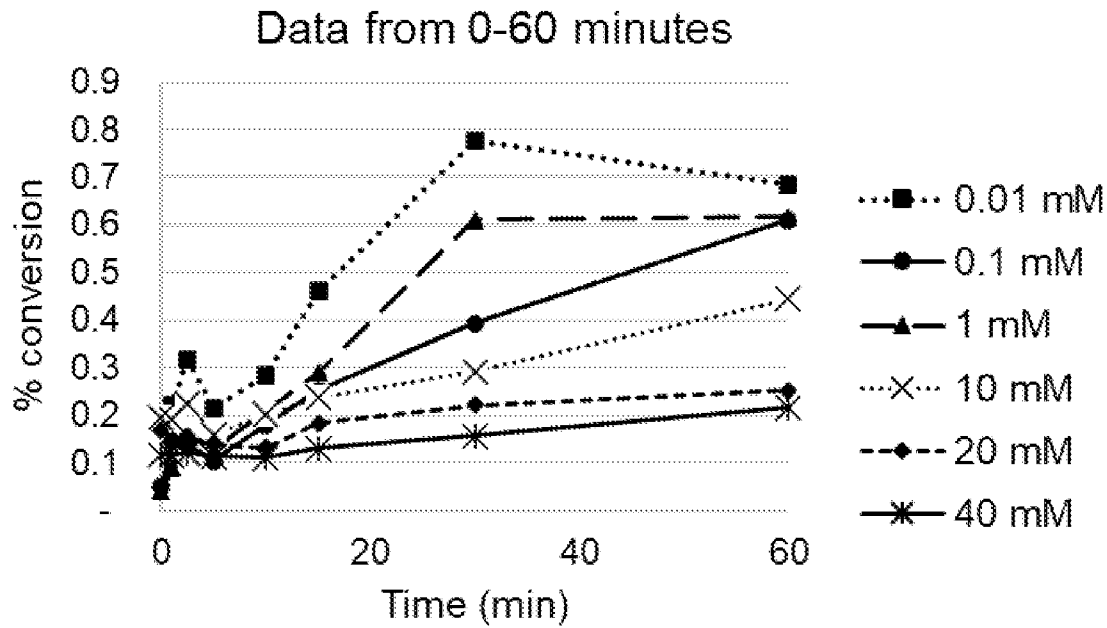


FIG. 5A

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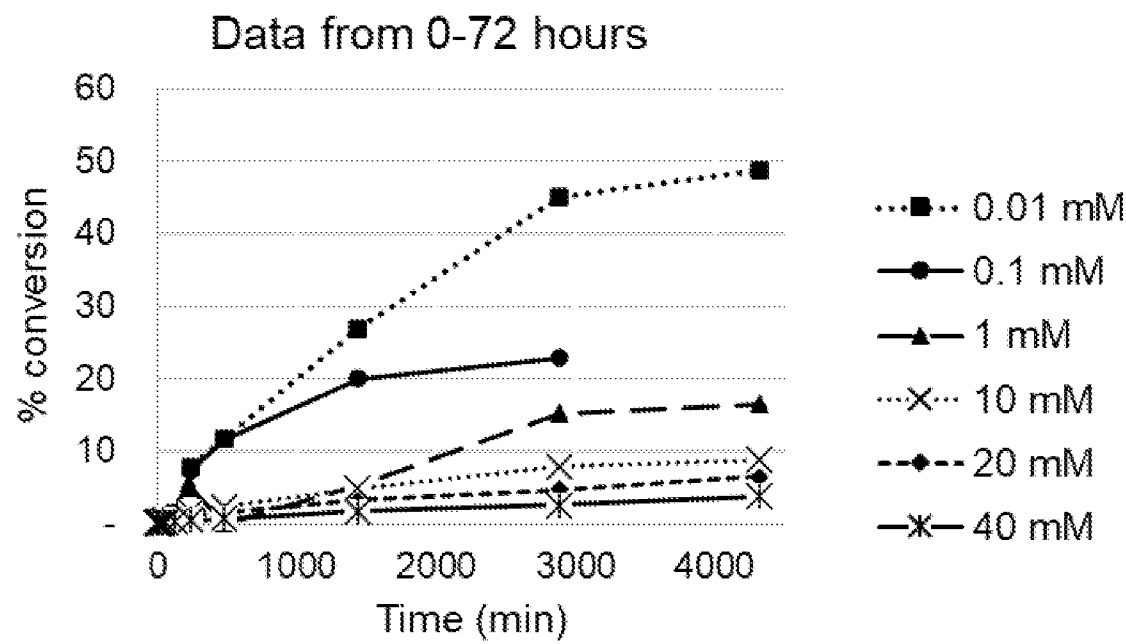


FIG. 5B

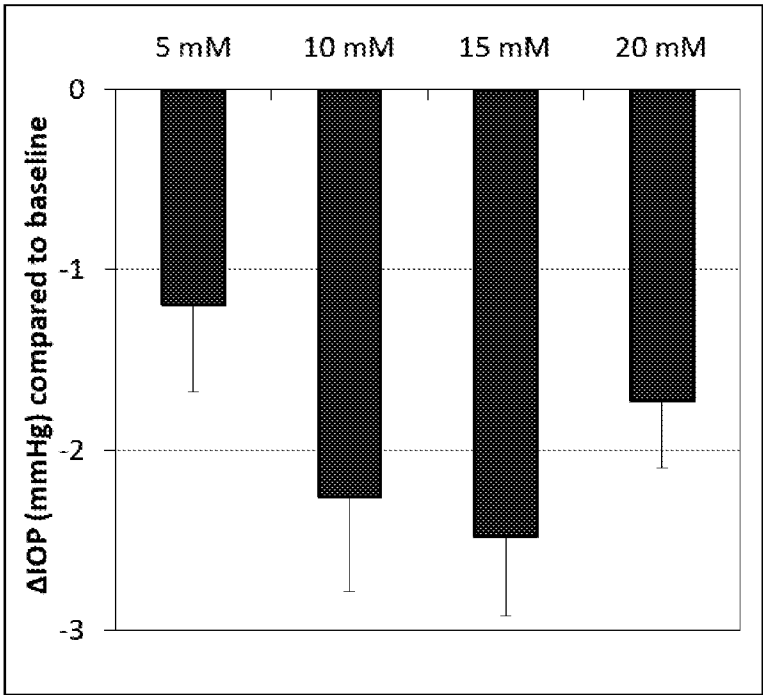


FIG. 6

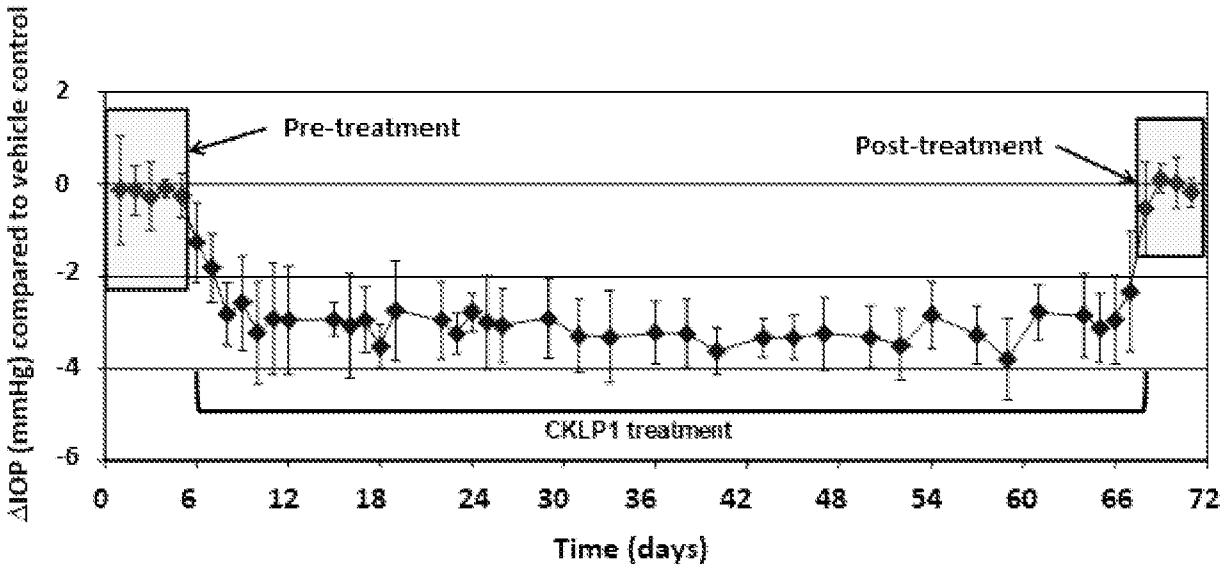


FIG. 7A

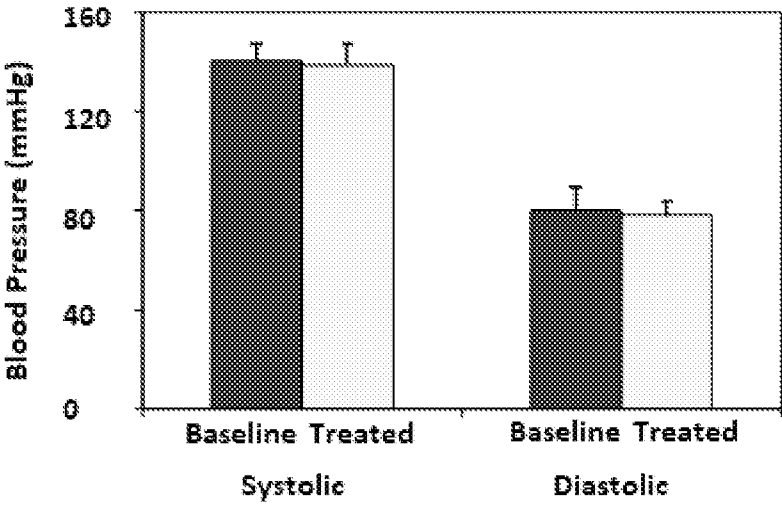


FIG. 7B

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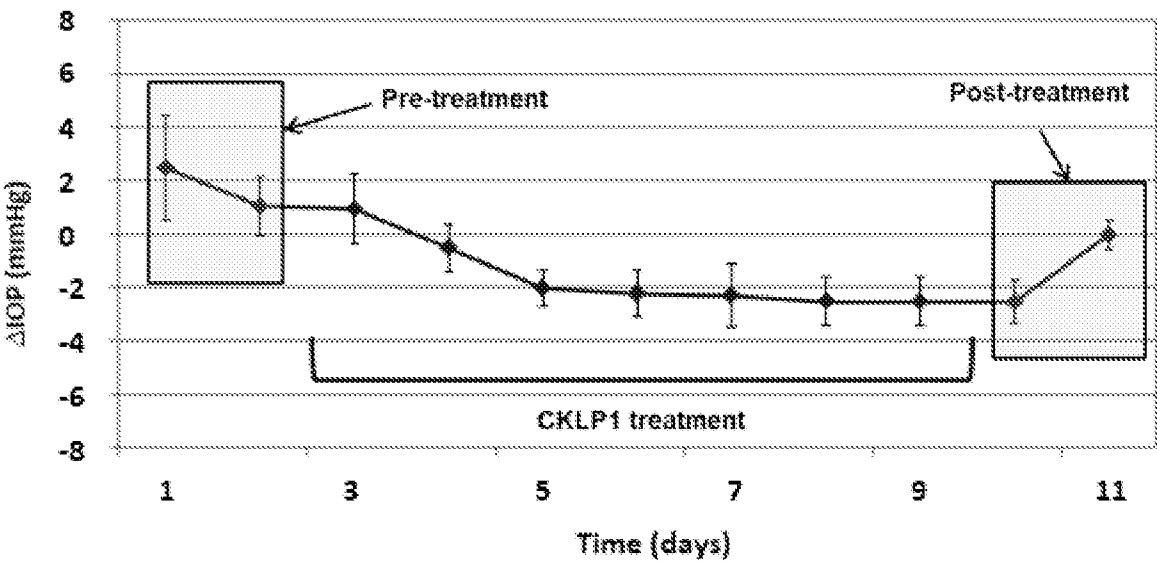


FIG. 8A

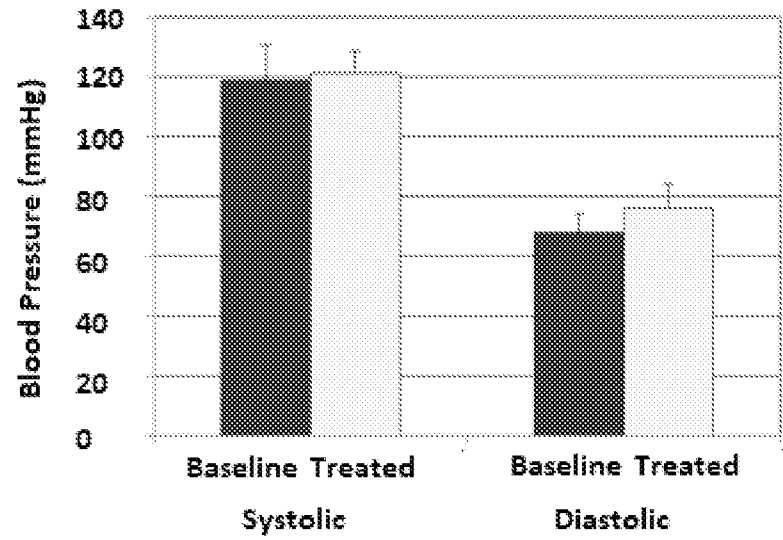


FIG. 8B

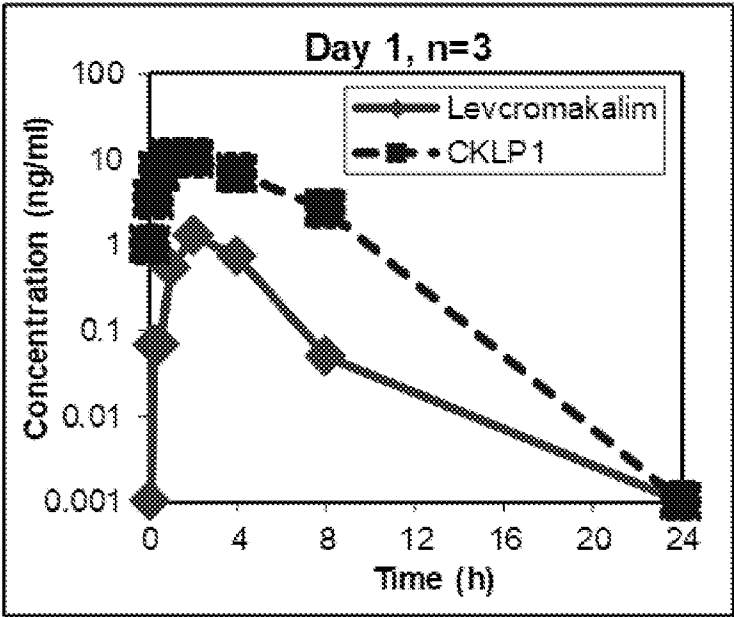


FIG. 9A

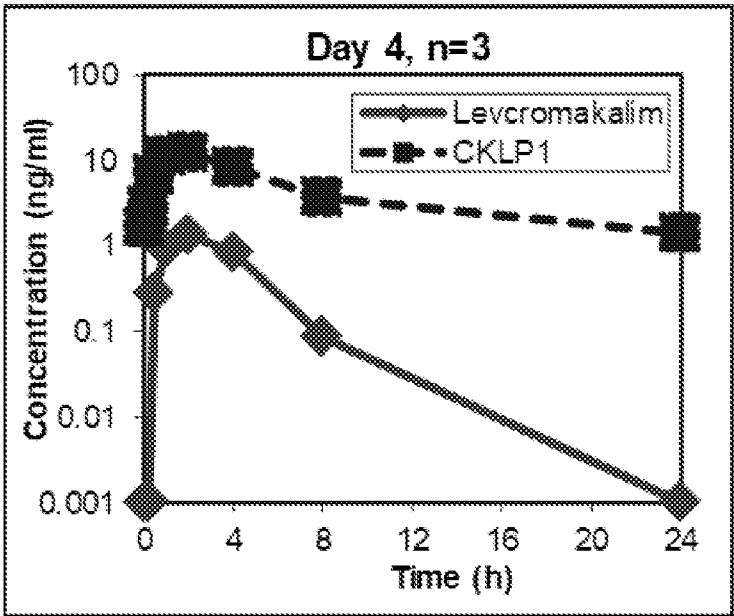


FIG. 9B

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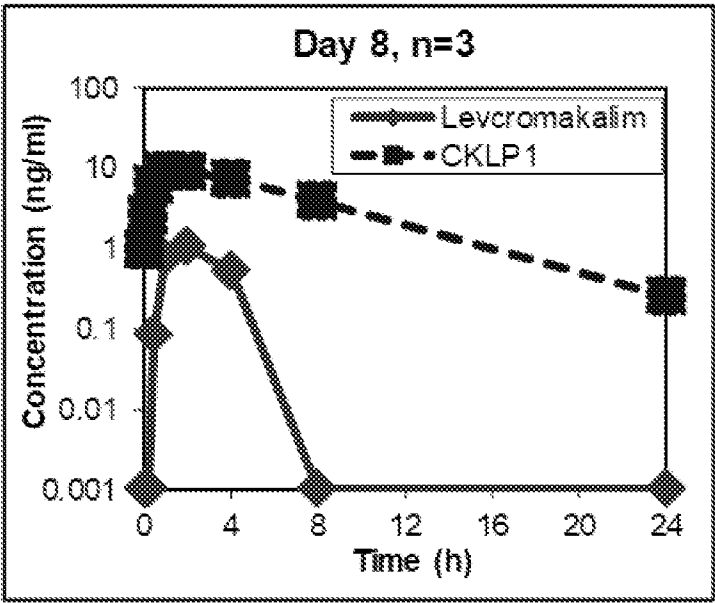


FIG. 9C

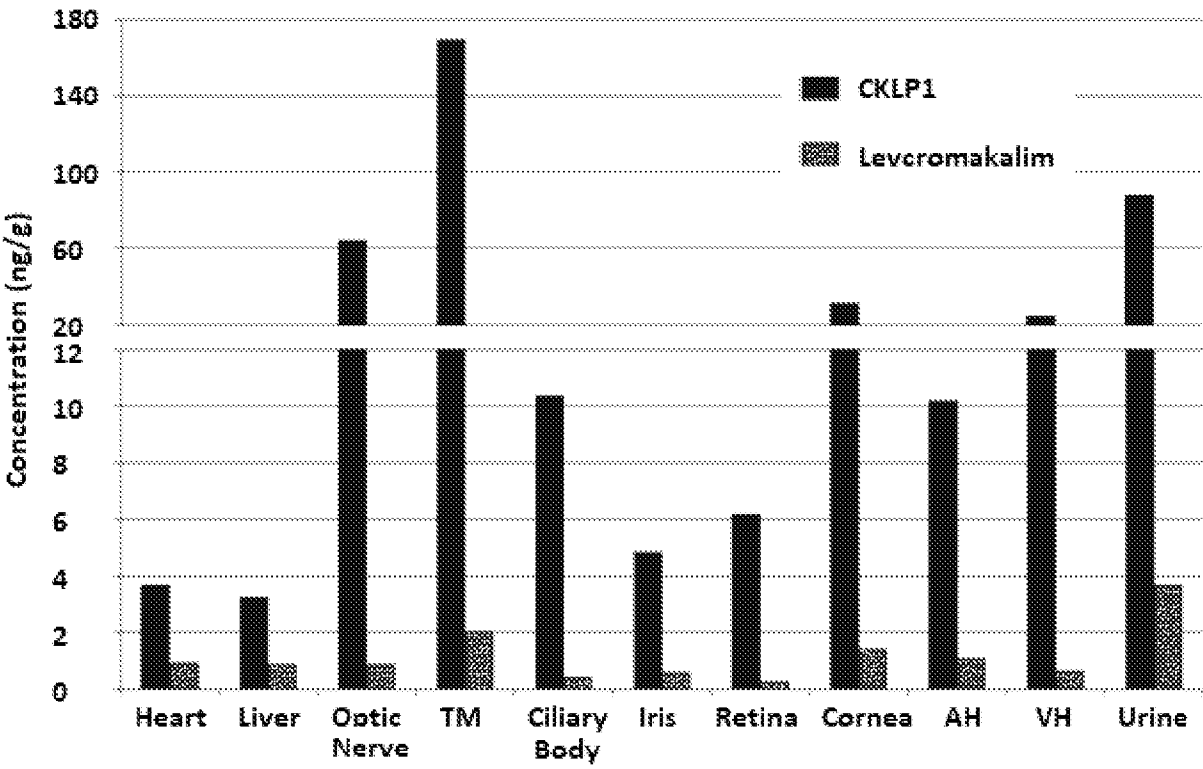


FIG. 10

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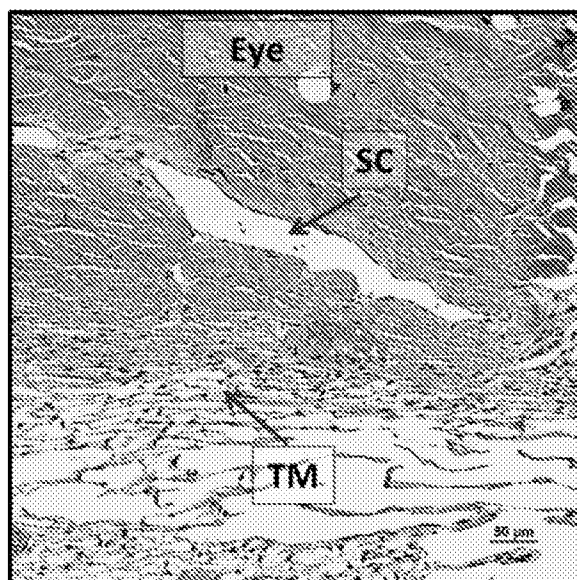


FIG. 11A

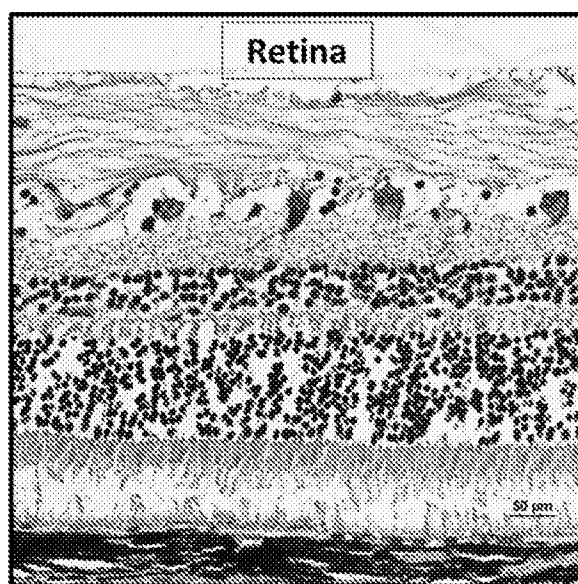


FIG. 11B

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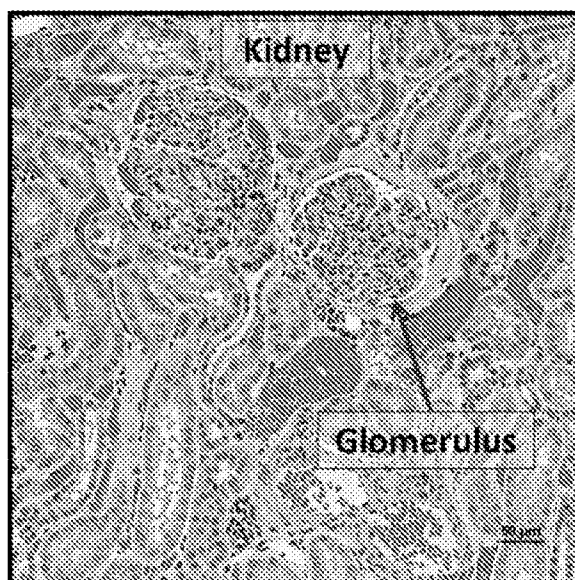


FIG. 11C

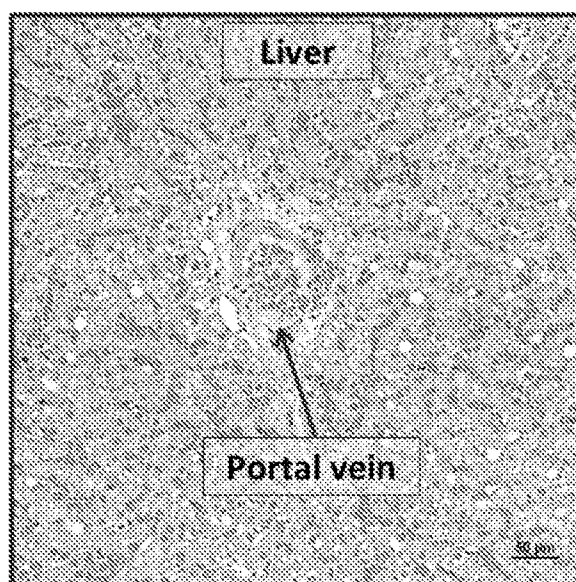
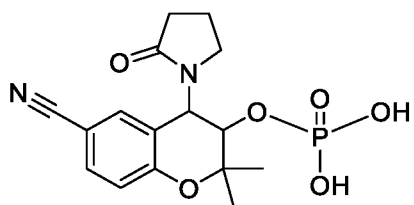
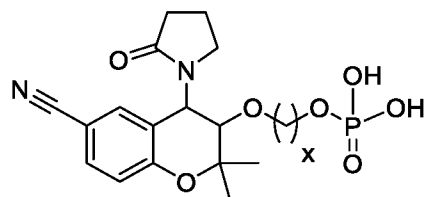


FIG. 11D

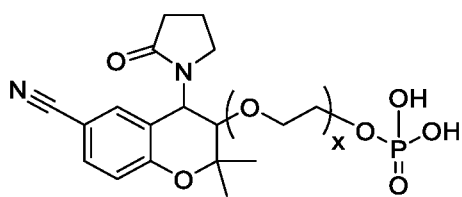
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CKLP1
Formula I



Formula II



Formula III

FIG. 12