Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 2931039 C 2022/07/12

(11)(21) 2 931 039

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(86) Date de dépôt PCT/PCT Filing Date: 2014/11/20
(87) Date publication PCT/PCT Publication Date: 2015/05/28
(45) Date de délivrance/Issue Date: 2022/07/12
(85) Entrée phase nationale/National Entry: 2016/05/18
(86) N° demande PCT/PCT Application No.: CA 2014/000841
(87) N° publication PCT/PCT Publication No.: 2015/074137
(30) Priorité/Priority: 2013/11/20 (US61/906,694)

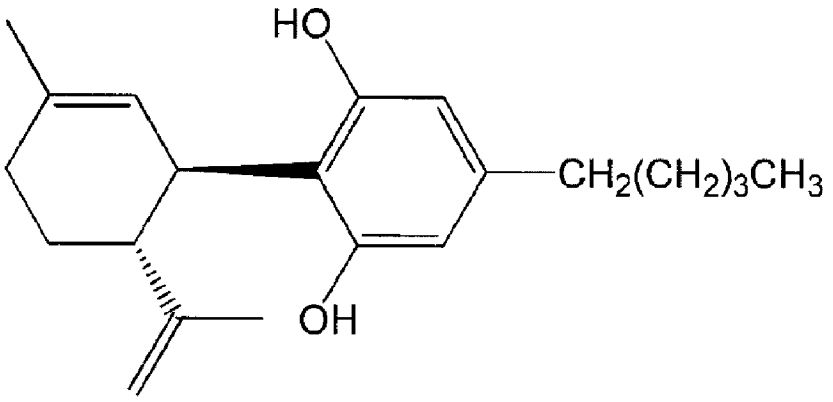
(51) Cl.Int./Int.Cl. *A61K 31/05* (2006.01),
A61K 31/015 (2006.01), *A61K 31/09* (2006.01),
A61P 25/02 (2006.01), *A61P 27/02* (2006.01),
A61P 29/00 (2006.01), *C07C 13/32* (2006.01),
C07C 39/23 (2006.01), *C07C 43/23* (2006.01)

(72) Inventeurs/Inventors:
LYNCH, MARY, CA;
KELLY, MELANIE, CA

(73) Propriétaire/Owner:
PANAG PHARMA INC., CA

(74) Agent: BERESKIN & PARR LLP/S.E.N.C.R.L.,S.R.L.

(54) Titre : COMPOSITIONS ET METHODES DE TRAITEMENT DE L'INFLAMMATION OCULAIRE ET DE LA DOULEUR
(54) Title: COMPOSITIONS AND METHODS FOR TREATMENT OF OCULAR INFLAMMATION AND PAIN



(57) Abrégé/Abstract:
The disclosure provides methods of treatment of ocular inflammation or neuropathic pain in a subject in need thereof, comprising administering to the subject in need thereof a CB2 target agent, a cannabimimetic agent (such as a non-psychotropic cannabimimetic agent) or a combination thereof. The agent is optionally a cannabinoid, such as a non-psychotropic cannabinoid or a synthetic cannabinoid. In certain embodiments, the non-psychotropic phytocannabinoid is β-caryophyllene or cannabidiol [CBD] and the synthetic cannabinoid is HU-433, HU-308, or a modified CBD such as CBD-DMH. The disclosure also provides ocular pharmaceutical compositions containing the CB2 target agents or cannabimimetic agents such as non-psychotropic cannabimimetic agents described herein.

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 May 2015 (28.05.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/074137 A1

(51) International Patent Classification:
A61K 31/05 (2006.01) *A61P 29/00* (2006.01)
A61K 31/015 (2006.01) *C07C 13/32* (2006.01)
A61K 31/09 (2006.01) *C07C 39/23* (2006.01)
A61P 25/02 (2006.01) *C07C 43/23* (2006.01)
A61P 27/02 (2006.01)

(21) International Application Number:
PCT/CA2014/000841

(22) International Filing Date:
20 November 2014 (20.11.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/906,694 20 November 2013 (20.11.2013) US

(72) Inventors; and
(71) Applicants : **LYNCH, Mary** [CA/CA]; 1942 Bloomingdale Terrace, Halifax, Nova Scotia B3H 4E5 (CA). **KELLY, Melanie** [CA/CA]; 12 Saraguay Place, Halifax, Nova Scotia B3P 2N9 (CA).

(74) Agent: **BERESKIN & PARR LLP/S.E.N.C.R.L., S.R.L.**; Scotia Plaza, 40 King Street West, 40th Floor, Toronto, Ontario M5H 3Y2 (CA).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

Published:
— *with international search report (Art. 21(3))*

WO 2015/074137 A1

(54) Title: COMPOSITIONS AND METHODS FOR TREATMENT OF OCULAR INFLAMMATION AND PAIN

(57) Abstract: The disclosure provides methods of treatment of ocular inflammation or neuropathic pain in a subject in need thereof, comprising administering to the subject in need thereof a CB2 target agent, a cannabimimetic agent (such as a non-psychotropic cannabimimetic agent) or a combination thereof. The agent is optionally a cannabinoid, such as a non-psychotropic cannabinoid or a synthetic cannabinoid. In certain embodiments, the non-psychotropic phytocannabinoid is β-caryophyllene or cannabidiol [CBD] and the synthetic cannabinoid is HU-433, HU-308, or a modified CBD such as CBD-DMH. The disclosure also provides ocular pharmaceutical compositions containing the CB2 target agents or cannabimimetic agents such as non-psychotropic cannabimimetic agents described herein.

COMPOSITIONS AND METHODS FOR TREATMENT OF OCULAR INFLAMMATION AND PAIN

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of priority from co-pending U.S. provisional application no. 61/906,694 filed on November 20, 2013 .

FIELD OF THE DISCLOSURE

[0002] The disclosure provides compositions and methods for treating ocular pain and inflammation.

BACKGROUND

[0003] There is a need for novel treatments for pain and inflammation. The current agents are inadequate and can cause unacceptable side effects. Additionally, the growing concern about the potential for addiction with opioid pain treatment further supports the need for new pain therapies. In particular, there is a need for new products for the treatment of ocular neuropathic pain and inflammation (e.g. uveitis).

[0004] Cannabinoids have been used for systemic treatment of pain and inflammation. All of the cannabinoids currently sold for human use also exhibit cannabinoid receptor type 1 (CB1) effects which are associated with, for example, hypothermia, catalepsy, hypolocomotion and psychoactive effects so these agents are associated with sedation and other effects that may limit, for example, systemic dosing.

[0005] CBD-DMH, like its parent molecule, cannabidiol (CBD), is non-psychotropic and exhibits analgesic and anti-inflammatory effects in animal models. However, CBD-DMH is reported to be more than 10-fold more potent than CBD. The structure of CBD and CBD-DMH have been previously described (Mechoulam et al., 2002; Fride et al., 2004).

[0006] HU-308 is a synthetic cannabinoid compound that binds and activates the CB2 receptor specifically (Hanus 1999). An enantiomeric derivative of HU-308, named HU-433, is also a CB2 agonist. HU-433 has been shown to have 2-3 orders of magnitude greater potency in both *in vitro*

Date Reçue/Date Received 2021-04-05

and *in vivo* systems. It shows no psychoactivity. The chemical structures of HU-308 and HU-433 were previously described in PCT Publication No. WO 2010/041253.

[0007] Without being bound by theory, cannabis synergy arises from constituent combination effects (Berenbaum 1989; McPartland and Russo 2001; Russo 2011). This may occur via several mechanisms including but not limited to: multi-target effects (receptor agonism or antagonism, anti-oxidant, modulation of endogenous endocannabinoid synthesis or metabolism, etc.), improved pharmacokinetic properties of compounds via modulation of solubility, bioavailability, as well as potential bacteriostatic activity (Wagner and Ulrich-Merzenich 2009; Russo 2011).

[0008] CBD synergy with other phytocannabinoids and terpenoids from Cannabis has been reported specifically with regard to the treatment of inflammation and pain (Russo, 2011).

[0009] Inflammatory eye diseases represent a particular challenge due to pain and risk of blindness. The conditions encompass intraocular inflammation (e.g. uveitis, uveoretinitis, proliferative vitreoretinopathy) as well as extraocular inflammation, including corneal inflammation and neuropathology. Collectively, ocular inflammation contributes significantly to the global incidence of blinding eye disease and can be a debilitating condition with high medical and economic burden on populations.

Neuropathic Pain

[0010] Neuropathic pain is generated by pathology in the peripheral or central nervous system. A large number of disorders can give rise to neuropathic pain. This may range from nerves being cut (trauma or surgery) or damaged by viruses, ischemic and metabolic injury or complex genetic disorders to name a few. Neuropathic pain may arise from local damage to neural tissues as well as tissues remote to initial trauma and may also arise as a result of chronic inflammatory disease. Pharmacological management is one of the most used pain treatment options but results are poor with many patients obtaining inadequate relief with currently available agents. There is therefore a need for new agents for treatment of neuropathic pain. Neuropathic pain may

affect any part of the body including the eye for which there are no adequate treatments at present.

Intraocular Inflammation and Pain

[0011] Uveitis is a term used to describe any intraocular inflammation within the eye from the uvea (iris, ciliary body and choroid) to the sclera, retina and optic nerve. It involves both infectious and non-infectious conditions, which can be localized within the eye or associated with systemic inflammatory and autoimmune diseases, including reactive arthritis and multiple sclerosis. The most common form of uveitis, anterior uveitis, with inflammation of the iris and ciliary body, is additionally associated with considerable pain and photophobia (Jabs, Nussenblatt et al. 2005; Lee and Dick 2012). Untreated uveitis can lead to permanent loss of vision. Severe uveitis is treated aggressively to mitigate the damage caused by inflammation. However, currently utilized agents, including the "gold-standard" corticosteroids, anti-metabolites, biologic response modifiers and non-steroidal anti-inflammatory agents, suffer from significant side-effects and in some cases escalating costs (i.e. biologics). A search for newer efficacious, safe and/or cost-effective anti-inflammatory and immunomodulatory agents, suitable for acute and chronic use, either as sole treatments or in combination, and delivered locally to the eye, is a priority for the future treatment of ocular inflammation in order to prevent loss of vision.

Extraocular Inflammation and pain

[0012] Corneal neuropathic hyperalgesia involves a dysfunctional corneal pain system and is associated with significant discomfort and persistent heightened sensitivity of the cornea (peripheral sensitization) in the absence of overt trauma or noxious stimuli (reviewed in Belmonte et al., 2004; Rosenthal & Borsook, 2012; Rosenthal et al., 2009). Ongoing excitation of corneal nerves, following corneal damage or irritation, results in the release of neuropeptides and inflammatory mediators that augment the inflammatory reaction (neurogenic inflammation) leading to hyperalgesia. Corneal hypersensitivity, neuroinflammation, pain and photophobia are reported in patients following refractive surgery and chemical/toxic exposure, including repetitive use of benzalkonium chloride-preserved eye drops. Corneal neuropathic pain is also a central pathogenic feature of eye disorders that are

collectively referred to as dry eye, and include non-infectious immunological causes such as Sjogren syndrome and systemic lupus as well as infections with Herpes Zoster (reviewed in Rosenthal & Borsook, 2012; Yawn et al., 2013). Up to 20% of adults aged 45 or older are affected by dry eye disease presenting a major health concern with significant economic and societal implications (reviewed in Friedman, 2013; Pflugfelder, 2008). In many cases dry eye disease is refractory to treatment and lacking in a clear association between symptoms and signs. For example, while inflammatory corneal hyperalgesia, as a result of ocular surface desiccation (evaporation dry eye), is the most common form of corneal hyperalgesia, many patients who report dry eye symptoms do not show signs of dry eyes (reduced tears), or superficial corneal erosions. Contrasted are others who have insufficient tear quantity and quality who are asymptomatic. Furthermore, neuropathic disease can sometimes precede alterations in tear film dynamics (Rosenthal & Borsook, 2012; Rosenthal et al., 2009).

[0013] Current agents prescribed for corneal neuropathic pain include a wide variety of distinct compounds such as but not limited to, opioids, non-steroidal anti-inflammatory drugs, sodium channel blockers (local anesthetics), anti-convulsants, tricyclic anti-depressants and GABAergic agents. However, present pharmacotherapy remains inadequate and the complex nature of corneal neuropathic pain is highlighted by the fact that no single known treatment appears to be effective in managing symptoms. Furthermore, the undesirable side-effects of many currently prescribed agents limit the therapeutic window for treatment. Corneal inflammatory neuropathic pain therefore represents a significant unmet therapeutic need (Rosenthal & Borsook, 2012; Rosenthal et al., 2009).

[0014] CBD, or CBD in combination with other endocannabinoid system modulators, has proven clinical and pre-clinical efficacy in the treatment of neuropathic pain resulting from nerve injury and disease (Hsieh et al., 2011; Ward et al., 2011; reviewed in Rahn and Hohmann 2009; Hohman & Suplita, 2006).

SUMMARY

[0015] The present disclosure provides anti-inflammatory and immunomodulatory agents, suitable for acute and chronic use, either as sole treatments or in combination, and for delivery locally to the eye. Agents are optionally used for treatment (including prevention) of ocular inflammation optionally preventing associated pain and loss of vision.

[0016] Cannabinoids, such as the CB2 agonist, HU-308, CBD and HU-433 possess anti-inflammatory properties. The present disclosure provides methods for ocularly administering such compounds for reducing ocular inflammation and pain in a subject. Non-psychotropic phytocannabinoids, (e.g. β-caryophyllene, cannabidiol [CBD]), and synthetic cannabinoids (e.g. HU-433, HU-308, CBD-DMH) are useful ocularly for the treatment of ocular inflammation and neuropathic pain. Without being bound by theory, these products are directed at the endocannabinoid system (ECS). The ECS is a complex and sophisticated network that is part of the body's pain and immune defence network. There are two main receptor types in the ECS. These are the CB1 and the CB2 receptors respectively. The CB2 receptors are located primarily in the peripheral tissues (e.g. skin, eye, skeleton, viscera) and in neural glial cells (brain immune defence cells). The ECS is an emerging useful target for treating pain and inflammation.

[0017] Accordingly, the present disclosure includes a method of treating ocular inflammation or ocular neuropathic pain in a subject in need thereof, comprising administering ocularly to the subject a CB2 target agent, a cannabimimetic agent or a combination thereof, optionally wherein the cannabimimetic agent is a non-psychotropic cannabimimetic agent.

[0018] In an embodiment, the subject is administered a CB2 target agent, and the CB2 target agent is a CB2 agonist agent, a CB2 partial agonist agent, a CB2 positive allosteric modulator or a combination thereof. In another embodiment, the CB2 agonist agent is HU-433, HU-308 or β-caryophyllene; the CB2 partial agonist agent is CBD; and the CB2 positive allosteric modulator is CBD-DMH. In a further embodiment of the present disclosure, the subject is ocularly administered CBD-DMH.

[0019] In an embodiment, the CB2 target agent or the cannabimimetic agent is a cannabinoid. In another embodiment, the cannabinoid is a non-psychotropic cannabinoid, optionally wherein the non-psychotropic cannabinoid is a phytocannabinoid, a synthetic cannabinoid or a combination thereof.

[0020] In a further embodiment, the phytocannabinoid is β-caryophyllene, cannabidiol or a combination thereof; and the synthetic cannabinoid is HU-433, HU-308, a modified CBD or a combination thereof, optionally wherein the modified CBD is CBD-DMH.

[0021] In an embodiment of the present disclosure, the method is a method of treating ocular inflammation. In an embodiment, the ocular inflammation is caused by an eye disease. In another embodiment, the eye disease causes intraocular inflammation. Optionally the eye disease is uveitis, uveoretinitis or proliferative vitreoretinopathy. In another embodiment, the eye disease causes extraocular inflammation. Optionally, the eye disease is corneal inflammation or neuropathology.

[0022] In an embodiment of the present disclosure, the subject has an eye disease that causes pain and loss of vision, and the agent reduces the pain and/or reduces the loss of vision.

[0023] In another embodiment of the present disclosure, the method is a method of treating ocular neuropathic pain. In an embodiment, the ocular neuropathic pain is visceral ocular neuropathic pain.

[0024] In an embodiment, the subject is a mammal, optionally a human.

[0025] The present disclosure also includes an ocular pharmaceutical composition comprising a CB2 target agent, a cannabimimetic agent or a combination thereof and a carrier suitable for ocular administration to an eye, optionally wherein the cannabimimetic agent is a non-psychotropic cannabimimetic agent.

[0026] In an embodiment, the composition comprises a CB2 target agent, and the CB2 target agent is a CB2 agonist agent, a CB2 partial agonist agent, a CB2 positive allosteric modulator or a combination thereof. In another embodiment, the CB2 agonist agent is HU-433, HU-308 or β-caryophyllene; the CB2 partial agonist agent is CBD; and the CB2 positive allosteric

modulator is CBD-DMH. In a further embodiment of the present disclosure, the composition comprises CBD-DMH.

[0027] In an embodiment, the CB2 target agent or the cannabimimetic agent is a cannabinoid. In another embodiment, the cannabinoid is a non-psychotropic cannabinoid, optionally wherein the non-psychotropic cannabinoid is a phytocannabinoid, a synthetic cannabinoid or a combination thereof.

[0028] In a further embodiment, the phytocannabinoid is β-caryophyllene, cannabidiol or a combination thereof; and the synthetic cannabinoid is HU-433, HU-308, a modified CBD or a combination thereof, optionally wherein the modified CBD is CBD-DMH.

[0029] In an embodiment, the carrier comprises a liposome. In another embodiment, the carrier comprises an oil-in-water emulsion formulation.

[0030] The present disclosure also includes a method of treating ocular inflammation or ocular neuropathic pain in a subject in need thereof, comprising administering to the subject in need thereof a CB2 agonist agent or non-psychotropic cannabimimetic agent.

[0031] In one embodiment, the agent is a cannabinoid. Optionally, the cannabinoid is a non-psychotropic cannabinoid, such as a phytocannabinoid, or a synthetic cannabinoid. In one embodiment, the non-psychotropic phytocannabinoid is β-caryophyllene or cannabidiol [CBD] and the synthetic cannabinoid is HU-433, HU-308 or CBD-DMH or a combination of two or more of the foregoing.

[0032] In one embodiment, the neuropathic pain is visceral ocular neuropathic pain.

[0033] In one embodiment, the inflammation is caused by the subject having an eye disease. In an embodiment, the eye disease causes intraocular inflammation. In another embodiment, the eye disease causes extraocular inflammation. In yet another embodiment, the eye disease causes pain and loss of vision, and the agent reduces the pain and/or reduces the loss of vision.

[0034] In one embodiment, the eye disease is uveitis, uveoretinitis or proliferative vitreoretinopathy. In another embodiment, the eye disease is corneal inflammation or neuropathology.

[0035] In one embodiment, the CB2 agonist agent or non-psychotropic cannabimimetic agent is delivered locally to the eye.

[0036] In one embodiment, the subject is a mammal, optionally a human.

[0037] The present disclosure also includes an ocular pharmaceutical composition comprising a CB2 agonist agent or a non-psychotropic cannabimimetic agent and a carrier suitable for administration to eye.

[0038] In one embodiment, the composition comprises an agent that is a cannabinoid, optionally a non-psychotropic cannabinoid or a synthetic cannabinoid. The non-psychotropic cannabinoid is optionally a phytocannabinoid. In one embodiment, the non-psychotropic phytocannabinoid is β-caryophyllene or CBD and the synthetic cannabinoid is HU-433, HU-308, CBD-DMH, or a combination of two or more of the foregoing.

[0039] In one embodiment, the carrier comprises a liposome, optionally a cyclodextrin liposome.

[0040] Other features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating embodiments of the disclosure are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041] The disclosure will now be described in relation to the drawings in which:

[0042] Figure 1 shows representative intravital microscopy (IVM) images of iridial microcirculation in rat eye showing adherent leukocytes at 6 hours after intravitreal injection of: (A) saline, and (B) lipopolysaccharide (LPS). Scale Bar = 100 μm. Arrows indicate adherent leukocytes.

[0043] Figure 2 shows representative intravital microscopy images in rat eye showing adherent leukocytes at 6 hours after intravitreal injection of (A) LPS; and (B) LPS + HU-433 (0.1 $mg \cdot kg^{-1}$) showing that administration of HU-433 ameliorates the effects of LPS demonstrated by few adherent leukocytes. White arrows in Figure 1A indicate adherent leukocytes.

[0044] Figure 3 is a bar graph of dose-response for i.v. administration (0.001-1 mg/kg) of cannabinoid agonist, HU-433, on leukocyte adhesion in iridial venules in control and LPS-treated animals (n = 3-7 per group). Values are represented as number of adherent leucocytes/mm^2 endothelium and are shown as mean +SEM. $P<0.01$ for HU-433 dose of 0.1 mg/kg.

[0045] Figure 4 is a bar graph showing the average percent decrease in leukocyte-endothelium adhesion after intravitreal LPS injection in the presence of various doses of the CB2 receptor agonist, HU-433, given i.v. at doses of 0.01-1 mg/kg compared to LPS treatment alone (n = 3 - 7 per group). Values represent means.

[0046] Figure 5 shows representative still images of intravital microscopy of the iridial microcirculation in CD1 eyes at 5 hours after intravitreal LPS injection in the following groups: (A) control (saline injection); (B) LPS injection + vehicle control (Saline + DMSO); (C) LPS + CBD-DMH; and (D) an image of a control eye on lowest magnification. Arrows indicate adherent leukocytes. Scale Bar = 100 µm.

[0047] Figure 6 depicts a bar graph of IVM measurements examining the mean number of adherent leukocytes for the groups in Figure 5: Control (n = 5), LPS + vehicle (n = 4), LPS + CBD-DMH (n = 4). ** $P<0.01$ compared to the LPS + vehicle group. *** $P<0.001$ compared to the LPS + vehicle group. Values represent mean ± SEM.

[0048] Figure 7 shows results of proliferative retinopathy (PVR) evaluation in C57Blk6 mice injected with dispase (0.2 U; 2 µl) and treated with daily ip injections (7 days) of cannabinoid ligands: Vehicle, CBD-DMH (10 mg/kg), CBD (10 mg/kg), and CBD (10 mg/kg) + β-Caryophyllene (βC; 20 mg/kg). (A) Clinical evaluation of PVR. The severity of the PVR was determined on a scale of 0-5, with 0 (no disease) to 5 (completely regenerated eye). (B) Histopathologic score

in PVR (or control) mice was assessed using H&E staining and was evaluated with the scoring system of 0 (no disease) to 4 (severely damaged ocular tissue). The evaluation was based on the degree of retinal damage, the infiltration of inflammatory cells, presence/absence of exudates and formation of granulomas. (C) Average microglia (MG) count per retinal section/animal. Data are shown as mean ± SEM *P<0.05.

[0049] Figure 8 shows representative images of Iba1 staining of activated microglia for C57Blk6 mice injected with dispase (0.2 U; 2 μl) and treated with daily ip injections (7 days) of cannabinoid ligands: top left image: Control + Vehicle; top right image: PVR + Vehicle; lower left image: PVR + CBD-DMH; and lower right image: PVR + CBD + βC.

[0050] Figure 9 is a plot comparing number of blinks to an ocular topical application of 1 μM capsaicin for cauterized vs. sham. Increased blinking in cauterized eye indicates higher level of pain.

[0051] Figure 10 shows plots showing that chemical cauterization causes corneal hypersensitivity to capsaicin: (A) Mean number of blinks recorded over 1 minute after single ocular topical application of 1 μM capsaicin. Cauterized eye showed a statistically significant increase in blinks when compared to the sham control (n=6, p<0.05); and (B) Data from Figure 10A plotted as individual points to demonstrate corneal hypersensitization.

[0052] Figure 11 is a plot of results showing that ocular topical treatment with 5% CBD-DMH reduces hypersensitivity in a comparable matter to ocular topical NSAID. Mean number of blinks recorded over 1 minute after a single ocular topical application of 1 μM capsaicin. Cauterized eye was treated with either 3 doses of vehicle, 5%CBD-DMH or topical NSAID (0.1% Napafenac ophthalmic suspension).

[0053] Figure 12 is a plot of results showing that ocular topical treatment with 5% CBD-DMH eliminates corneal hypersensitivity produced by chemical cauterization. Mean number of blinks recorded over 1 minute after single ocular topical application of 1 μM capsaicin. Cauterized eye treated with 3 doses of 5%CBD-DMH eliminated hypersensitivity to capsaicin (n=8, p>0.05).

[0054] Figure 13 shows plots showing the results of *in vitro* studies of CBD and CBD-DMH: A: HEK 293A cells transiently transfected with hCB2 were treated with 0.001 – 10 μM of the indicated compound ± 1 μM CBD-DMH or CBD for 10 min. Following 10 min treatment, cells were fixed with 4% paraformaldehyde and used in In-cell™ western assays for the detection of phosphorylated and total extracellular signal regulated kinase (ERK) according to the methods described in Laprairie et al. (2014 J Biol Chem); B: HEK 293A cells transiently transfected with hCB2 were treated with 0.001 – 10 μM of the indicated compound ± 1 μM CBD-DMH or CBD for 10 min. Following 10 min treatment, cells were fixed with 4% paraformaldehyde and used in In-cell™ western assays for the detection of phosphorylated and total PLCβ3 according to the methods described in Laprairie et al. (2014 J Biol Chem); C: HEK-CRE reporter cells stably expressing firefly luciferase under the regulatory control of a promoter containing tandem cAMP-response elements and transiently transfected with hCB2 were treated with 10 μM forskolin for 30 min followed by 0.001 – 10 μM of the indicated compound ± 1 μM CBD-DMH or CBD for an additional 30 min. Following 30 min treatment cells were lysed and cAMP activity was measured at 405 nm (RLU, relative light units). Concentration-response curves were fit using non-linear regression analysis (GraphPad Prism, version 5.0). Data are displayed as the mean ± S.E.M from 4 independent experiments.

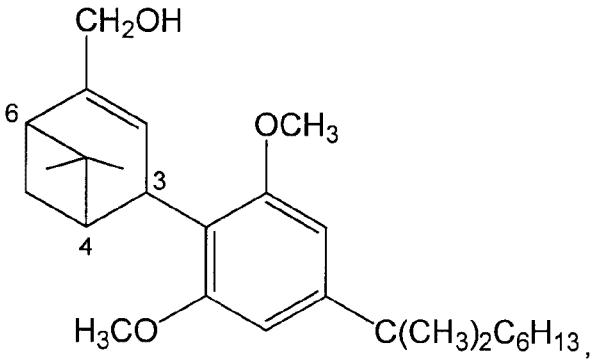
DETAILED DESCRIPTION

[0055] The disclosure relates to the use of a CB2 target agent, a cannabimimetic agent or a combination thereof, optionally a non-psychotropic cannabimimetic agent for treatment of ocular inflammation or ocular neuropathic pain in a subject. For example, the disclosure provides methods of treatment of ocular inflammation or ocular neuropathic pain in a subject in need thereof, comprising administering ocularly to the subject in need thereof a CB2 target agent or a cannabimimetic agent, optionally a non-psychotropic cannabimimetic agent. The agent is optionally a cannabinoid, such as a non-psychotropic cannabinoid or a synthetic cannabinoid. In certain embodiments, the non-psychotropic phytocannabinoid is a phytocannabinoid such as β-caryophyllene or cannabidiol [CBD] and the synthetic cannabinoid is HU-433, HU-308 or CBD-

DMH. A combination of two or more of the foregoing may also be used for treatment. The CB2 target agent is optionally a CB2 agonist agent, a CB2 partial agonist agent or a CB2 positive allosteric modulator. The disclosure also provides ocular pharmaceutical compositions containing the CB2 target agents and/or cannabimimetic agents such as non-psychotropic cannabimimetic agents.

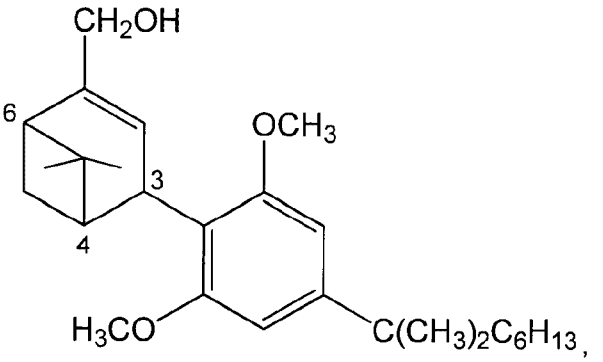
I. Definitions

[0056] The term "HU-433" as used herein refers to a synthetic cannabinoid agonist of the chemical structure:



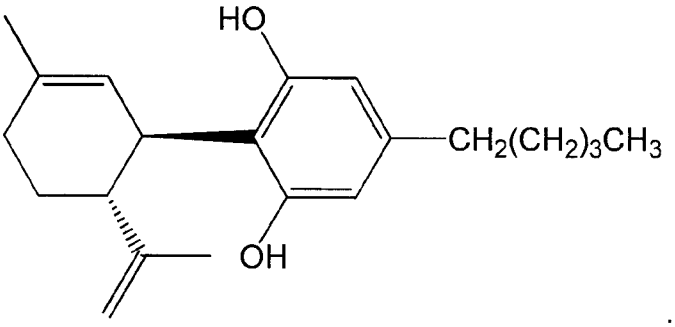
wherein the CIP configurations of the positions marked "3", "4" and "6" in the above chemical structure are R, R and R, respectively.

[0057] The term "HU-308" as used herein refers to a synthetic cannabinoid agonist of the chemical structure:

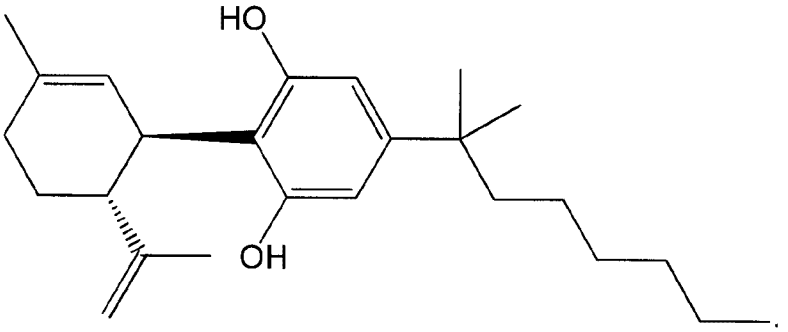


wherein the CIP configurations of the positions marked "3", "4" and "6" in the above chemical structure are S, S and S, respectively.

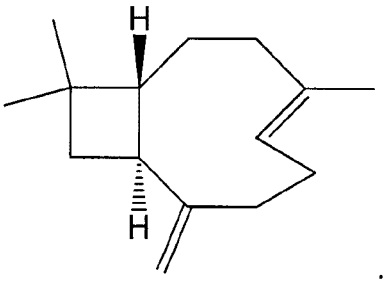
[0058] The terms "cannabidiol" or "CBD" as used herein refer to a non-psychotropic phytocannabinoid of the chemical structure:



[0059] The term "CBD-DMH" as used herein refers to a synthetic cannabinoid of the chemical structure:



[0060] The terms "β-caryophyllene", "βc" or "Beta-C" as used herein refer to a non-psychotropic phytocannabinoid of the chemical structure:



[0061] In embodiments of the present disclosure, the compounds described herein have at least one asymmetric center. Where compounds possess more than one asymmetric center, they may exist as diastereomers. It is to be further understood that while the stereochemistry of the compounds may be as shown in any given compound listed herein, such compounds may also contain certain amounts (e.g. less than 20%, optionally less than 10%, optionally less than 5%, optionally less than 1%) of compounds having alternate

stereochemistry. It will be appreciated that, for example, (+)-CBD and modified (+)-CBDs are known to be psychoactive; i.e. they may bind to the CB1 receptor.

[0062] The term "subject" as used herein includes all members of the animal kingdom including mammals, and suitably refers to humans.

II. Pharmaceutical Compositions

[0063] The present disclosure includes a composition comprising a CB2 target agent and/or a cannabimimetic agent such as a non-psychotropic cannabimimetic agent. Such agents are suitably formulated into ocular pharmaceutical compositions for ocular administration to subjects in a biologically compatible form suitable for ocular administration to an eye.

[0064] For example, solubility profile, partition coefficient, pH rate profile, pK_a, stability in pharmaceutical solvents, drug-excipient interaction and effect of moisture, temperature, light and oxygen on an agent such as Beta-C, CBD, CBD-DMH or other modified CBDs are determined. Optionally, all excipients used in the formulation are "Generally Regarded as Safe" (GRAS) and are approved by Food and Drug Administration (FDA) and Health Canada for ocular delivery. Biopharmaceutical characterization, analytical methods development, optimization and validation are also determined. Accordingly, the present disclosure includes an ocular pharmaceutical composition comprising a CB2 target agent, a cannabimimetic agent (such as a non-psychotropic cannabimimetic agent) or a combination thereof and a carrier suitable for ocular administration to an eye.

[0065] The selection of a suitable agent such as a non-psychotropic phytocannabinoid and/or synthetic cannabinoid derivative for use in the compositions of the disclosure can be made by a person skilled in the art.

[0066] For example, both CBD and β-caryophyllene are useful as agents to treat pain and inflammation; they lack psychoactivity, and have a broad safety margin. Also useful for treating pain and inflammation is the CBD derivative, CBD dimethyl heptyl (CBD-DMH), a CBD analogue (also sometimes referred to herein as an example of a "modified CBD"). The synthetic cannabinoid HU-308 has shown useful anti-inflammatory action in pre-clinical models of uveitis and proliferative vitreoretinopathy and in

experimental endotoxemia, where it decreases intestinal leukocyte adherence, improves intestinal capillary perfusion, reduces release of pro-inflammatory cytokines and reduces soluble adhesion molecule levels.

[0067] The inventors have obtained reduced inflammation in experimental models of ocular inflammation and pain. HU-433 is more potent than HU-308 in reducing ocular inflammation in experimental uveitis as well as mitigating inflammation in experimental models of sepsis. Models of neuropathic pain and painful inflammatory conditions of the eye are tested to show useful anti-pain and anti-inflammatory activity of HU-433.

[0068] It will be appreciated by a person skilled in the art that certain agents may fall under both the term "CB2 target agent" and the term "cannabimimetic agent" as those terms are used herein. For example, CBD-DMH is a CB2 positive allosteric modulator which is one example of a CB2 target agent as that term is used herein. CBD-DMH is also an example of a cannabimimetic agent as that term is used herein.

[0069] In an embodiment of the present disclosure, the active agent in the ocular pharmaceutical composition is a CB2 target agent. As used herein the term "CB2 target agent" refers to an agent that binds, activates and/or increases the activation of the CB2 receptor. Optionally, the CB2 target agent is a CB2 agonist agent, a CB2 partial agonist agent, a CB2 positive allosteric modulator or a combination thereof. It will be appreciated by a person skilled in the art that the term "CB2" as used herein in terms such as "CB2 target agent", "CB2 agonist agent", "CB2 partial agonist agent", "CB2 positive allosteric modulator" and the like refers to the CB2 receptor.

[0070] For example, the CB2 agonist agent can be HU-433, HU-308 or β-caryophyllene. For example, the CB2 partial agonist agent can be CBD. For example, the CB2 positive allosteric modulator can be CBD-DMH.

[0071] In an embodiment, the CB2 target agent or the cannabimimetic agent (such as a non-psychotropic cannabimimetic agent) is a cannabinoid. In another embodiment, the cannabinoid is a non-psychotropic cannabinoid. For example, the non-psychotropic cannabinoid can be a phytocannabinoid, a synthetic cannabinoid or a combination thereof.

[0072] In an embodiment of the present disclosure, the phytocannabinoid is β-caryophyllene, cannabidiol or a combination thereof. For example, the phytocannabinoid can be β-caryophyllene. For example, the phytocannabinoid can be cannabidiol. For example, the phytocannabinoid can be a combination of β-caryophyllene and cannabidiol.

[0073] In another embodiment of the present disclosure, the synthetic cannabinoid is HU-433, HU-308, a modified CBD (such as CBD-DMH) or combinations thereof. For example, the synthetic cannabinoid can be HU-433. For example, the synthetic cannabinoid can be HU-308. For example, the synthetic cannabinoid can be a modified CBD such as CBD-DMH or another synthetic cannabinoid that is a modified CBD with comparable activity to CBD-DMH. In an embodiment, the modified CBD is CBD-DMH. In another embodiment, the synthetic cannabinoid is a combination of HU-433, HU-308 and/or a modified CBD, optionally CBD-DMH.

[0074] The selection of a carrier suitable for ocular administration to an eye can be made by a person skilled in the art.

[0075] For example, phytocannabinoids, including THC and CBD, are typically poorly water-soluble, amorphous, highly viscous, and unstable in acidic solutions and when exposed to heat, air and light (Thumma, Majumdar et al. 2008). Beta-C and CBD-DMH also share most of these characteristics. Despite these properties, THC and CBD as well as other cannabinoids have been formulated for systemic administration, but with poor oral bioavailability. The inventors provide herein formulations for compounds such as Beta-C, CBD, CBD-DMH and HU-433 that can, for example act locally with minimal or no systemic effect. For example, the ocular pharmaceutical compositions of the present disclosure may be suitable for ocular topical, periocular or intravitreal administration to an eye.

[0076] Biopharmaceutical characterization of these ocular drug delivery systems shows the extent of, e.g. Beta-C, CBD, CBD-DMH and HU-433 absorption following application. Plasma samples are collected and analyzed using the validated LC/MS assay methods to determine the ocular pharmacokinetics and distribution in multiple species (including rabbits and

pigs). In addition, *in vitro* ocular permeability (www.absorption.com/ocular) and the potential ocular irritation of the chemicals and excipient used are determined using the Draize rabbit eye test (Draize, Woodard et al. 1944); the standard method for evaluating the ocular irritation/corrosion potential of a substance for regulatory purposes.

[0077] The eye presents a unique opportunity for localized direct drug delivery including corneal and transscleral delivery (periocular) of phytocannabinoid-based drugs, such as CBD, modified CBDs (e.g. CBD-DMH) and combinations thereof (e.g. CBD + Beta-C).

[0078] In anterior segment painful and/or inflammatory eye diseases such as uveitis and corneal neuropathic pain, drugs can be applied in various vehicles (emulsions, gels, liquid drops, etc.) to the cornea as ocular formulations or introduced via the periocular route from a conjunctival drug or posterior juxtascleral depot to reach anterior segment tissue structures and aqueous humor, and posterior structures (retina, optic nerve, retinal pigment epithelium, choroid and vitreous), respectively (Conway, 2008).

[0079] Liposomal encapsulation of cannabinoids and other compounds described herein can, for example enhance bioavailability and ocular efficacy compared to systemic drug injection. For example, non-psychotropic phytocannabinoid therapies suitable for ocular surface contact and periocular (transscleral) application in inflammatory ocular disease provide, for example a useful immunomodulatory therapy with fewer side effects than currently utilized immunosuppressive agents.

[0080] Liposomal formulations are established, safe and efficacious drug carriers for the delivery of poorly soluble lipophilic drugs (Agarwal et al., 2014). For example, they have been used in the formulation of drugs for controlled extended delivery with resultant increases in clinical efficacy in comparison to drug alone. For example, liposomes have been used to deliver a phytocannabinoid (see, for example: Sczcesniak et al., 2006).

[0081] It will be appreciated by a person skilled in the art that liposome formulations that are useful for delivery of a phytocannabinoid such as Δ^9-THC may also be useful for delivery of other compounds such as the

cannabinoids and other compounds described herein of the ocular pharmaceutical compositions of the present disclosure.

[0082] Accordingly, in an embodiment, the carrier suitable for ocular administration to an eye comprises a liposome.

[0083] Optionally, lipid components in the liposome formulations are phospholipids and cholesterol; excipients are tocopherol, antioxidants, viscosity-inducing agents and/or preservatives. The selection of suitable components can be made by a person skilled in the art.

[0084] For example, the phospholipids can be phosphatidylcholines, lysophosphatidylcholines, phosphatidylserines, phosphatidylethanolamines, phosphatidyl-glycerols, phosphatidylinositols or combinations thereof. Optionally, the phospholipid comprises, consists essentially of or consists of dipalmitoylphosphatidylcholine. Optionally, the phospholipids are provided in admixtures with modifying agents selected from the group consisting of cholesterol, stearyl amines, stearic acid, and tocopherols.

[0085] In an embodiment, the phospholipid and cholesterol are present in a molar ratio of from 20:1 to 1:1. In another embodiment, the phospholipid and cholesterol are present in a molar ratio of from 10:1 to 5:4. In a further embodiment, the phospholipid and cholesterol are present in a molar ratio of from 9:1 to 6:4. Optionally, the phospholipid and cholesterol are present in a molar ratio of 9:1 or 7:3 or 6:4. For example, the phospholipid and cholesterol are present in a molar ratio of 9:1. For example, the phospholipid and cholesterol are present in a molar ratio of 7:3. For example, the phospholipid and cholesterol are present in a molar ratio of 6:4.

[0086] In an embodiment, the ocular pharmaceutical composition contains the CB2 target agent and/or the cannabimimetic agent in an amount of from 0.01% to 10% by weight, based on the weight of the total composition.

[0087] Using a combined delivery platform with cyclodextrin complexation and liposomal incorporation can avoid the use of organic solvents to solubilize hydrophobic compounds and enables entrapment of the lipophilic phytocannabinoid complex into the aqueous core of liposomes. This approach therefore may not only increase drug solubility and stability but may

also bypass the accelerated drug release that can occur following the more usual incorporation of hydrophobic drug into the liposomal lipid component (Maestrelli et al., 2010; 2005). Accordingly, the ocular pharmaceutical compositions of the present disclosure, for example those comprising CBD, modified CBD (e.g. CBD-DMH) and CBD combinations may also be delivered using drug-in cyclodextrin liposomal formulations. For example, a combined formulation approach of cyclodextrin complexation and entrapment in liposomes may be used to deliver ocular formulations of CBD and CBD combinations. Alternatively, use of the "double-loaded technique" can be exploited to load drug-cyclodextrin into the aqueous core of liposomes and drug alone into the lipid phase of liposomes providing, for example, a fast onset and an extended duration of action (Maestrelli et al., 2010). Another advantage associated with the use of cyclodextrin in the liposomal formulation for phytocannabinoid delivery may be that cyclodextrin complexation can improve drug permeation for ocular routes (Loftsson & Duchene, 2007; Loftsson & Stefansson, 2002). Accordingly, optionally, the carrier suitable for ocular administration to an eye comprises a cyclodextrin liposome.

[0088] In certain *in vivo* studies of the present disclosure, an oil-in-water emulsion was used to deliver phytocannabinoids and cannabinoids to the eye. Such emulsions comprised soya bean oil in either a viscous (>20% oil) or less viscous (<20% oil) formulation. A block co-polymer surfactant (Pluronic^{TM} 668) was also used in some of the tested formulations.

[0089] Accordingly, in another embodiment, the carrier suitable for ocular administration to an eye comprises an oil-in-water emulsion formulation.

[0090] For example, the oily phase of the oil-in-water emulsion formulation comprises an oil, which may be a vegetable oil such as but not limited to soya bean oil. In an embodiment, the oil comprises, consists essentially of or consists of soya bean oil. Optionally, the oil comprises one or more medium chain triglyceride (MCT) oils (i.e. a triglyceride oil in which the carbohydrate chain has 8-12 carbons) or combinations of an MCT oil and a vegetable oil. MCT oils are available commercially. Examples of such MCT oils include TCR (trade name of Societe Industrielle des Oleagineaux, France for a mixture of triglycerides

wherein about 95% of the fatty acid chains have 8 or 10 carbons) and MIGLYOL™ 812 (a mixed triester of glycerine and of caprylic and capric acids).

[0091] The oil-in-water emulsion formulations of the present disclosure also comprise an emulsifier. Suitable emulsifiers include a phospholipid or a mixture of phospholipids. For example, purified egg yolk phospholipids, soybean oil phospholipids or other purified phospholipid mixtures may be useful emulsifiers.

[0092] Additionally, the oil-in-water emulsion formulations of the present disclosure include a surfactant. For example, the surfactant can be a non-ionic alkylene oxide condensate of an organic compound which contains one or more hydroxyl groups. Suitable surfactants include, but are not limited to TYLOXAPOL™, compounds sold under the trade name TWEEN™, and PLURONIC™ F-68 (a copolymer of polyoxyethylene and polyoxypropylene). The TYLOXAPOL and TWEEN surfactants are FDA approved for human use.

[0093] The aqueous component of the oil-and-water emulsion formulations of the present disclosure is the continuous phase of the emulsion and may be water, saline or any other suitable aqueous solution which can, for example, yield an isotonic and pH controlled preparation.

[0094] The oil-in-water emulsion formulations of the present disclosure, for example used in the ocular pharmaceutical compositions of cannabinoids may comprise from 0.5 to 50% oil, from 0.1 to 10% emulsifier and from 0.05 to 5% surfactant. Optionally, in order to obtain a non-viscous composition, the concentration of the non-aqueous phase should generally not exceed 25%. For more viscous formulations this concentration is increased. The agent is optionally present in an amount of 0.05 to 5% by weight of the composition.

[0095] Both corneal and transscleral drug delivery in the eye can, for example, avoid the complications associated with invasive intraocular injections and also take advantage of the relatively high permeability of sclera structures to macromolecules (Hughes et al., 2005; Lobo et al., 2012; Ranta & Urtti, 2006). Additionally, use of viscous solutions or nanoparticles and liposomes has been effectively utilized via both corneal and transscleral routes to obtain sustain drug delivery in ocular structures for up to 2 weeks (Conway, 2008; Souto et al., 2010; Natarajan et al., 2012).

[0096] The inventors show that synergistic combination therapies with other cannabis constituents, for example those that act at CB2 receptors can produce anti-inflammatory and analgesic effects.

[0097] Another embodiment of the invention relates to formulations containing HU-433, a potent CB2 analog, CBD-DMH a potent CBD derivative and/or other modified CBDs. Products designed to treat neuropathic pain and uveitis are usefully provided as with the other embodiments discussed herein. These cannabinoid agents such as HU-433 and CBD-DMH can provide useful CB2 action for treatment of ocular neuropathic pain and uveitis.

[0098] Accordingly, the disclosure provides an ocular formulation of cannabinoids (e.g. Beta-caryophyllene [also referred to herein as Beta-C or βc], Cannabidiol [CBD], cannabidiol-dimethylheptyl [CBD-DMH] or other modified CBDs, HU-308 and HU-433, individually or in combinations of two or more of the foregoing) for treatment of ocular diseases.

[0099] The disclosure also includes an ocular pharmaceutical composition comprising a CB2 target agent, a cannabimimetic agent (such as a non-psychotropic cannabimimetic agent) or a combination thereof and a carrier suitable for ocular administration to an eye of the present disclosure for use for the ocular treatment of ocular inflammation or ocular neuropathic pain in a subject. It will be appreciated that the embodiments for such ocular pharmaceutical compositions for use can be varied as discussed herein for the ocular pharmaceutical compositions of the present disclosure and the methods and uses of the present disclosure, as appropriate.

[00100] For example, in an embodiment, the disclosure provides a phytocannabinoid formulation (e.g. CBD derivatives, or a combination of CBD + ß-caryophyllene) for administration to the cornea and/or other ocular depots for treatment of eye diseases causing inflammation in a subject, such as intraocular (uveitis) or extraocular (corneal neuropathic hyperalgesia).

[00101] Combination ocular therapies of CBD or CBD derivatives with ß-caryophyllene, a CB_2 agonist, can enhance the efficacy of CBD in the treatment of inflammatory and neuropathic eye disease.

III. Methods and Uses

[00102] Without being bound by theory, cannabimimetics, optionally cannabimimetics that target CB2 such as phytocannabinoids that target CB2 (for example, CBD which is a CB2 partial agonist) and synthetic cannabinoids that target CB2 (for example, modified CBDs such as CBD-DMH which is a CB2 positive allosteric modulator) may, for example be effective in reducing markers of inflammation. For example, such compounds may reduce pro-inflammatory cytokine signaling, oxidative stress and/or inhibit activated immune cells (microglia); all of which are also features of tissue damage seen in experimental models of acute and chronic ocular inflammation, and which are exacerbated in animals lacking CB2 receptors.

[00103] The anti-inflammatory and immunomodulatory ocular effects of CBD in experimental models were achieved with doses of 5-10 mg/kg of CBD, which is comparable to that of therapeutic doses utilized in humans to alleviate neuropathic pain and spasticity associated with multiple sclerosis (Oreja-Guevara, 2012a,b). The inventors provide the first studies specifically addressing the use of CBD for ocular inflammation and pain.

[00104] There is a substantive therapeutic window for efficacy and excellent tolerability, respectively, for the phytocannabinoid, CBD, in the treatment of inflammatory eye diseases. Without being bound by theory, CBD appears to exert its actions via modulation of the endocannabinoid system as well as non-endocannabinoid system targets that can collectively modulate cellular signaling pathways involved in inflammation and pain. CBD is not psychotropic and its versatile pharmacology underscores its usefulness for combinations with other anti-inflammatory and immunomodulatory agents, including the terpenoid, β-caryophyllene, which acts at CB2. These pharmacological properties of CBD therefore can, for example provide useful combination phyto-therapeutic products (i.e. CBD and/or CBD derivatives (also referred to herein as modified CBDs) + ß-caryophyllene) for enhanced actions. The delivery platform of this formulation is optionally based on liposomal formulations, optimized for the eye.

[00105] The invention provides the first disclosure of ß-caryophyllene used in the eye in humans. ß-caryophyllene is useful, for example, for combination therapy with CBD for ocular inflammatory and neuropathic

disease. An additional advantage can, for example be that the physicochemical properties of ß-caryophyllene are similar to CBD such that both of these compounds are readily delivered together using the proposed drug, for example in cyclodextrin or liposome preparations.

[00106] The inventors demonstrate herein the anti-inflammatory and analgesic properties of novel ocular formulations such as those comprising CBD and other cannabinoids in experimental models of ocular inflammatory disease. The disclosure thus provides methods of treatment of inflammation by administering cannabinoids to the eye of a subject.

[00107] Experimental models of uveitis and corneal hyperalgesia are used to show the local delivery of CBD formulations (e.g. CBD, combination CBD + ß-caryophyllene) and cannabinoids (CBD-DMH, HU-308, HU-433) for the treatment of ocular inflammation and pain. These models are established and the inventors have considerable experience with their use for pharmacological studies of various agents, including cannabinoids, as well as preclinical studies of ocular cannabinoid drug delivery and tolerability.

[00108] Accordingly, the present disclosure includes a method of treating ocular inflammation or ocular neuropathic pain in a subject in need thereof, comprising administering ocularly to the subject in need thereof a CB2 target agent, a cannabimimetic agent or a combination thereof. Optionally, the method is a method of treating ocular inflammation. In another embodiment, the method is a method of treating ocular neuropathic pain. In a further embodiment, the method is a method of treating ocular inflammation and ocular neuropathic pain.

[00109] The present disclosure also includes an ocular use of a CB2 target agent, a cannabimimetic agent or a combination thereof for treatment of ocular inflammation or ocular neuropathic pain in a subject in need thereof. Optionally, the use is for treatment of ocular inflammation. In another embodiment of the disclosure, the use is for treatment of ocular neuropathic pain. In a further embodiment, the use is for treatment of ocular inflammation and ocular neuropathic pain.

[00110] The present disclosure further includes a use of a CB2 target agent, a cannabimimetic agent or a combination thereof for preparation of an

ocular medicament for treatment of ocular inflammation or ocular neuropathic pain in a subject in need thereof. Optionally, the use is for preparation of a medicament for treatment of ocular inflammation. In another embodiment, the use is for preparation of a medicament for treatment of ocular neuropathic pain. In a further embodiment, the use is for preparation of a medicament for treatment of ocular inflammation and ocular neuropathic pain.

[00111] It will be appreciated by a person skilled in the art that in embodiments of the methods and uses of the present disclosure, the CB2 target agent and the cannabimimetic agent (such as a non-psychotropic cannabimimetic agent) can be varied as discussed herein for the embodiments of the compositions of the present disclosure.

[00112] In an embodiment, the ocular neuropathic pain is visceral ocular neuropathic pain. In another embodiment, the ocular inflammation is caused by the subject having an eye disease.

[00113] In an embodiment, the eye disease causes intraocular inflammation. Optionally, the eye disease is uveitis, uveoretinitis or proliferative vitreoretinopathy. In another embodiment, the eye disease causes extraocular inflammation. Optionally, the eye disease is corneal inflammation or neuropathology.

[00114] In another embodiment, the eye disease causes pain and loss of vision, and the agent reduces the pain and/or reduces the loss of vision.

[00115] The dosage of the CB2 target agent and/or the cannabimimetic agent (such as the non-psychotropic cannabimimetic agent) can vary depending on many factors such as the pharmacodynamic properties of these compounds, the mode of administration, the age, health and weight of the subject, the nature and extent of the ocular inflammation or ocular neuropathic pain, the frequency of the treatment, the type of concurrent treatment, if any, and the clearance rate of the compound in the subject to be treated. One of skill in the art can determine the appropriate dosage based on the above factors. For example, the CB2 target agent and/or the cannabimimetic agent such as a phytocannabinoid (e.g. CBD, CBD + ß-caryophyllene) and synthetic cannabinoid-containing ocular formulations (e.g. HU-433, HU-308, CBD-

DMH) can be delivered via the cornea and transscleral routes (periocular) at various doses, optionally 0.1-10% w/v.

[00116] Dosing regimens include single dose treatments as well as multiple dosing. The CB2 target agent and/or the cannabimimetic agent may be administered initially in a suitable dosage that may be adjusted as required, depending on the clinical response.

[00117] Optionally, the agent is administered topically to the eye; i.e. the agent is for ocular topical use. In another embodiment, the agent is administered intravitreally to the eye; i.e. the agent is for intravitreal use. In a further embodiment of the present disclosure, the agent is administered periocularly to the eye; i.e. the agent is for periocular use.

[00118] The following non-limiting examples are illustrative of the present disclosure:

EXAMPLES

[00119] This data has been generated using several different animal models as explained in the methods sections. These can be divided into ocular inflammation models and ocular neuropathic pain models.

Example 1: Effects of the CB2 Receptor Agonist, HU-433 on Endotoxin-Induced Uveitis

I. Purpose

[00120] This study showed the anti-inflammatory role of the cannabinoid 2 receptor (CB2R) agonist, HU-433 on intraocular inflammation in an endotoxin-induced uveitis (EIU) model in rats.

II. Introduction

[00121] Tissue histology and immunohistochemistry: Ocular inflammation is accompanied by tissue edema, migration of immune cells to the sites of injury and pathology. Histology allows the tissue structure to be accessed for edema and structural dissolution, along with evidence of plasma extravasation (indicative of pathological changes in microvascular structure). Use of antibodies to proteins expressed by immune cells including neutrophils, macrophages and

microglia, allows identification of immune cell types recruited to sites of tissue damage in the anterior and posterior ocular tissues.

[00122] Intravital imaging for real-time quantitative measurement of leukocyte adhesion and migration: Tissue damage or injury results in alterations in capillary blood flow and microvascular structure, as well as adhesion and transmigration of immune cells (leukocytes) from the blood vessel to accumulate at the site of tissue injury (inflammation). This is a necessary host response to resolve injury, however escalation of the inflammatory response or persistent inflammatory responses can lead to tissue damage (Ley, Laudanna et al. 2007). Quantification of leukocytes adhering to the cells lining the lumen of blood vessels (endothelium) is carried out dynamically in the iridial microvasculature using intravital microscopy to directly visualize in real-time, or histologically in the post-mortem retina, leukocyte adhesion and diapedesis.

[00123] Assessment of pro-inflammatory markers (cytokines, adhesion molecules): The levels of adhesion molecules and pro-inflammatory mediators (cytokines) are analyzed by immunoassay of respective protein levels to provide assessment of immune status.

[00124] Approaches such as tissue histology/pathology, IVM and cytokine analysis provide a measure of the inflammatory response. Immunomodulatory and anti-inflammatory drugs reduce leukocyte adhesion and pro-inflammatory markers and tissue damage and promote inflammation resolution (Sanz and Kubes 2012).

III. Materials and Methods

[00125] The endotoxin-induced uveitis (EIU) model is a widely used animal model of human bacterially-derived uveitis, involving inflammation of the uveal tract. The uveal tract comprises the middle layer of the eye, including the iris, ciliary body and uvea.

[00126] EIU was induced in male Lewis rats by intravitreal injection of 100 ng of lipopolysaccharide (LPS, *Escherichia coli*) in saline. Treatments of the cannabinoid 2 receptor (CB2R) agonist, HU-433 were administered, in the presence and absence of the selective antagonist, AM630. Cannabinoid

treatments involved intravenous (i.v.) HU-433 (0.001-1 mg/kg), AM630 (2.5 mg/kg i.v.) and AM630 + HU-433, administered 15 minutes after intravitreal injection of LPS. Intravital microscopy (IVM) was used to observe leukocyte-endothelial adhesion each hour after induction of EIU for a duration of 6 hours.

IV. Results and Discussion

[00127] Data in Figure 1 was collected from experiments using an animal model of ocular inflammation called endotoxin-induced uveitis. This model has been shown to cause inflammation within the eye. The level of inflammation is quantified by counting the number of adherent leukocytes in the iris microcirculation. Leukocytes must adhere to the microvasculature for more than 30 s (measured as adherent leukocytes per mm^2). Imaging was conducted in a minimum of 4 quadrants within the eye, 4 vessels each quadrant, 6 hours after inflammation was induced.

[00128] Figure 1A is a representative image of the iris microcirculation after an injection of saline into the eye (control); leukocytes are the white dots within the black vasculature. Figure 1B is a representative image of the iris microcirculation after injection of lipopolysaccharide (LPS) into the eye. LPS is an inflammatory agent derived from gram-negative bacteria. LPS causes a significant increase in the number of leukocytes adhering to the vasculature compared to the saline injection.

[00129] HU-433 at doses of 0.01 and 0.1 mg/kg (Figures 2-4) significantly ($p<0.01$) reduced leukocyte-endothelial adhesion (inflammation) 6 hours after induction of EIU. This decrease in leukocyte adhesion was abolished when animals were treated with the CB2R antagonist AM630 prior to treatment with HU-308 in EIU. Use of the CB2R antagonist alone caused a significant increase in the number of adherent leukocytes to the microvasculature ($p<0.01$).

[00130] Figure 2A is a representative image of inflammation within the iris which can be compared to after treatment with HU-433 (Figure 2B).

[00131] Figure 3 is the dose response curve of HU-433 used to treat ocular inflammation in the present study. It was demonstrated (Figure 3) that HU-433 (0.1 mg/kg) was able to significantly ($p<0.05$) reduce the number of

adherent leukocytes in the iris microcirculation. This data is also depicted as the average decrease of adherent leukocytes compared to LPS alone with different doses of HU-433 (Figure 4).

[00132] CB2R activation by using the cannabinoid, HU-433 reduces leukocyte recruitment to the iris and decreases local release of inflammatory mediators during acute EIU. Drugs targeting the CB2R are useful as therapeutics for uveitis and decreasing acute ocular inflammation.

Example 2: Effects of administration of the synthetic cannabinoid, CBD-DMH on LPS Induced Uveitis

I. Materials and Methods

[00133] **Tested compound:** CBD-DMH

[00134] **Subjects:** Two different EIU experimental groups were examined in BALB/c Mice:

Group (A): Intravital microscopy (IVM) at 5 hours after intravitreal injection of saline(control)

Group (B): IVM at 5 hours after induction of EIU and i.v. administration of drug vehicle control (1 time, 0.2 mL 30% ethanol in saline right after intravitreal injection)

Group (C): IVM at 5 hours after induction of EIU and i.v. administration of cannabinoid (1 time, 0.2 mL 10 mg/kg CBD-DMH right after intravitreal injection).

[00135] **Intravitreal injection of LPS to induce uveitis:** The strain of animals chosen for these experiments was based on preliminary testing conducted and published literature (see, for example: Toguri et al., 2014). The strain of mice chosen was BALB/c and Lewis rats were used. Animals were anesthetised prior to induction of uveitis. Mice were anesthetized with 5% isoflurane in 100% oxygen. Rats were anesthetized with 65 $mg \cdot kg^{-1}$ of sodium pentobarbital. Depth of anesthesia was monitored via toe pinch test. The head of the animal was immobilized, and the sclera of the left eye was punctured with a 30-gauge needle at the dorsonasal quadrant at approximately the level of the equator. Mice received a total of 250 ng of LPS (*E. coli* 026:B6; Sigma-

Aldrich, Oakville, ON, Canada) in 2 µl of sterile 0.9% saline. Rats received a total of 100 ng of LPS in 5 µl of sterile 0.9% saline. Intravitreal injections were made under microscopic control with a Hamilton syringe (Hamilton Company, Reno, Nevada, USA), with a 30 G1/6 needle. To avoid touching the lens or causing any damage to the eye, the tip of the needle was directed towards the posterior pole and only the bevelled tip (2-3 mm) entered the vitreal cavity. The needle was held in place after injection for 5 seconds to avoid leakage of the LPS from the site of injection (sclerostomy). Sclerostomy was closed by tissue adhesive to prevent any leakage. Animals with bleeding or swelling post injection were excluded from the study.

[00136] **In vivo imaging:** The technique of intravital microscopy (IVM) was used for *in vivo* investigation of leukocyte recruitment. The intravital fluorescence video microscope was focused on the iridial microcirculation, which allowed for imaging of the leukocyte-endothelial interactions. Throughout IVM, the animal's head was made stationary The iris was divided into four equal quadrants by drawing two superficial lines, lengthwise and widthwise. IVM was carried out at each of these quadrants. In each video, leukocyte recruitment was observed and recorded for 30 seconds each. Data analysis was conducted off-line.

[00137] **IVM analysis:** Several videos of each quadrant were recorded for 30 seconds. Leukocyte adhesion was the parameter analyzed. Adherent leukocytes was defined as the number of leukocytes during the 30 s observation period that did not detach from the cylindrical endothelial surface. The number of adherent leukocytes within each vessel segment was calculated by measuring the diameter and length of vessel segment studied, assuming a cylindrical geometry of blood vessel. Adherent leukocytes were expressed as number of cells per mm^2 of endothelial surface.

[00138] **IVM Data analysis:** Results were analyzed using the software Prism 5 (GraphPad Software, La Jolla, CA, USA). All data are expressed as means ± standard error mean (SEM). Groups were tested for significance using one-way analysis of variance (ANOVA) with a Dunnett's post hoc test, comparing all experimental groups to the vehicle treated group. Significance was considered at $p < 0.05$.

II. Results and Discussion

[00139] Figure 5 shows representative images of the microvasculature and adherent leukocytes: (A) saline injection; (B) LPS injection; and (C) a decrease in number of adherent leukocytes with CBD-DMH. Inflammation was quantified by measurement of adherent leukocytes to the endothelium 6 hours after LPS injection (Figure 5D).

[00140] Figure 6 depicts a bar graph of IVM measurements examining the mean number of adherent leukocytes for the groups of Figure 5.

Example 3: Effects of administration of CBD-DMH, CBD or a combination of CBD+ßC on a PVR-dispase model of PVR

I. Background

[00141] Following retinal detachment surgery or ocular trauma, 5-10% of patients may develop proliferative vitreoretinopathy (PVR) (Yanoof & Duker, 2009). There are currently no non-surgical treatments for PVR which can be classified in 3 main stages: an inflammatory stage with activation and migration of immune cells including neutrophils, macrophages and microglia, an early proliferative stage and a late proliferative stage. In the early inflammatory stage, the ocular trauma can cause retinal tears and folds and retinal detachment. Lack of resolution of the inflammation results in astrocyte proliferation and remodelling, epiretinal membrane formation and retinal detachment with resultant fibrosis.

[00142] Experimental PVR lesions can be generated using intravitreal injections of the proteolytic enzyme, dispase (3 µl of 0.1 - 0.3 U/µl dispase). This results in a chronic inflammatory response with the development of retinal tears and folds within 1-3 weeks post-injection (technique modified from Frenzel et al., 1998). The Dispase PVR model provides a useful model for chronic posterior ocular inflammation, astrogliosis and fibrosis.

II. Materials and Methods

[00143] **Animals:** C57Blk/6 male mice (20-25 g; Charles Rivers, QC, Canada) were used for the experiments. The animals were housed on a 12 hrs light/dark cycle, with unrestricted access to food and water. All

experiments were conducted in accordance with the standards and procedures of the Canadian Council on Animal Care and the Dalhousie University animal care committee.

[00144] **Intravitreal Injections:** The PVR was induced in C57Blk/6 animals with an intraocular injection of dispase (Sigma), a neutral protease which cleaves basement membrane, into the dorso-lateral quadrant of the left eye. Dispase was diluted to the concentration of 0.2 U/μl in a sterile Ringer saline solution. Intraocular injections (2 μl) were made under a microscope with a Hamilton syringe attached to a 30 G needle. Control animals received 2 μl of sterile Ringer saline solution.

[00145] **Drug Treatment:** Animals were treated with daily intraperitoneal injections of cannabinoid ligands: CBD-DMH (10 mg/kg), CBD (10 mg/kg) and CBD (10 mg/kg) + β-Caryophyllene (20 mg/kg), for a period of seven days. One week following the induction of PVR, mice were sacrificed by an i.p. overdose of sodium pentobarbital (250 mg/kg), eyes were inoculated and prepared for histological or immunohistochemical staining.

[00146] **Clinical Scoring:** The external morphology of the eyes was evaluated by clinical scoring at 7 days following the intraocular injection. The severity of the PVR was determined on a scale of 0-5, with 0 (no disease) to 5 (completely degenerated eye) as detailed in Table 1.

Table 1: Clinical scoring for evaluation of experimental murine PVR

Clinical Stage	Description
0	No clinical signs of the disease
0.5	Dilated iris vessels
1	Swollen blood vessels in the iris; sporadic abnormal miosis
2	Pupil partially covered with fibrin, hazy anterior chamber
3	Exudate in anterior chamber, but pupil still visible
4	Exudate with haemorrhage (opaque anterior chamber), completely obscured pupil
5	No exudate in anterior chamber, abnormal pupil configuration, degenerating iris

[00147] The data was analyzed by One-Way ANOVA analysis, followed by Kruskal-Wallis test. $p<0.05$ was considered significant.

[00148] **Histology:** The internal anatomy morphology of the eye was visualized by haematoxylin and eosin (H&E) staining. The severity of the disease was scored under the light microscope and was evaluated with the scoring system of 0 (no disease) to 4 (severely damaged ocular tissue) as detailed in Table 2.

Table 2: Histopathology scoring for experimental murine PVR

Histopathology	Description
0	No disease, normal retinal architecture
0.5	Mild inflammatory cell infiltration in the retina, no tissue damage
1	Infiltration, retinal folds and focal retinal detachments, few small granulomas in choroid & retina
2	Mod. infiltration, retinal folds, detachment, focal photoreceptor damage, granulomas, perivaculitis
3	Moderate to marked infiltration, extensive photoreceptor damage. Exudate with hemorrhage (opaque anterior chamber), completely obscured pupil
4	Severe inflammation and/or full thickness retinal damage with serous exudates and subretinal neovascularisation, large granulomatous lesions

[00149] **Immunohistochemistry:** Eyes were inoculated and immersed in 4% (paraformaldehyde (PFA) in 0.1 M phosphate buffer for 24 hrs. Then the eyes were transferred into 30% sucrose in phosphate buffered saline (PBS) for cryoprotection. Symmetrical sagittal sections (14 µm) of the whole eye were cut on a freezing microtome and collected on the microscope slides. For immunohistochemical staining, slides were washed in PBS (3x15min), and then were incubated for 1 hr at room temperature with 10% normal goat serum (Vector Labs). This step was followed by overnight incubation of sections, at 4°C, with the primary antibodies: anti-rabbit Iba1 (Wako Chemicals, CA; 1: 100), anti-rabbit glial fibrillary acidic protein (GFAP; astrocyte marker) (Chemicon, Temecula, CA 1:1000). Fluorescent-tagged antibodies CY^{TM3} goat anti-rabbit IgG (1:500, Jackson ImmunoResearch Laboratories) were used for visualization of Iba1 and GFAP. The microglia counts were performed under the fluorescence microscopy.

III. Results and Discussion

[00150] Proliferative vitreoretinopathy (PVR) is a model of ocular inflammation that occurs with both external and internal changes in the eye. This inflammation is caused by intraocular injection of dispase. Several different cannabinoid treatments were tested in this model. Inflammation was quantified by clinical scoring (Figure 7A), histology (Figure 7B) and immunohistochemistry (Figure 7C). Clinical scoring, histology and immunohistochemistry are explained herein under the PVR method.

[00151] CBD-DMH significantly decreased the clinical scores and histological scores received in the model of PVR indicating its ability to reduce ocular inflammation. Immunohistochemistry was used to study the activation of immune cells (microglia) in the retina.

[00152] CBD-DMH, CBD alone and CBD + βc were able to decrease the number of activated immune cells (Figure 7C). While not wishing to be limited by theory, this could provide evidence of a potential mechanism to how CBD-DMH, CBD, and CBD + βc decrease inflammation.

[00153] An increase in IBa1+ microglia is associated with neuroinflammation. Iba1 is specific to MG (Daisuke et al., 2001). Using the selective immunohistochemical label, IBa1, for activated retinal immune cells, microglia, it can be seen that control animals treated with no retinal pathology treated with drug vehicle, there is very sparse labelling for IBa1 positive (IBa1+) cells (Figure 8, top left). In contrast, in animals with experimental PVR, retinas treated with vehicle have extensive IBa1+ staining for activated microglia (Figure 8, top right). Iba1+ labeling is substantially reduced in animals with experimental PVR and treated with CBD-DMH (Figure 8, bottom left) and also (but to a lesser extent) with CBD + beta-C (Figure 8, bottom right). These results indicate that the synthetic cannabidiol derivative CBD-DMH and CBD + beta-C are able to reduce activated immune cells that contribute to the inflammatory response and pathology in PVR.

Example 4: Example 3: Effects of administration of CBD-DMH on corneal hyperalgesia

I. Background

[00154] The chemical cauterization model of corneal inflammation and hyperalgesia is an established model to look at corneal sensitization and pain. Chemical cauterization of the murine cornea using topical silver nitrate produces non-specific inflammation followed by chronic behavioral sensitization to subsequent chemical stimuli (modified from Wenk & Honda, 2003).

[00155] The corneal reflex blink test provides a behavioral assessment of corneal sensitization and hyperalgesia (decreased pain threshold). The hyperalgesia (defined as increased responsiveness to painful stimuli) is gauged by quantifying the number and frequency of a protective blinking response in the treated eye (stimulus-induced blinking) relative to control non-sensitized eyes (Wenk and Honda 2003). Anti-inflammatory agents and agents that act at targets on nociceptive nerves can reduce development of corneal sensitization and hyperalgesic activity (reduced protective blinking response in response to noxious irritant).

II. Results and Discussion

[00156] Using a model characterized by Wenk & Honda, 2003, chemical cauterization using silver nitrate application to the cornea was used to create a corneal hypersensitivity model. Hypersensitivity was determined by assessing blinks to an ocular topical application of 1 µM capsaicin. The blink response is one measure of the level of corneal hyperalgesia. Increased blinking in response to capsaicin in a cauterized eye indicates a higher level of pain (Figure 9). There was a significant increase in blinks to 1 µM capsaicin in the chemical cauterized eye when compared to the sham control eye (Figure 10). Ocular topical application of the NSAID Nevanac™ (Nepafenac ophthalmic suspension) eliminated this hypersensitivity (Figure 11).

[00157] Evaluation of CBD-DMH showed that it further eliminates this hypersensitivity, showing a statistically significant decrease in blinks to 1 µM capsaicin when in the chemical cauterized eye when compared to the sham control eye (Figure 12). Beta-C has also been tested in this model and appeared to also produce a reduction in hyperalgesia.

Summary of Examples 1-4

[00158] Table 3 provides a summary of models, treatments and doses used in the above-described studies of the disclosure.

Table 3

Figure	Model	Treatment	Dose
Figure 1	Endotoxin-induced Uveitis	LPS + HU-433	1, 0.1, 0.01, 0.001 mg/kg
Figure 2	Endotoxin-induced Uveitis	LPS + HU-433	0.1 mg/kg
Figure 3	Endotoxin-induced Uveitis	LPS + HU-433	1, 0.1, 0.01, 0.001 mg/kg
Figure 4	Endotoxin-induced Uveitis	LPS + HU-433	1, 0.1, 0.01, 0.001 mg/kg
Figure 5	Experimental Uveitis	LPS+CBD-DMH	
Figure 6	Experimental Uveitis	LPS+CBD-DMH	
Figure 7	PVR	CBD-DMH CBD CBD+ßC	10 mg/kg 10 mg/kg 10 mg/kg + 20 mg/kg
Figure 8	PVR	CBD-DMH CBD+ßC	10 mg/kg 10 mg/kg + 20 mg/kg
Figure 9	Corneal Hyperalgesia	–	–
Figure 10	Chemical cauterization causes corneal hypersensitivity to capsaicin.		
Figure 11	Corneal Hyperalgesia	CBD-DMH	5% solution
Figure 12	Corneal Hyperalgesia	CBD-DMH	5% solution

Example 5: Other animal models of intraocular inflammation

[00159] Receptor knock-out models: Genetic receptor null models (murine) are available for the following receptor targets: CB_2; Receptor knock-outs (-/-) are used as controls for further validation of drug targets in models of ocular inflammation and neuropathic pain.

Example 6: *in vitro* analysis of CBD and CBD-DMH

I. Materials and Methods

[00160] Methods are modified from LaPrairie et al., 2014 a, b.

Cell Culture

[00161] HEK cells were maintained at 37°C, 5% CO_2 in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 10^4 $U \cdot mL^{-1}$ Pen/Strep.

Drugs

[00162] Drug stocks were made up in DMSO [CBD, CBD-DMH and CP 55,940] and diluted to final solvent concentrations of 0.1%. CBD and CP 55,940 were purchased from Tocris Bioscience (Bristol, UK).

[00163] CP 55,940 is a full (orthosteric) agonist of CB1 and CB2, which is commonly used in studies of the activity of compounds at these receptors. This agonist binds to CB1 and CB2 to maximally activate the receptor and G protein coupled signaling pathways with resultant alterations in downstream signaling molecules and functional changes.

On- and In-cell™ western

[00164] For In-cell™ western analyses, cells were fixed for 10 min at room temperature with 4% paraformaldehyde and washed three times with 0.1 M PBS for 5 min each. Cells were incubated with blocking solution (0.1 M PBS, 5% normal goat serum, 0.3% TritonX-100, in dH_2O) for 1 h at room temperature. Cells were treated with primary antibody diluted in antibody dilution buffer [0.1 M PBS, 1% (w/v) BSA, 0.3% TritonX-100, in dH_2O] overnight at 4°C. Primary antibody solutions were: pERK1/2(Tyr205/185) (1:200), ERK1/2 (1:200), pPLCβ3(S537) (1:500), PLCβ3 (1:1000), or β-actin (1:2000; Santa Cruz Biotechnology). Cells were washed three times with 0.1 M PBS for 5 min each. Cells were then incubated in $IR^{CW800dye}$ (1:500; Rockland Immunochemicals, Gilbertsville, PA, USA) for 1 h at room temperature. Cells were washed three times with 0.1 M PBS for 5 min each. Cells were allowed to air-dry overnight.

[00165] In-cell™ data were collected using the Odyssey Imaging system and software (version 3.0; Li-Cor, Lincoln, NE, USA).

Statistical analyses

[00166] Goodness of fit to non-linear regression models was tested in GraphPad (v. 5.0, Prism). Concentration-response curves (CRC) are shown in

each figure according to the model with the best fit. Pharmacological statistics were obtained from non-linear regression models. Statistical analyses were two-way analysis of variance (ANOVA), as indicated, using GraphPad. Homogeneity of variance was confirmed using Bartlett's test. The level of significance was set to $P < 0.001$ or < 0.01, as indicated. Results are reported as the mean ± the standard error of the mean (SEM) or mean and 95% confidence interval, as indicated, from at least 4 independent experiments.

II. Results and Discussion

[00167] The results of this study are shown in Figure 13A-C and Tables 4-6. The results indicate that CBD-DMH is a positive allosteric modulator (Christopoulos and Kenakin, 2002) of CB_2-dependent G protein signalling and enhances the potency and efficacy of the orthosteric CB2 agonist, CP55940, to activate CB2 coupled G protein signalling pathways (summarized in Tables 4-6). CBD-DMH does not activate CB2 in the absence of the orthosteric agonist, CP55940. In these assays, CBD is a partial agonist of CB_2-dependent G protein signalling (summarized in Tables 4-6).

[00168] The following tables show the mean EC_{50} and E_{Max}/E_{Min} values for the effects of CBD-DMH and CBD on CP55,940-dependent $G\alpha_{i/o}$ ERK phosphorylation, cAMP and $G\alpha_q$ PLCβ3 phosphorylation.

Table 4: ERK ($G\alpha_{i/o}$)

		EC_{50} (nM) ± SEM	E_{max} (%) ± SEM*
CB_2	CP55,940 + 1 μM CBD-DMH	135.70 ± 22.58	117.17 ± 12.01
	CP55,940 + 1 μM CBD	865.40 ± 6.62	97.36 ± 7.09
	CBD-DMH	–	–
	CBD	1286.00 ± 22.98	–
	CBD-DMH + 500 nM CP55,940	39.90 ± 64.98	113.11 ± 22.96
	CBD + 500 nM CP55,940	348.70 ± 78.69	46.83 ± 12.33

*Calculated as a percentage of the maximal response to the agonist CP 55,940

Table 5: PLCβ3 ($G\alpha_q$)

		EC_{50} (nM) ± SEM	E_{max} (%) ± SEM*
CB_2	CP55,940 + 1 μM CBD-DMH	185.30 ± 18.43	114.37 ± 17.06
	CP55,940 + 1 μM CBD	609.50 ± 5.93	95.98 ± 12.36
	CBD-DMH	–	–
	CBD	977.90 ± 7.80	51.68 ± 7.04

	CBD-DMH + 500 nM CP55,940	196.70 ± 9.24	102.01 ± 6.32
	CBD + 500 nM CP55,940	699.30 ± 11.80	43.59 ± 3.98

*Calculated as a percentage of the maximal response to the agonist CP 55,940

Table 6: cAMP

		EC_{50} (nM) ± SEM	E_{min} (%) ± SEM†
CB_2	CP55,940 + 1 μM CBD-DMH	48.27 ± 37.49	153.24 ± 23.13
	CP55,940 + 1 μM CBD	31.39 ± 31.37	103.01 ± 12.64
	CBD-DMH	–	–
	CBD	237.30 ± 47.55	928.15 ± 24.61
	CBD-DMH + 500 nM CP55,940	241.85 ± 48.33	475.19 ± 11.91
	CBD + 500 nM CP55,940	353.96 ± 49.37	423.98 ± 88.16

†Calculated as a percentage of the maximal inhibition of cAMP in response to the agonist CP 55,940

[00169] While the present disclosure has been described with reference to what are presently considered to be the examples, it is to be understood that the disclosure is not limited to the disclosed examples. Changes in form and substitution of equivalents are contemplated as circumstances might suggest or render expedient. These changes are to be understood within the spirit and scope of the appended claims. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

Date Reçue/Date Received 2021-04-05

REFERENCES

Agarwal R., Iezhitsa I., Agarwal P., Abdul Nasir N.A., Razali N., Alyautdin R., Ismail N.M., Liposomes in topical ophthalmic drug delivery: an update. Drug Deliv. 2014 Aug 12:1-17.

Belmonte, C., M. C. Acosta and J. Gallar (2004). "Neural basis of sensation in intact and injured corneas." Exp Eye Res **78**(3): 513-525.

Berenbaum, M. C. (1989). "What is synergy?" Pharmacol Rev **41**(2): 93-141.

Conway, B. R. (2008). "Recent patents on ocular drug delivery systems." Recent Pat Drug Deliv Formul **2**(1): 1-8.

Christopoulos, A. and T. Kenakin (2002). "G protein-coupled receptor allosterism and complexing." Pharmacol Rev **54**(2): 323-374.

Daisuke Ito, Kortaro Tanaka, Shigeaki Suzuki, Tomohisa Dembo, and Yasuo Fukuuchi, "Enhanced Expression of Iba1, Ionized Calcium-Binding Adapter Molecule 1, After Transient Focal Cerebral Ischemia In Rat Brain" Stroke. 2001;32:1208-1215.

Draize, J. H., G. Woodard and H. O. Calvery (1944). "Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes." J Pharmacol and Exp Therapeutics **82**: 377-390.

Frenzel, E. M., K. A. Neely, A. W. Walsh, J. D. Cameron and D. S. Gregerson (1998). "A new model of proliferative vitreoretinopathy." Invest Ophthalmol Vis Sci **39**(11): 2157-2164.

Fride E, Feigin C, Ponde DE, Breuer A, Hanus L, Arshavsky N, Mechoulam R. (2004). "(+)-Cannabidiol analogues which bind cannabinoid receptors but exert peripheral activity only." Eur J Pharmacol **506(2)**: 179-188.

Friedman, N. J. (2010). "Impact of dry eye disease and treatment on quality of life." Curr Opin Ophthalmol **21**(4): 310-316.

Hanus, L., A. Breuer, S. Tchilibon, S. Shiloah, D. Goldenberg, M. Horowitz, R. G. Pertwee, R. A. Ross, M. R and E. Fride (1999). "HU-308: A specific agonist for CB2, a peripheral cannabinoid receptor." Proc Nat Acad Sci **96**: 14228-14233.

Hohmann, A. G. and R. L. Suplita, 2nd (2006). "Endocannabinoid mechanisms of pain modulation." AAPS J **8**(4): E693-708.

Hsieh, G. C., M. Pai, P. Chandran, B. A. Hooker, C. Z. Zhu, A. K. Salyers, E. J. Wensink, C. Zhan, W. A. Carroll, M. J. Dart, B. B. Yao, P. Honore and M. D. Meyer (2011). "Central and peripheral sites of action for CB(2) receptor mediated analgesic activity in chronic inflammatory and neuropathic pain models in rats." Br J Pharmacol **162**(2): 428-440.

Hughes, P. M., O. Olejnik, J. E. Chang-Lin and C. G. Wilson (2005). "Topical and systemic drug delivery to the posterior segments." Adv Drug Deliv Rev **57**(14): 2010-2032.

Jabs, D. A., R. B. Nussenblatt and J. T. Rosenbaum (2005). "Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop." Am J Ophthalmol **140**(3): 509-516.

Laprairie RB, Bagher AM, Kelly MEM, Denovan-Wright EM (2014a). Cannabidiol is a negative allosteric modulator of the type 1 cannabinoid receptor (Brit. J. Pharmacol. Submitted).

Laprairie RB, Bagher AM, Kelly MEM, Dupré DJ, Denovan-Wright EM (2014b). Type 1 Cannabinoid Receptor Ligands Display Functional Selectivity in a Cell Culture Model of Striatal Medium Spiny Projection Neurons. J Biol Chem E-pub ahead of print.

Lee, R. W. and A. D. Dick (2012). "Current concepts and future directions in the pathogenesis and treatment of non-infectious intraocular inflammation." Eye (Lond) **26**(1): 17-28.

Ley, K., C. Laudanna, M. I. Cybulsky and S. Nourshargh (2007). "Getting to the site of inflammation: the leukocyte adhesion cascade updated." Nat Rev Immunol **7**(9): 678-689.

Lobo, C. (2012). "Pseudophakic cystoid macular edema." Ophthalmologica **227**(2): 61-67.

Loftsson, T. and D. Duchene (2007). "Cyclodextrins and their pharmaceutical applications." Int J Pharm **329**(1-2): 1-11.

Loftsson, T. and E. Stefánsson (2002). "Cyclodextrins in eye drop formulations: enhanced topical delivery of corticosteroids to the eye." Acta Ophthalmol Scand **80**(2): 144-150.

Maestrelli, F., M. L. Gonzalez-Rodriguez, A. M. Rabasco, C. Ghelardini and P. Mura (2010). "New "drug-in cyclodextrin-in deformable liposomes" formulations to improve the therapeutic efficacy of local anaesthetics." Int J Pharm **395**(1-2): 222-231.

Maestrelli, F., M. L. Gonzalez-Rodriguez, A. M. Rabasco and P. Mura (2005). "Preparation and characterisation of liposomes encapsulating ketoprofen-cyclodextrin complexes for transdermal drug delivery." Int J Pharm **298**(1): 55-67.

McPartland, J. M. and E. B. Russo (2001). "Cannabis and cannabis extracts, greater than the sum of their parts?" J Cannabis Ther **1(3-4)**: 103-132.

Mechoulam, R. and Hanus, L. (2002). "Cannabidiol: an overview of some chemical and pharmacological aspects. Part I: chemical aspects." Chem Phys Lipids **121(1-2)**: 35-43.

Natarajan J.V., Ang M., Darwitan A., Chattopadhyay S., Wong T.T., Venkatraman S.S., Nanomedicine for glaucoma: liposomes provide sustained release of latanoprost in the eye. Int J Nanomedicine. 2012;7:123-31.

Oreja-Guevara C., Treatment of spasticity in multiple sclerosis: new perspectives regarding the use of cannabinoids. Rev Neurol. 2012a Oct 1;55(7):421-30.

Oreja-Guevara C., Clinical efficacy and effectiveness of Sativex, a combined cannabinoid medicine, in multiple sclerosis-related spasticity. Expert Rev Neurother. 2012b Apr;12(4 Suppl):3-8.

Pflugfelder, S. C. (2008). "Prevalence, burden, and pharmacoeconomics of dry eye disease." Am J Manag Care **14**(3 Suppl): S102-106.

Rahn, E. J. and A. G. Hohmann (2009). "Cannabinoids as pharmacotherapies for neuropathic pain: from the bench to the bedside." Neurotherapeutics **6**(4): 713-737.

Ranta, V. P. and A. Urtti (2006). "Transscleral drug delivery to the posterior eye: prospects of pharmacokinetic modeling." Adv Drug Deliv Rev **58**(11): 1164-1181.

Rosenthal, P., I. Baran and D. S. Jacobs (2009). "Corneal pain without stain: is it real?" Ocul Surf **7**(1): 28-40.

Rosenthal, P. and D. Borsook (2012). "The corneal pain system. Part I: the missing piece of the dry eye puzzle." Ocul Surf **10**(1): 2-14.

Russo, E. B. (2011). "Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects." Br J Pharm **163**: 1344-1364.

Sanz, M. J. and P. Kubes (2012). "Neutrophil-active chemokines in in vivo imaging of neutrophil trafficking." Eur J Immunol **42**(2): 278-283.

Souto, E. B., S. Doktorovova, E. Gonzalez-Mira, M. A. Egea and M. L. Garcia (2010). "Feasibility of lipid nanoparticles for ocular delivery of anti-inflammatory drugs." Curr Eye Res **35**(7): 537-552.

Szczesniak, A. M., M. E. Kelly, S. Whynot, P. N. Shek and O. Hung (2006). "Ocular hypotensive effects of an intratracheally delivered liposomal delta9-tetrahydrocannabinol preparation in rats." J Ocul Pharmacol Ther **22**(3): 160-167.

Thumma, S., S. Majumdar, M. A. Elsohly, W. Gul and M. A. Repka (2008). "Preformulation studies of a prodrug of Delta9-tetrahydrocannabinol." AAPS PharmSciTech **9**(3): 982-990.

Toguri, J. T., C. Lehmann, R. B. Laprairie, A. M. Szczesniak, J. Zhou, E. M. Denovan-Wright and M. E. Kelly (2014). "Anti-inflammatory effects of cannabinoid CB(2) receptor activation in endotoxin-induced uveitis." Br J Pharmacol **171**(6): 1448-1461.

Wagner, H. and G. Ulrich-Merzenich (2009). "Synergy research: approaching a new generation of phytopharmaceuticals." Phytomedicine **16**(2-3): 97-110.

Ward, S. J., M. D. Ramirez, H. Neelakantan and E. A. Walker (2011). "Cannabidiol prevents the development of cold and mechanical allodynia in paclitaxel-treated female C57Bl6 mice." Anesth Analg **113**(4): 947-950.

Wenk, H. N. and C. N. Honda (2003). "Silver nitrate cauterization: characterization of a new model of corneal inflammation and hyperalgesia in rat." Pain **105**(3): 393-401.

WO 2010041253 A1: Bab, I., R. Mechoulam, A. Breuer and N. Mussai. "Compositions comprising cb receptor agonists, uses thereof and methods for their preparation. " Published: Apr 15, 2010.

Yanoof M and Duker JS, (2009). Opthalmology. Mosby Elsevier.

Yawn, B. P., P. C. Wollan, J. L. St Sauver and L. C. Butterfield (2013). "Herpes zoster eye complications: rates and trends." Mayo Clin Proc **88**(6): 562-570.

CLAIMS:

1. Use of a CB2 target agent in the treatment of ocular inflammation or ocular neuropathic pain in a subject in need thereof, wherein the CB2 target agent comprises a positive allosteric modulator that is CBD-DMH, and the CB2 target agent is formulated for ocular use.

2. The use of claim 1, wherein the CBD-DMH is used in combination with a CB2 agonist agent, a CB2 partial agonist agent, or a combination thereof.

3. The use of claim 2, wherein the CB2 agonist agent is HU-433, HU-308 or β-caryophyllene; the CB2 partial agonist agent is CBD; and the CB2 positive allosteric modulator is CBD-DMH.

4. The use of claim 1, wherein the CB2 target agent is used in combination with a cannabimimetic agent.

5. The use of claim 4, wherein the cannabimimetic agent is a cannabinoid.

6. The use of claim 5, wherein the cannabinoid is a non-psychotropic cannabinoid, optionally wherein the non-psychotropic cannabinoid is a phytocannabinoid, a synthetic cannabinoid or a combination thereof.

7. The use of claim 6, wherein the phytocannabinoid is β-caryophyllene, cannabidiol or a combination thereof; and the synthetic cannabinoid is HU-433, HU-308, a modified CBD or a combination thereof.

8. The use of any one of claims 1 to 7, wherein the use is for treatment of ocular inflammation.

9. The use of any one of claims 1 to 8, wherein the ocular inflammation is caused by an eye disease.

10. The use of claim 9, wherein the eye disease causes intraocular inflammation, optionally wherein the eye disease is uveitis, uveoretinitis or proliferative vitreoretinopathy.

11. The use of claim 9, wherein the eye disease causes extraocular inflammation, optionally wherein the eye disease is corneal inflammation or neuropathology.

12. The use of any one of claims 1 to 7, wherein the subject has an eye disease that causes pain and loss of vision, and the agent reduces the pain and/or reduces the loss of vision.

13. The use of any one of claims 1 to 7, wherein the use is for treatment of ocular neuropathic pain.

14. The use of any one of claims 1 to 7 and 13, wherein the ocular neuropathic pain is visceral ocular neuropathic pain.

15. The use of any one of claims 1 to 14, wherein the subject is a mammal.

16. The use of claim 15, wherein the mammal is a human.

17. An ocular pharmaceutical composition comprising a CB2 target agent and a carrier suitable for ocular administration to an eye, wherein the CB2 target agent comprises a positive allosteric modulator that is CBD-DMH.

18. The composition of claim 17, wherein the CB2 target agent further comprises a CB2 agonist agent, a CB2 partial agonist agent, or a combination thereof.

19. The composition of claim 18, wherein the CB2 agonist agent is HU-433, HU-308 or β-caryophyllene; the CB2 partial agonist agent is CBD; and the CB2 positive allosteric modulator is CBD-DMH.

20. The composition of claim 17, wherein the composition further comprises a cannabimimetic agent.

21. The composition of claim 20, wherein the cannabimimetic agent is a cannabinoid.

22. The composition of claim 21, wherein the cannabinoid is a non-psychotropic cannabinoid, optionally wherein the non-psychotropic cannabinoid is a phytocannabinoid, a synthetic cannabinoid or a combination thereof.

23. The composition of claim 22, wherein the phytocannabinoid is β-caryophyllene, cannabidiol or a combination thereof; and the synthetic cannabinoid is HU-433, HU-308, a modified CBD or a combination thereof.

24. The composition of any one of claims 17 to 23, wherein the carrier comprises a liposome.

25. The composition of any one of claims 17 to 23, wherein the carrier comprises an oil-in-water emulsion formulation.

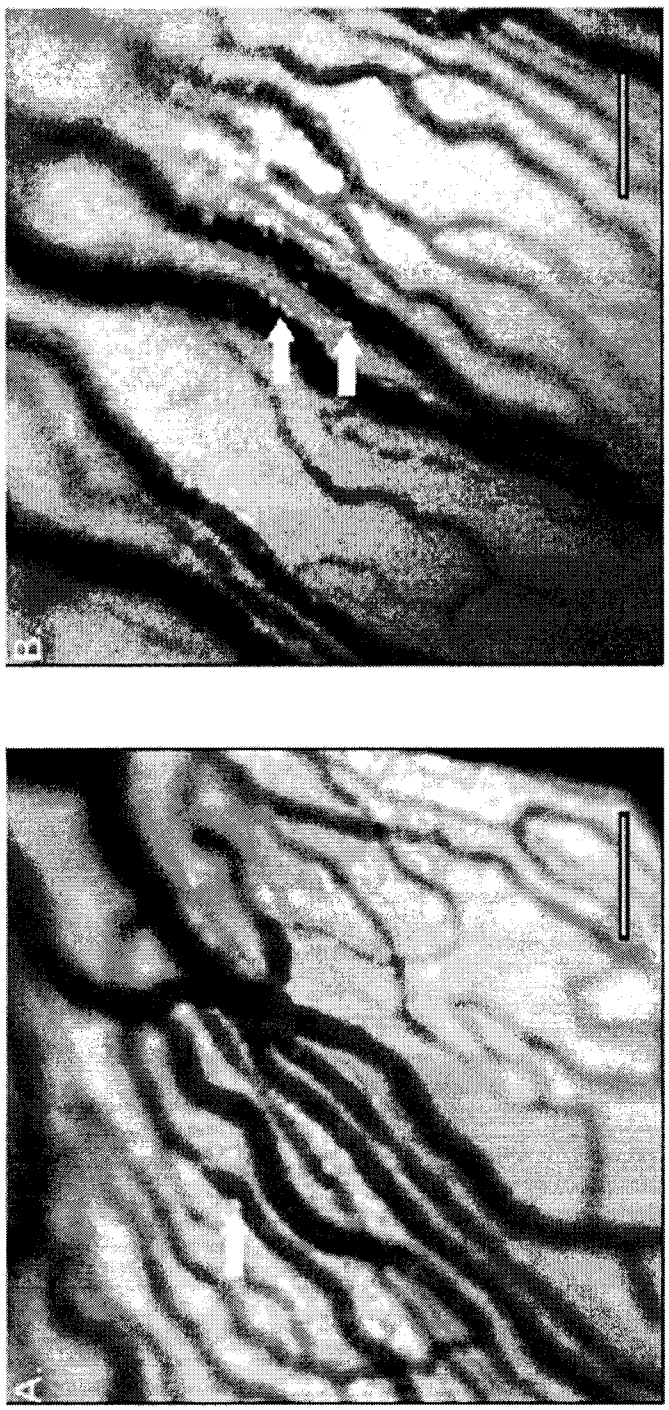


FIG. 1

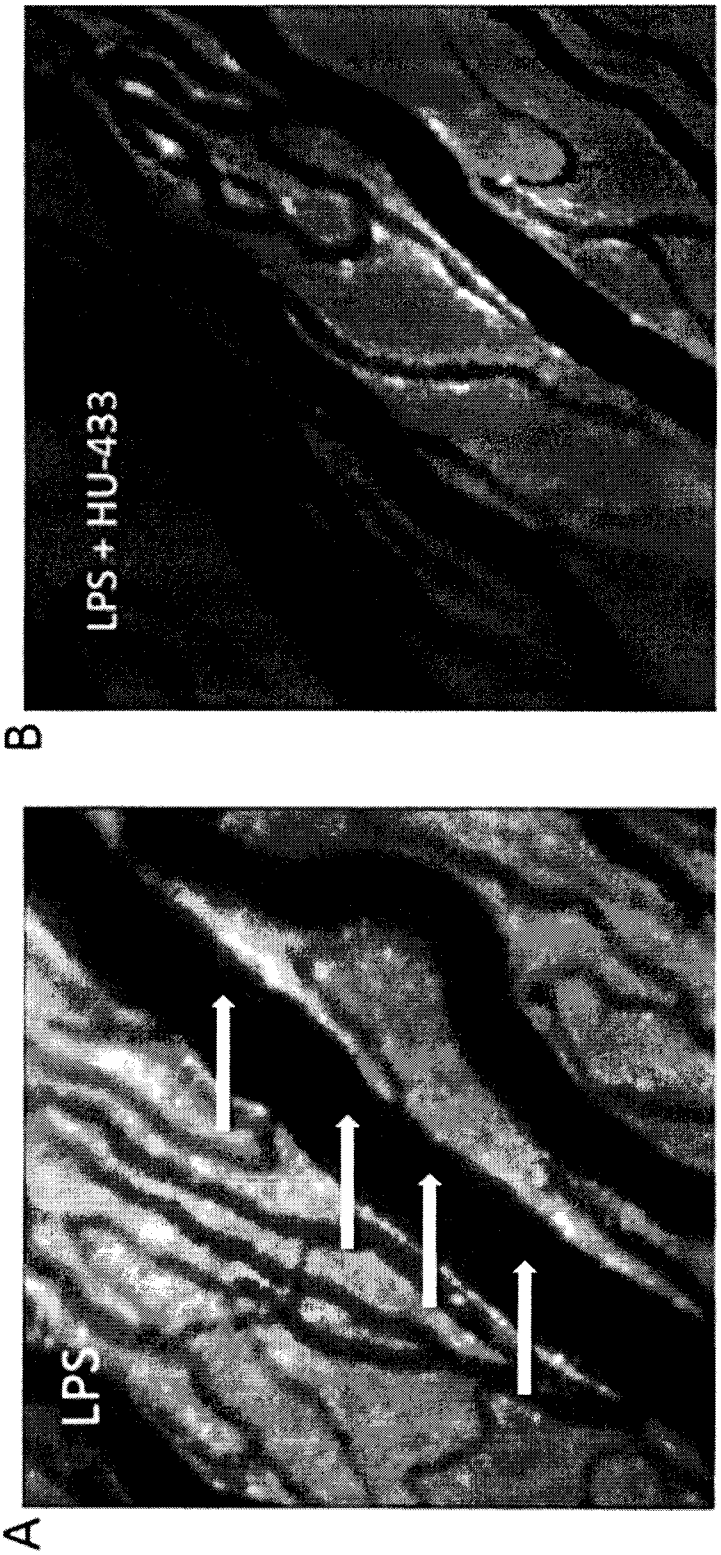


FIG. 2


Control
LPS
LPS + HU-433 [0.001mg/kg]
LPS + HU-433 [0.01mg/kg]
LPS + HU-433 [0.1mg/kg]
LPS + HU-433 [1mg/kg]
N of adherent leukocytes/
mm² endothelium
400
300
200
100
0

FIG. 3

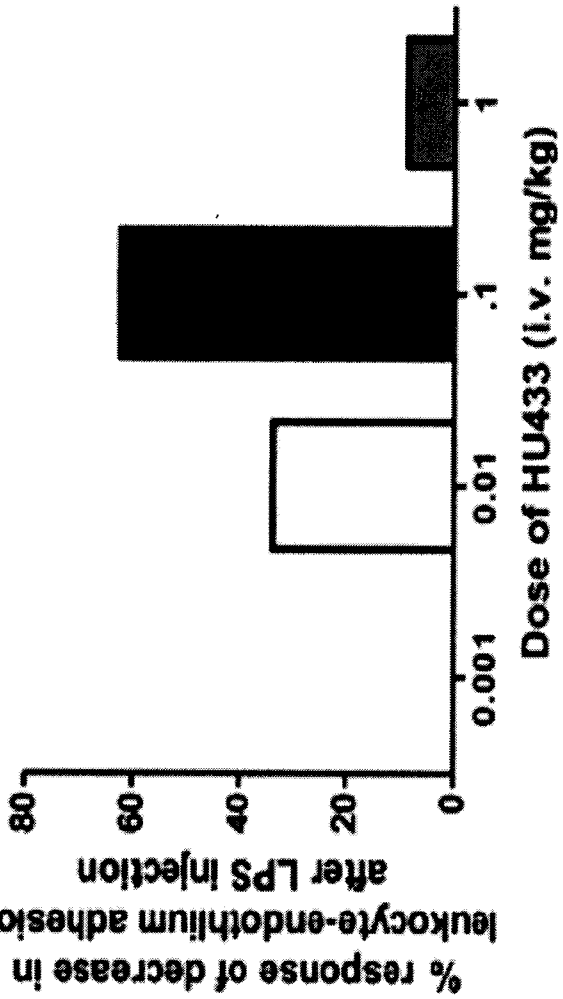

% response of decrease in
leukocyte-endothilium adhesion
after LPS injection
0
20
40
60
80
0.001
0.01
.1
1
Dose of HU433 (i.v. mg/kg)

FIG. 4

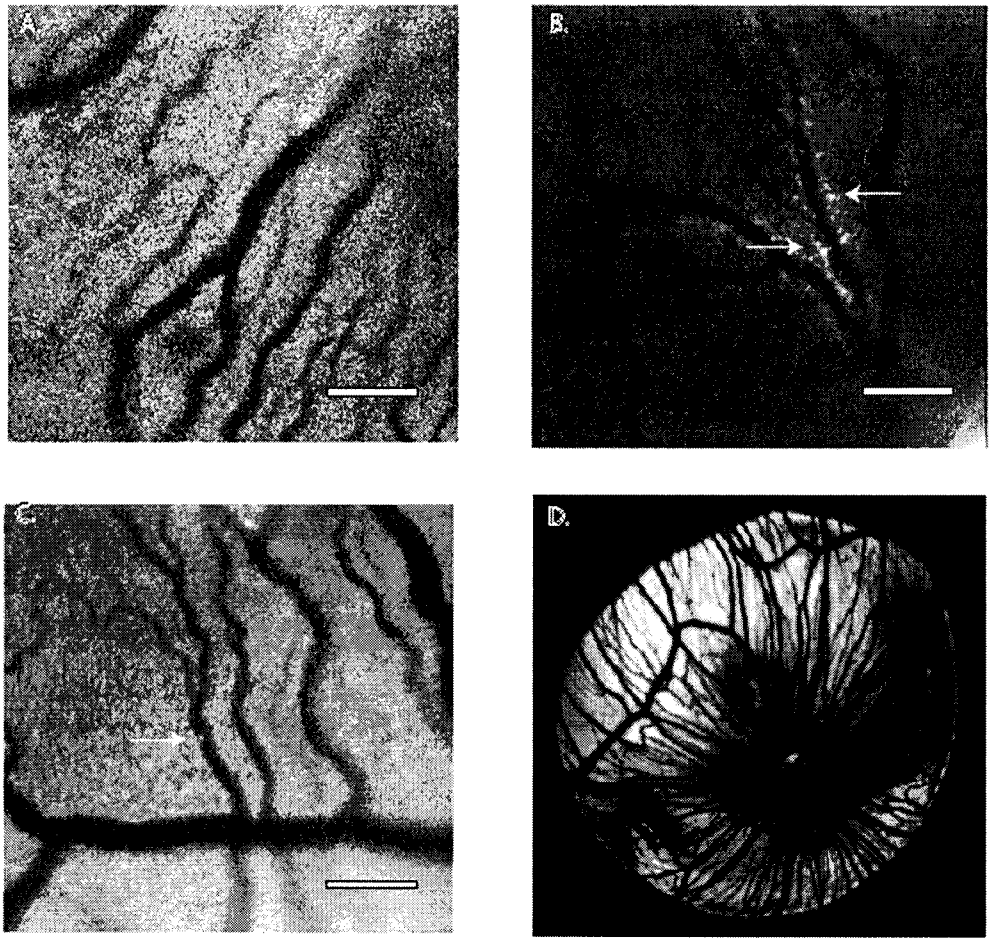


FIG. 5

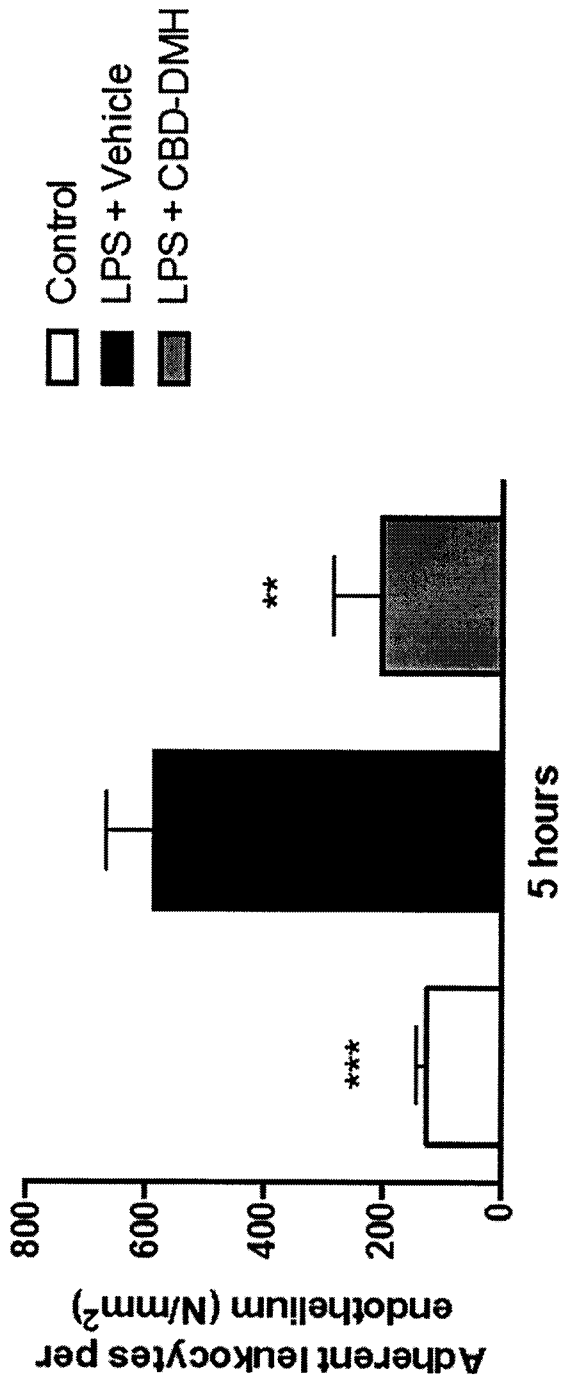
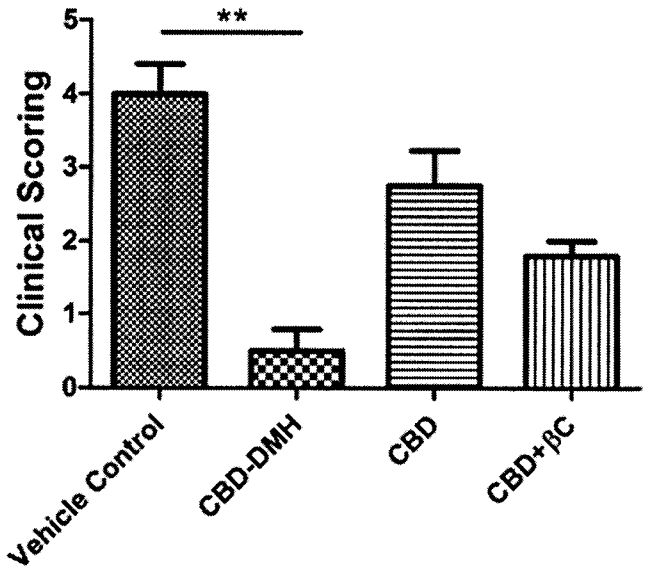

Control
LPS + Vehicle
LPS + CBD-DMH
Adherent leukocytes per endothelium (N/mm²)
800
600
400
200
0
***
**
5 hours

FIG. 6

A


**
Clinical Scoring
5
4
3
2
1
0
Vehicle Control
CBD-DMH
CBD
CBD+βC

B

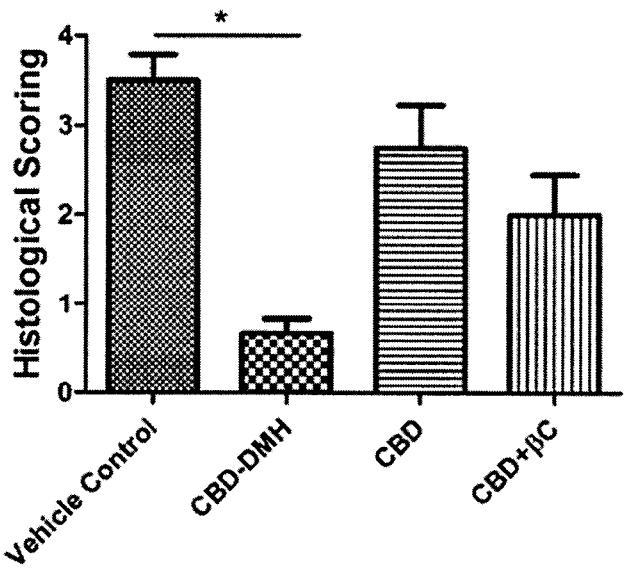

*
Histological Scoring
4
3
2
1
0
Vehicle Control
CBD-DMH
CBD
CBD+βC

FIG. 7

C

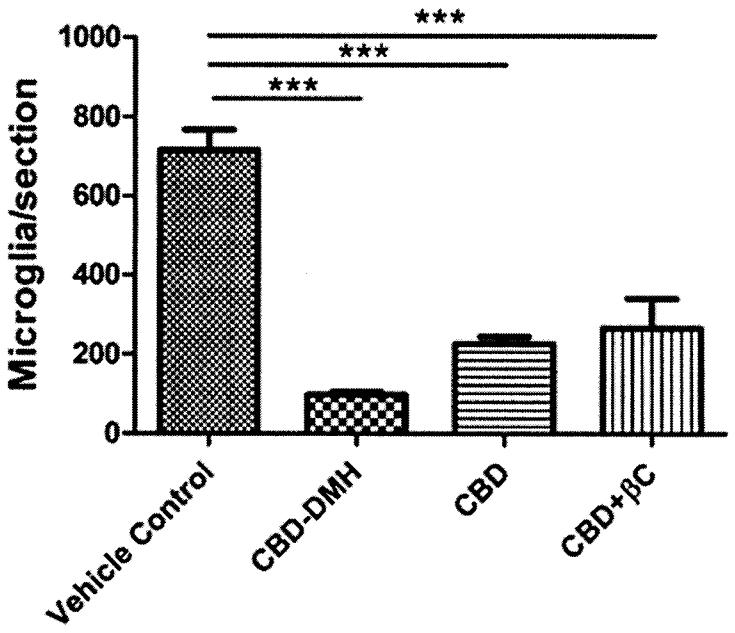

***
***
***
1000
800
600
400
200
0
Microglia/section
Vehicle Control
CBD-DMH
CBD
CBD+βC

FIG. 7 (cont.)

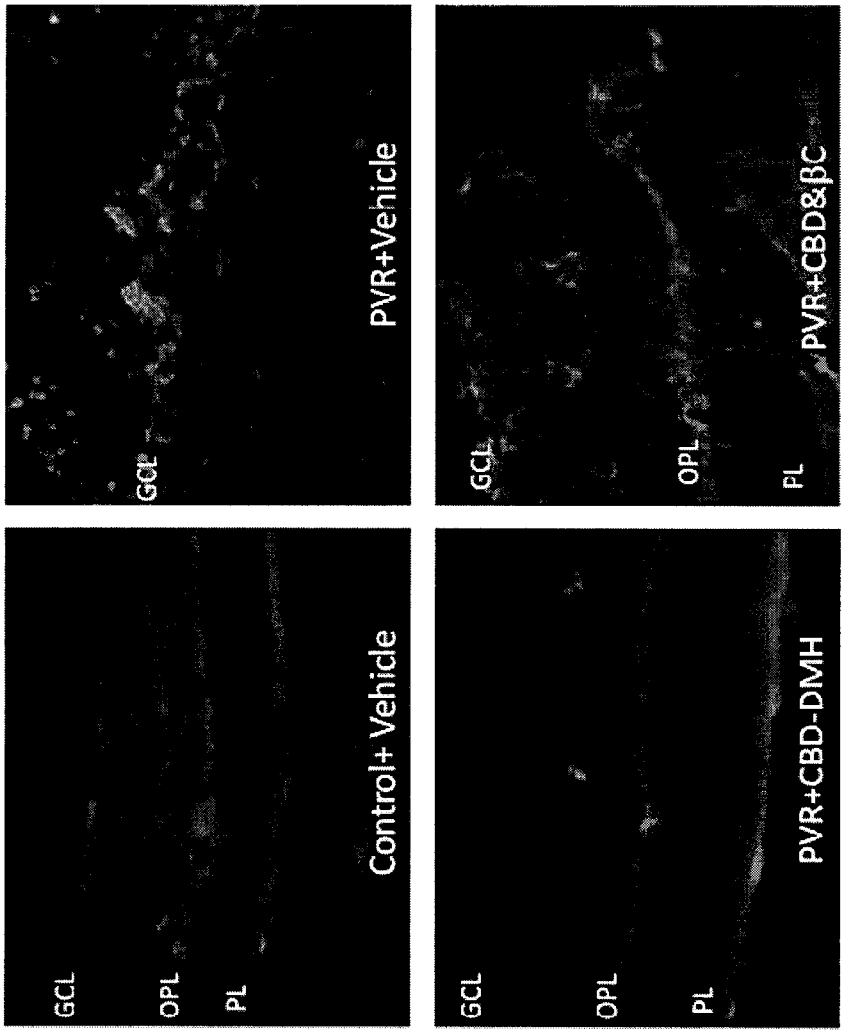

GCL
OPL
PL
Control+ Vehicle
GCL
PVR+Vehicle
GCL
OPL
PL
PVR+CBD-DMH
GCL
OPL
PL
PVR+CBD&βC

FIG. 8

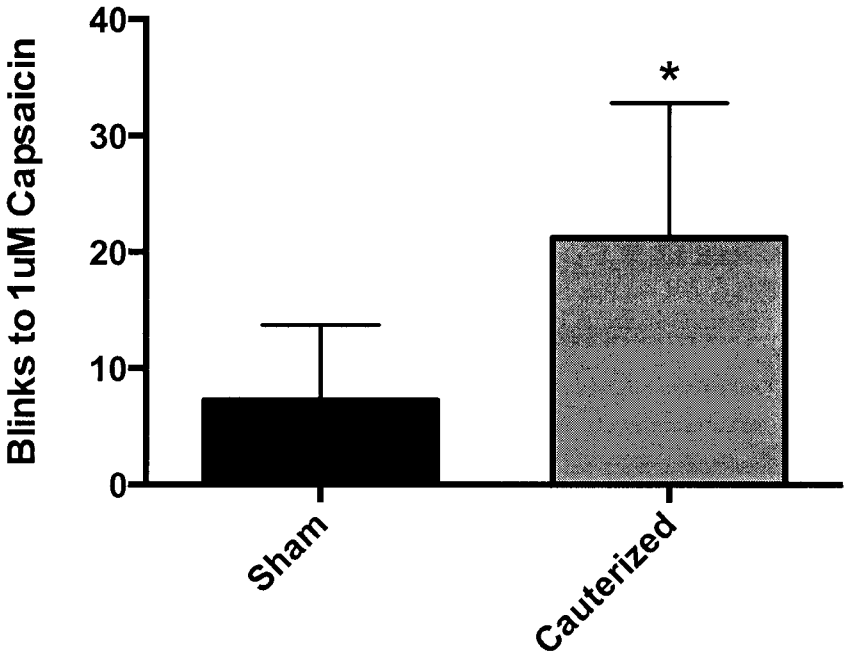


FIG. 9

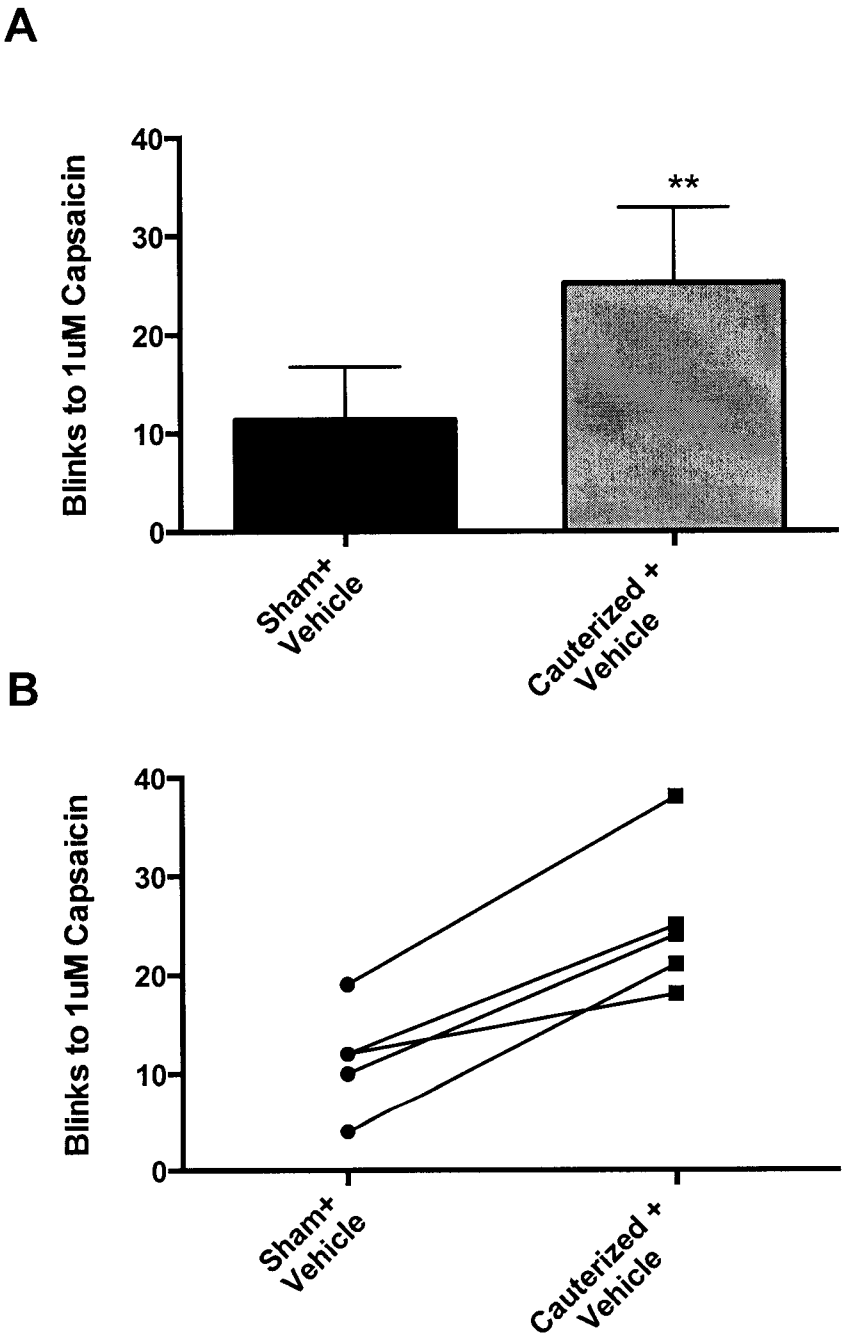

A
Blinks to 1uM Capsaicin
40
30
20
10
0
**
Sham+ Vehicle
Cauterized + Vehicle
B
Blinks to 1uM Capsaicin
40
30
20
10
0
Sham+ Vehicle
Cauterized + Vehicle

FIG. 10

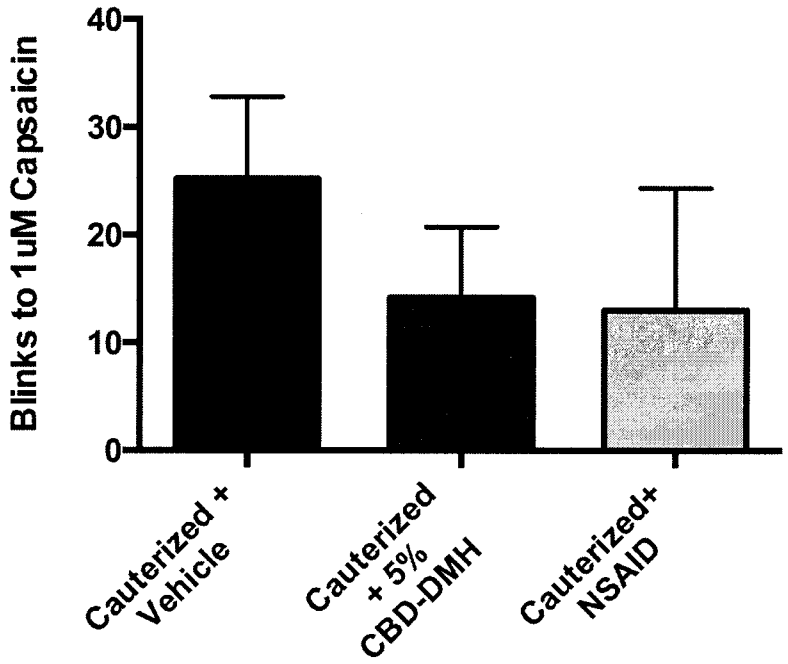

Blinks to 1uM Capsaicin
40
30
20
10
0
Cauterized + Vehicle
Cauterized + 5% CBD-DMH
Cauterized+ NSAID

FIG. 11

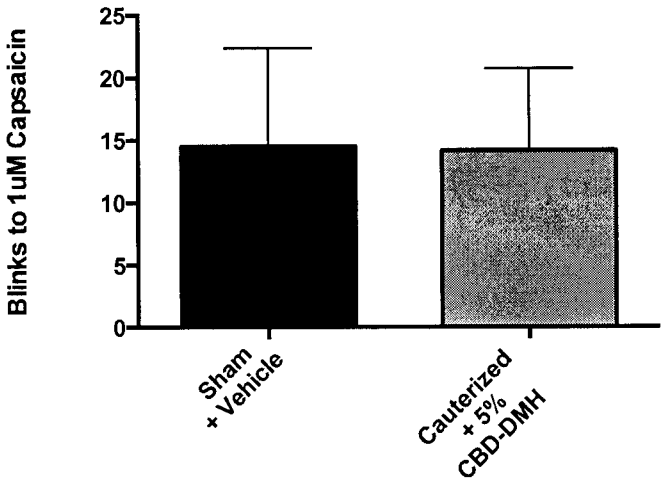
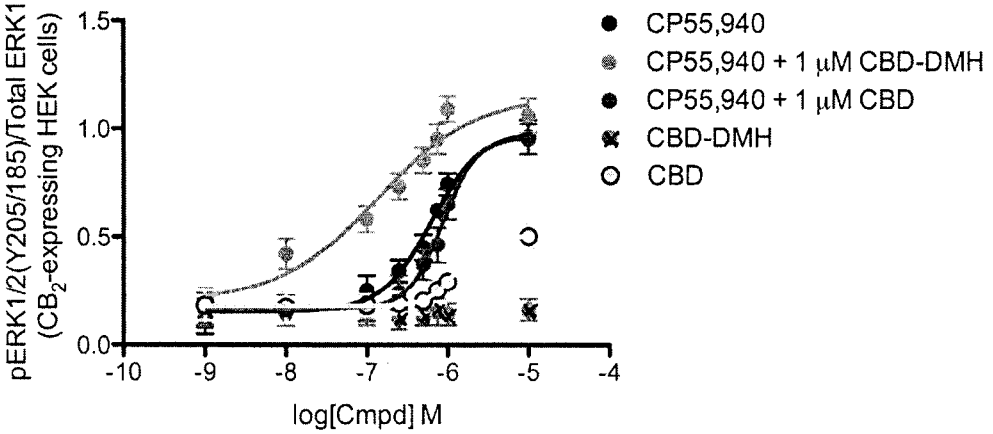

Blinks to 1uM Capsaicin
25
20
15
10
5
0
Sham + Vehicle
Cauterized + 5% CBD-DMH

FIG. 12

A


pERK1/2(Y205/185)/Total ERK1/2
(CB_2-expressing HEK cells)
1.5
1.0
0.5
0.0
-10
-9
-8
-7
-6
-5
-4
log[Cmpd] M
CP55,940
CP55,940 + 1 μM CBD-DMH
CP55,940 + 1 μM CBD
CBD-DMH
CBD

B

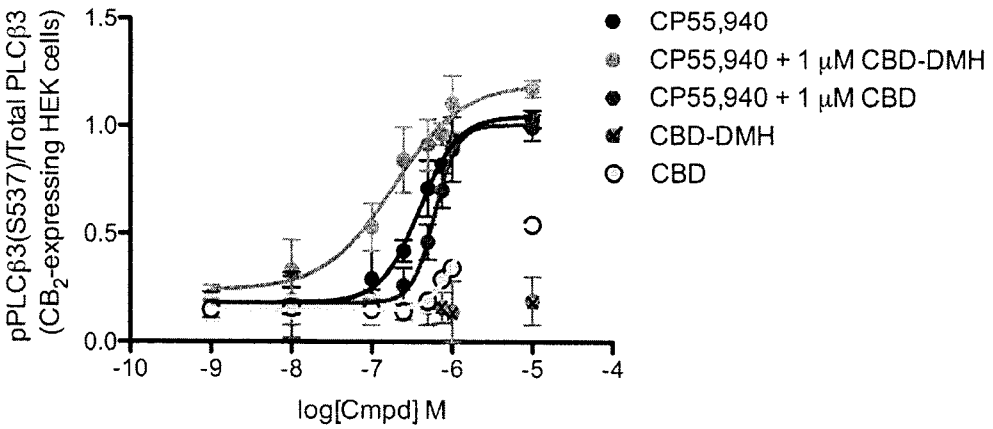

pPLCβ3(S537)/Total PLCβ3
(CB_2-expressing HEK cells)
1.5
1.0
0.5
0.0
-10
-9
-8
-7
-6
-5
-4
log[Cmpd] M
CP55,940
CP55,940 + 1 μM CBD-DMH
CP55,940 + 1 μM CBD
CBD-DMH
CBD

FIG. 13

C

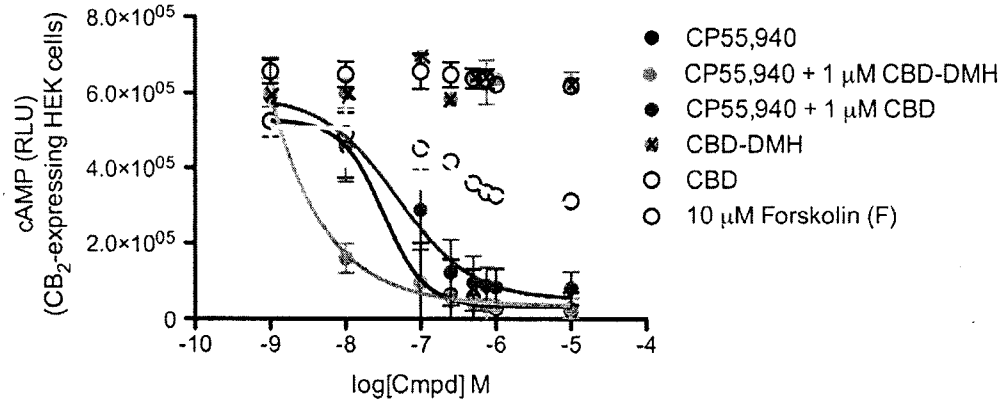
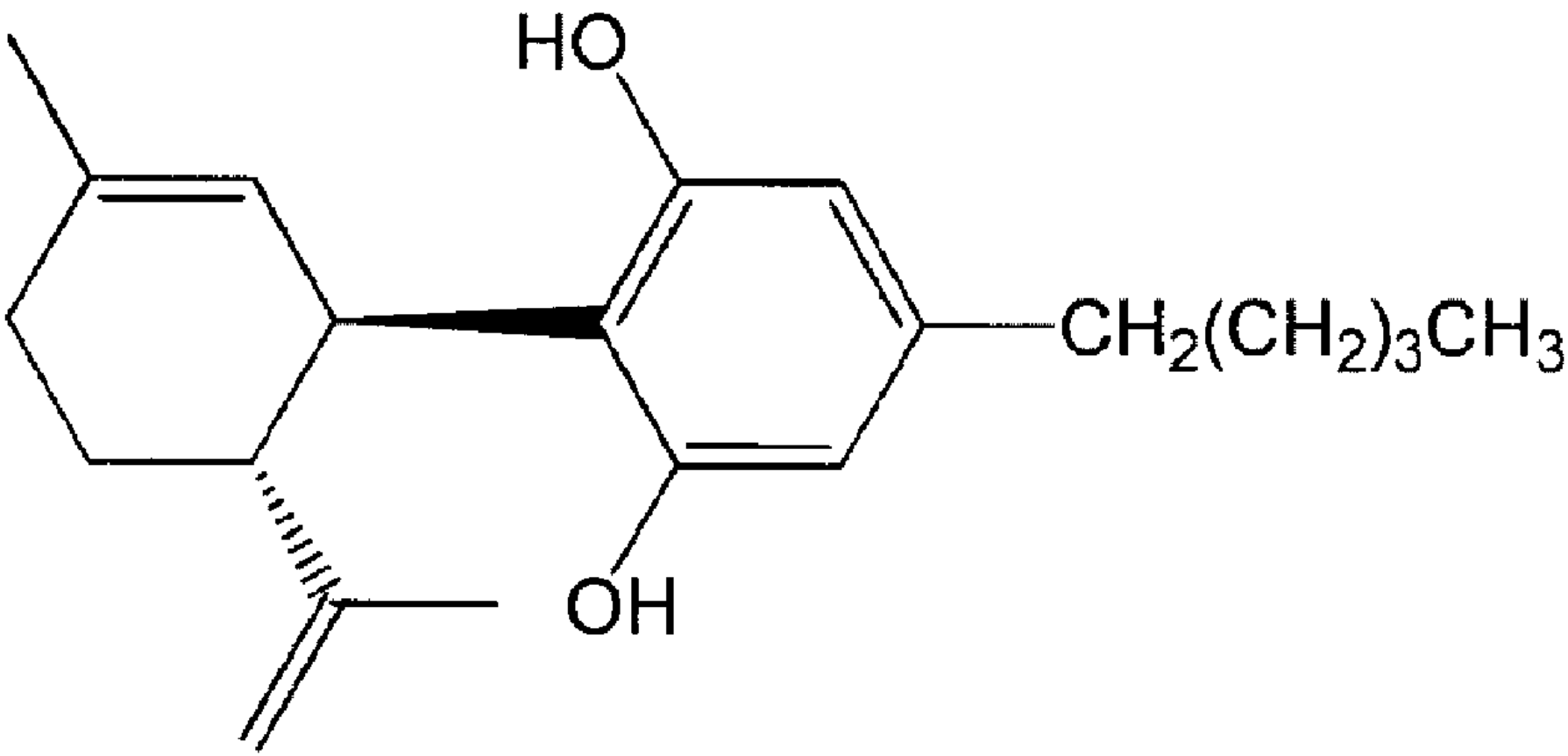

cAMP (RLU)
(CB2-expressing HEK cells)
8.0×10^05
6.0×10^05
4.0×10^05
2.0×10^05
0
-10
-9
-8
-7
-6
-5
-4
log[Cmpd] M
CP55,940
CP55,940 + 1 μM CBD-DMH
CP55,940 + 1 μM CBD
CBD-DMH
CBD
10 μM Forskolin (F)

FIG. 13 (cont.)


HO
OH
CH2(CH2)3CH3