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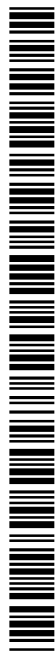


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WO 02/094311 A1

(54) Title: USE OF PROTEASOME INHIBITORS TO TREAT DRY EYE DISORDERS

(57) Abstract: Proteasome inhibitors are useful for treating dry eye disorders and other disorders requiring the wetting of the eye.

Use of Proteasome Inhibitors to Treat Dry Eye Disorders

The present invention is directed to the treatment of dry eye disorders.

5 In particular, the present invention is directed to the use of proteasome inhibitors in the treatment of dry eye and other disorders requiring the wetting of the eye in mammals.

Background of the Invention

10

Dry eye, also known generically as *keratoconjunctivitis sicca*, is a common ophthalmological disorder affecting millions of Americans each year. The condition is particularly widespread among post-menopausal women due to hormonal changes following the cessation of fertility. Dry eye may afflict an individual with varying severity. In mild cases, a patient may experience burning, a feeling of dryness, and persistent irritation such as is often caused by small bodies lodging between the eye lid and the eye surface. In severe cases, vision may be substantially impaired. Other diseases, such as Sjogren's disease and *cicatricial pemphigoid* manifest dry eye complications.

20

Although it appears that dry eye may result from a number of unrelated pathogenic causes, all presentations of the complication share a common effect, that is the breakdown of the pre-ocular tear film, which results in dehydration of the exposed outer surface and many of the symptoms outlined above (Lemp, *Report of the National Eye Institute/Industry Workshop on Clinical Trials in Dry Eyes*, The CLAO Journal, volume 21, number 4, pages 221-231 (1995)).

25

Practitioners have taken several approaches to the treatment of dry eye. One common approach has been to supplement and stabilize the ocular tear film using so-called artificial tears instilled throughout the day. Other

30

approaches include the use of ocular inserts that provide a tear substitute or stimulation of endogenous tear production.

Examples of the tear substitution approach include the use of buffered, isotonic saline solutions, aqueous solutions containing water soluble polymers that render the solutions more viscous and thus less easily shed by the eye. Tear reconstitution is also attempted by providing one or more components of the tear film such as phospholipids and oils. Phospholipid compositions have been shown to be useful in treating dry eye; see, e.g., McCulley and Shine, *Tear film structure and dry eye*, Contactologia, volume 20(4), pages 145-49 (1998); and Shine and McCulley, *Keratoconjunctivitis sicca associated with meibomian secretion polar lipid abnormality*, Archives of Ophthalmology, volume 116(7), pages 849-52 (1998). Examples of phospholipid compositions for the treatment of dry eye are disclosed in U.S. Patent Nos. 4,131,651 (Shah et al.), 4,370,325 (Packman), 4,409,205 (Shively), 4,744,980 and 4,883,658 (Holly), 4,914,088 (Glonek), 5,075,104 (Gressel et al.), 5,278,151 (Korb et al.), 5,294,607 (Glonek et al.), 5,371,108 (Korb et al.) and 5,578,586 (Glonek et al.). U.S. Patent No. 5,174,988 (Mautone et al.) discloses phospholipid drug delivery systems involving phospholipids, propellants and an active substance.

U.S. Patent No. 3,991,759 (Urquhart) discloses the use of ocular inserts in the treatment of dry eye. Other semi-solid therapy has included the administration of carrageenans (U.S. Patent No. 5,403,841, Lang) which gel upon contact with naturally occurring tear film.

Another approach involves the provision of lubricating substances in lieu of artificial tears. For example, U.S. Patent No. 4,818,537 (Guo) discloses the use of a lubricating, liposome-based composition, and U.S. Patent No. 5,800,807 (Hu et al.) discloses compositions containing glycerin and propylene glycol for treating dry eye.

Aside from efforts directed primarily to the alleviation of symptoms associated with dry eye, methods and compositions directed to treatment of the dry eye condition have also been pursued. For example, U.S. Patent No. 5,041,434 (Lubkin) discloses the use of sex steroids, such as conjugated
5 estrogens, to treat dry eye conditions in post-menopausal women; U.S. Patent No. 5,290,572 (MacKeen) discloses the use of finely divided calcium ion compositions to stimulate pre-ocular tear film production; and U.S. Patent No. 4,966,773 (Gressel et al.) discloses the use of microfine particles of one or more retinoids for ocular tissue normalization.

10 Although these approaches have met with some success, problems in the treatment of dry eye nevertheless remain. The use of tear substitutes, while temporarily effective, generally requires repeated application over the course of a patient's waking hours. It is not uncommon for a patient to have
15 to apply artificial tear solution ten to twenty times over the course of the day. Such an undertaking is not only cumbersome and time consuming, but is also potentially very expensive. Transient symptoms of dry eye associated with refractive surgery have been reported to last in some cases from six weeks to six months or more following surgery.

20 The use of ocular inserts is also problematic. Aside from cost, they are often unwieldy and uncomfortable. Further, as foreign bodies introduced in the eye, they can be a source of contamination leading to infections. In situations where the insert does not itself produce and deliver a tear film,
25 artificial tears must still be delivered on a regular and frequent basis.

The 20S proteasome is a complex of several enzymes that functions to degrade certain cellular proteins. Besides its involvement in the removal of misfolded or otherwise abnormal proteins, the proteasome also constitutes an
30 integral part of several signaling mechanisms. One of these pathways involves inflammatory responses mediated by the nuclear transcription factor NF- κ B. In its quiescent state NF- κ B exists as a heterodimer with the protein

I κ -B α , which masks the nuclear localization signals and DNA binding domain of the former protein. Under inflammatory conditions I κ -B α is phosphorylated, causing a conformational change which results in its tagging with multiple copies of the ubiquitin protein. Ubiquitinated I κ -B α is recognized and degraded
5 by the proteasome, which liberates NF- κ B. The free protein is translocated to the nucleus, where it binds to the appropriate DNA sequence and upregulates the production of several inflammatory mediators, such as COX-2, iNOS, IL-1, and TNF- α . Inhibiting the activity of the proteasome may therefore represent a potential strategy for reducing excessive inflammation.

10
Some recent literature reports suggest that patients suffering from dry eye syndrome disproportionately exhibit the hallmarks of excessive inflammation in relevant ocular tissues, such as the lacrimal and meibomian glands. The use of the following types of pharmaceutically active compounds
15 to treat dry eye patients has been disclosed: steroids [e.g. U.S. Patent No. 5,958,912; Marsh, et al., *Topical nonpreserved methylprednisolone therapy for keratoconjunctivitis sicca in Sjogren syndrome*, Ophthalmology, 106(4): 811-816 (1999); Pflugfelder, et. al. U.S. Patent No. 6,153,607], cytokine release inhibitors (Yanni, J.M.; et. al. WO 0003705 A1), cyclosporine A
20 [Tauber, J. *Adv. Exp. Med. Biol.* **1998**, 438 (Lacrimal Gland, Tear Film, and Dry Eye Syndromes 2), 969], and 15-HETE (Yanni et. al., US Patent No. 5,696,166). Additionally, while proteasome inhibitors, have been disclosed as antiinflammatory agents for the treatment of various non-ocular (e.g., septic shock, ischemia-reperfusion injury, cancer, and graft rejection; see US patent
25 6,083,903, WO 0043000, WO 0064863, WO 9922729, WO 9915183, WO 9835691, and WO 9632105) and ocular diseases (glaucoma and damage due to ocular surgery; see Jpn. Kokai Tokkyo Koho JP 10036289 A2, Chemical Abstracts Accession Number 128:226254), the use of proteasome inhibitors for the treatment of dry eye syndrome has not been previously
30 suggested or disclosed.

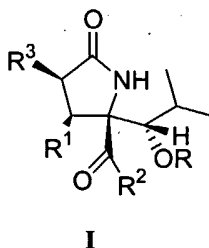
Summary of the Invention

The present invention is directed to methods for the treatment of dry eye and other disorders requiring the wetting of the eye, including symptoms of dry eye associated with refractive surgery such as LASIK surgery. According to the methods of the present invention, proteasome inhibitors are administered to a patient suffering from dry eye or other disorders requiring wetting of the eye. The proteasome inhibitors are preferably administered topically to the eye.

Detailed Description of the Invention

Many proteasome inhibitors are known. Proteasome inhibitors useful in the methods of the present invention are those of formulas (I) – (III).

Formula (I):



wherein:

R is H, alkyl, or acyl;

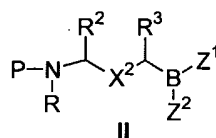
R¹ is OR⁴, where R⁴ is H or acyl, and R² is SR⁵, where R⁵ is alkyl, cycloalkyl, aryl, alkenyl, alkynyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl, all optionally containing one or more alkyl, halo, amino, acylamino, aminoacyl, hydroxyl, acyloxy, alkoxy, aryloxy, carboxyl, alkoxy carbonyl, or acyl groups; or

R¹ is O and R² is bonded to R¹ to form a β-lactone ring; and

R^3 is CH_3 , C_2H_5 , *n*- or *i*- C_3H_7 , or *n*- or *i*- C_4H_9 .

Formula (II):

5



wherein:

10 P is morpholinylcarbonyl or 2- or 8-quinolinyl-, 2-quinoxaliny-, 2- or 3-pyridyl-, piperazinyl-, 3-furanyl-, or 3-pyrrolylcarbonyl or -sulfonyl;

X^2 is CONH, CH_2NH , $CH(OH)CH_2$, $CH(OH)CH(OH)$, $CH(OH)CH_2NH$, $CH:CH$, $COCH_2$, SO_2NH , SO_2CH_2 , or $CH(OH)CH_2CONH$;

15

R is H or alkyl;

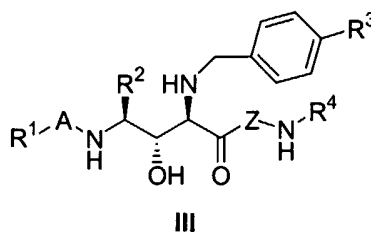
R^2 and R^3 are the same or different and are H, alkyl, cycloalkyl, aryl, heterocyclyl, CH_2R^5 , or alkyl-chalcogen;

20

R^5 is aryl, aralkyl, alkaryl, cycloalkyl, or heterocyclyl; and

Z^1 and Z^2 are the same or different and are alkyl, hydroxy, alkoxy, or aryloxy.

25 Formula (III):



wherein:

A and Z represent a bond or an optionally substituted amino acid moiety;

5 R¹ is H, an amino protecting group (such as *t*-butoxycarbonyl or 9-fluorenylmethylcarbonyl), or a group R⁵Y, where R⁵ is H or a heterocyclalkyl group and Y is CO, NHCO, NHCS, SO₂, OCO, or OCS;

10 R² represents the side chain of a natural amino acid or is alkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, trimethylsilylmethyl, 2-thienylmethyl, or styrylmethyl;

R³ is halo, alkyl, alkoxy, or hydroxyalkoxy; and

15 R⁴ is (2*R*)-hydroxyindan-(1*S*)-yl, (S)-2-hydroxy-1-phenylethyl, or 2-hydroxybenzyl, the phenyl rings of each being optionally substituted in the 4-position with a methoxy group.

20 The compounds of formulas (I) – (III) are known and can be made by known methods. For example, the synthesis of compounds included in formula (I) is disclosed in Adams, *et.al.*, *J. Am. Chem. Soc.*, 121:9967 (1999). The synthesis of compounds included in formula (II) is disclosed in US Patent Number 6,083,903. The synthesis of compounds included in formula (III) is disclosed in WO 0064863.

25 Included within the scope of the present invention are the individual enantiomers of the compounds of the present invention, as well as their racemic and non-racemic mixtures. The individual enantiomers can be enantioselectively synthesized from the appropriate enantiomerically pure or enriched starting material by means such as those described below.
30 Alternatively, they may be enantioselectively synthesized from racemic/non-racemic or achiral starting materials. (*Asymmetric Synthesis*; J. D. Morrison

and J. W. Scott, Eds.; Academic Press Publishers: New York, 1983-1985, volumes 1-5; *Principles of Asymmetric Synthesis*; R.E. Gawley and J. Aube, Eds.; Elsevier Publishers: Amsterdam, 1996). They may also be isolated from racemic and non-racemic mixtures by a number of known methods, e.g. by purification of a sample by chiral HPLC (*A Practical Guide to Chiral Separations by HPLC*; G. Subramanian, Ed.; VCH Publishers: New York, 1994; *Chiral Separations by HPLC*; A.M. Krstulovic, Ed.; Ellis Horwood Ltd. Publishers, 1989), or by enantioselective hydrolysis of a carboxylic acid ester sample by an enzyme (Ohno, M.; Otsuka, M. Organic Reactions, volume 37, page 1 (1989)). Those skilled in the art will appreciate that racemic and non-racemic mixtures may be obtained by several means, including without limitation, nonenantioselective synthesis, partial resolution, or even mixing samples having different enantiomeric ratios. Departures may be made from such details within the scope of the accompanying claims without departing from the principles of the invention and without sacrificing its advantages. Also included within the scope of the present invention are the individual isomers substantially free of their respective enantiomers.

The term "acyl" represents a group that is linked by a carbon atom that has a double bond to an oxygen atom and a single bond to another carbon atom.

The term "alkyl" includes straight or branched chain aliphatic hydrocarbon groups that are saturated and have 1 to 10 carbon atoms. The alkyl groups may be interrupted by one or more heteroatoms, such as oxygen, nitrogen, or sulfur, and may be substituted with other groups, such as halogen, hydroxyl, aryl, cycloalkyl, aryloxy, or alkoxy. Preferred straight or branched alkyl groups include methyl, ethyl, propyl, isopropyl, butyl and *t*-butyl.

The term "cycloalkyl" includes straight or branched chain, saturated or unsaturated aliphatic hydrocarbon groups which connect to form one or more rings, which can be fused or isolated. The rings may be substituted with other

groups, such as halogen, hydroxyl, aryl, aryloxy, alkoxy, or lower alkyl. Preferred cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

5 The term "heterocycloalkyl" refers to cycloalkyl rings that contain at least one heteroatom such as O, S, or N in the ring, and can be fused or isolated. The rings may be substituted with other groups, such as halogen, hydroxyl, aryl, aryloxy, alkoxy, or lower alkyl. Preferred heterocycloalkyl groups include pyrrolidinyl, tetrahydrofuranyl, piperazinyl, and
10 tetrahydropyranyl.

The term "alkenyl" includes straight or branched chain hydrocarbon groups having 1 to 10 carbon atoms with at least one carbon-carbon double bond, the chain being optionally interrupted by one or more heteroatoms.
15 The chain hydrogens may be substituted with other groups, such as halogen. Preferred straight or branched alkenyl groups include allyl, 1-butenyl, 1-methyl-2-propenyl and 4-pentenyl.

The term "cycloalkenyl" includes straight or branched chain, saturated
20 or unsaturated aliphatic hydrocarbon groups which connect to form one or more non-aromatic rings containing a carbon-carbon double bond, which can be fused or isolated. The rings may be substituted with other groups, such as halogen, hydroxyl, alkoxy, or lower alkyl. Preferred cycloalkenyl groups include cyclopentenyl and cyclohexenyl.

25 The term "heterocycloalkenyl" refers to cycloalkenyl rings which contain one or more heteroatoms such as O, N, or S in the ring, and can be fused or isolated. The rings may be substituted with other groups, such as halogen, hydroxyl, aryl, aryloxy, alkoxy, or lower alkyl. Preferred heterocycloalkenyl
30 groups include pyrrolidinyl, dihydropyranyl, and dihydrofuranyl.

The term "carbonyl group" represents a carbon atom double bonded to an oxygen atom, wherein the carbon atom has two free valencies.

The term "aminocarbonyl" represents a free or functionally modified amino group bonded from its nitrogen atom to the carbon atom of a carbonyl group, the carbonyl group itself being bonded to another atom through its carbon atom.

The term "lower alkyl" represents alkyl groups containing one to six carbons (C₁-C₆).

The term "halo" or "halogen" represents fluoro, chloro, bromo, or iodo.

The term "aryl" refers to carbon-based rings which are aromatic. The rings may be isolated, such as phenyl, or fused, such as naphthyl. The ring hydrogens may be substituted with other groups, such as lower alkyl, halogen, free or functionalized hydroxy, trihalomethyl, etc. Preferred aryl groups include phenyl, 3-(trifluoromethyl)phenyl, 3-chlorophenyl, and 4-fluorophenyl.

The term "heteroaryl" refers to aromatic hydrocarbon rings which contain at least one heteroatom such as O, S, or N in the ring. Heteroaryl rings may be isolated, with 5 to 6 ring atoms, or fused, with 8 to 10 atoms. The heteroaryl ring(s) hydrogens or heteroatoms with open valency may be substituted with other groups, such as lower alkyl or halogen. Examples of heteroaryl groups include imidazole, pyridine, indole, quinoline, furan, thiophene, pyrrole, tetrahydroquinoline, dihydrobenzofuran, and dihydrobenzindole.

The terms "aryloxy", "heteroaryloxy", "alkoxy", "cycloalkoxy", "heterocycloalkoxy", "alkenyloxy", "cycloalkenyloxy", "heterocycloalkenyloxy", and "alkynyloxy" represent an aryl, heteroaryl, alkyl, cycloalkyl, heterocycloalkyl, alkenyl, cycloalkenyl, heterocycloalkenyl, or alkynyl group, respectively, attached through an oxygen linkage.

The terms "alkoxycarbonyl", "aryloxycarbonyl", "heteroaryloxycarbonyl", "cycloalkoxycarbonyl", "heterocycloalkoxycarbonyl", "alkenyloxycarbonyl", "cycloalkenyloxycarbonyl", "heterocycloalkenyloxycarbonyl", and "alkynyloxycarbonyl" represent an alkoxy, aryloxy, heteroaryloxy, cycloalkoxy, heterocycloalkoxy, alkenyloxy, cycloalkenyloxy, heterocycloalkenyloxy, or alkynyloxy group, respectively, bonded from its oxygen atom to the carbon of a carbonyl group, the carbonyl group itself being bonded to another atom through its carbon atom.

Preferred for purposes of the present invention are those compounds of formula I wherein:

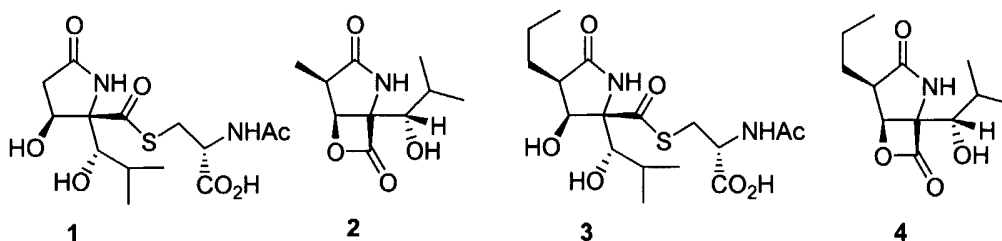
R is H, CH₃CO, C₂H₅CO, Me₃CCO, or PhCO;

R¹ is OR⁴, where R⁴ is H, CH₃CO, C₂H₅CO, Me₃CCO, or PhCO, and R² is SR⁵, where R⁵ is as defined above; or

R¹ is O and R² is bonded to R¹ to form a β-lactone ring; and

R³ is CH₃, C₂H₅, *n*- or *i*-C₃H₇, or *n*- or *i*-C₄H₉.

Among the most preferred compounds of the present invention are the following compounds 1-4 (where Ac = an acetyl group):



The syntheses of these compounds are disclosed in: Corey *et al.*, *Total Synthesis and Biological Activity of Lactacystin, Omuralide, and Analogs*. Chem. Pharm. Bull., volume 47, pages 1-10 (1999); and Adams *et al.*, *A*

Novel and Efficient Synthesis of a Highly Active Analogue of clasto-Lactacystin β -Lactone. J. Am. Chem. Soc., volume 121, pages 9967-9976 (1999).

5 According to the methods of the present invention, a composition comprising one or more proteasome inhibitors and a pharmaceutically acceptable carrier for topical ophthalmic administration or implantation into the conjunctival sac or anterior chamber of the eye is administered to a mammal in need thereof. The compositions are formulated in accordance
10 with methods known in the art for the particular route of administration desired.

 The compositions administered according to the present invention comprise a pharmaceutically effective amount of one or more proteasome
15 inhibitors. As used herein, a "pharmaceutically effective amount" is one which is sufficient to reduce or eliminate signs or symptoms of dry eye or other disorders requiring the wetting of the eye. Generally, for compositions intended to be administered topically to the eye in the form of eye drops or eye ointments, the total amount of proteasome inhibitor will be about 0.0001
20 to 10 percent weight/volume ("% w/v"). For preferred topically administrable ophthalmic compositions, the total amount of proteasome inhibitor will be about 0.001-1% w/v.

 The present invention is particularly directed to compositions useful in
25 treating dry eye. Preferably, such compositions will be formulated as solutions, suspensions and other dosage forms for topical administration. Aqueous solutions are generally preferred, based on ease of formulation, as well as a patient's ability to easily administer such compositions by means of instilling one to two drops of the solutions in the affected eyes. However, the
30 compositions may also be suspensions, viscous or semi-viscous gels, or other types of solid or semi-solid compositions. Suspensions may be preferred for proteasome inhibitors which are sparingly soluble in water.

The compositions of the present invention may also contain a surfactant. Various surfactants useful in topical ophthalmic formulations may be employed. The surfactant(s) may provide additional chemical stabilization of the compounds of formulas (I) – (III) and may further provide for the physical stability of the compounds. In other words, the surfactants may aid in preventing chemical degradation of the compounds of formulas (I) – (III) and also prevent the compounds from binding to the containers in which their compositions are packaged. As used herein, “an effective concentration of surfactant(s)” refers to a concentration that enhances the chemical and physical stability of the compounds of formulas (I) – (III). Examples of surfactants include, but are not limited to: Cremophor[®] EL, polyoxyl 20 ceto stearyl ether, polyoxyl 40 hydrogenated castor oil, polyoxyl 23 lauryl ether and poloxamer 407 may be used in the compositions. A preferred surfactant is polyoxyl 40 stearate. The concentration of surfactant will vary, depending on the concentration of the compound(s) of formulas (I) – (III) and optional ethanol present in the formulation. In general, however, the surfactant(s) concentration will be about 0.001 to 2.0% w/v. Preferred compositions of the present invention will contain about 0.1% w/v of polyoxyl 40 stearate.

The compositions of the present invention may also include various other ingredients, such as tonicity agents, buffers, preservatives, co-solvents and viscosity building agents.

Various tonicity agents may be employed to adjust the tonicity of the composition, preferably to that of natural tears for ophthalmic compositions. For example, sodium chloride, potassium chloride, magnesium chloride, calcium chloride, dextrose and/or mannitol may be added to the composition to approximate physiological tonicity. Such an amount of tonicity agent will vary, depending on the particular agent to be added. In general, however, the compositions will have a tonicity agent in an amount sufficient to cause the

final composition to have an ophthalmically acceptable osmolality (generally about 150 – 450 mOsm, preferably 250 – 350 mOsm).

An appropriate buffer system (e.g., sodium phosphate, sodium acetate, sodium citrate, sodium borate or boric acid) may be added to the compositions to prevent pH drift under storage conditions. The particular concentration will vary, depending on the agent employed. Preferably, however, the buffer will be chosen to maintain a target pH within the range of pH 6-7.5.

Antioxidants may be added to compositions of the present invention to protect the proteasome inhibitor compounds from oxidation during storage. Examples of such antioxidants include, but are not limited to, vitamin E and analogs thereof, ascorbic acid and derivatives, and butylated hydroxyanisole (BHA).

Compositions formulated for the treatment of dry eye-type diseases and disorders may also comprise aqueous carriers designed to provide immediate, short-term relief of dry eye-type conditions. Such carriers can be formulated as a phospholipid carrier or an artificial tears carrier, or mixtures of both. As used herein, "phospholipid carrier" and "artificial tears carrier" refer to aqueous compositions which: (i) comprise one or more phospholipids (in the case of phospholipid carriers) or other compounds, which lubricate, "wet," approximate the consistency of endogenous tears, aid in natural tear build-up, or otherwise provide temporary relief of dry eye symptoms and conditions upon ocular administration; (ii) are safe; and (iii) provide the appropriate delivery vehicle for the topical administration of an effective amount of one or more proteasome inhibitors. Examples of artificial tears compositions useful as artificial tears carriers include, but are not limited to, commercial products, such as Tears Naturale®, Tears Naturale II®, Tears Naturale Free®, and Bion Tears® (Alcon Laboratories, Inc., Fort Worth, Texas). Examples of phospholipid carrier formulations include those disclosed in U.S. Patent Nos. 4,804,539 (Guo et al.), 4,883,658 (Holly), 4,914,088 (Glonek), 5,075,104

(Gressel et al.), 5,278,151 (Korb et al.), 5,294,607 (Glonek et al.), 5,371,108 (Korb et al.), 5,578,586 (Glonek et al.); the foregoing patents are incorporated herein by reference to the extent they disclose phospholipid compositions useful as phospholipid carriers of the present invention.

5

Other compounds designed to lubricate, "wet," approximate the consistency of endogenous tears, aid in natural tear build-up, or otherwise provide temporary relief of dry eye symptoms and conditions upon ocular administration the eye are known in the art. Such compounds may enhance the viscosity of the composition, and include, but are not limited to:

10 monomeric polyols, such as, glycerol, propylene glycol, ethylene glycol; polymeric polyols, such as, polyethylene glycol, hydroxypropylmethyl cellulose ("HPMC"), carboxy methylcellulose sodium, hydroxy propylcellulose ("HPC"), dextrans, such as, dextran 70; water soluble proteins, such as gelatin; and

15 vinyl polymers, such as, polyvinyl alcohol, polyvinylpyrrolidone, povidone and carbomers, such as, carbomer 934P, carbomer 941, carbomer 940, carbomer 974P.

Other compounds may also be added to the ophthalmic compositions of the present invention to increase the viscosity of the carrier. Examples of

20 viscosity enhancing agents include, but are not limited to: polysaccharides, such as hyaluronic acid and its salts, chondroitin sulfate and its salts, dextrans, various polymers of the cellulose family; vinyl polymers; and acrylic acid polymers. In general, the phospholipid carrier or artificial tears carrier

25 compositions will exhibit a viscosity of 1 to 400 centipoises ("cps").

Topical ophthalmic products are typically packaged in multidose form. Preservatives are thus required to prevent microbial contamination during use. Suitable preservatives include: benzalkonium chloride, chlorobutanol,

30 benzododecinium bromide, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, polyquaternium-1, or other agents known to those skilled in the art. Such preservatives are typically employed at a level of from 0.001 to 1.0% w/v. Unit dose compositions of the present

invention will be sterile, but typically unpreserved. Such compositions, therefore, generally will not contain preservatives.

The preferred compositions of the present invention are intended for administration to a human patient suffering from dry eye or symptoms of dry eye. Preferably, such compositions will be administered topically. In general, the doses used for the above described purposes will vary, but will be in an effective amount to increase mucin production in the eye and thus eliminate or improve dry eye conditions. Generally, 1-2 drops of such compositions will be administered 1-10 times per day for the treatment of dry eye or other ocular disease or disorder. Preferably, 1-2 drops of the compositions will be administered 1-4 times per day.

A representative eye drop formulation is provided in Example 1 below.

Example 1

Ingredient	Amount (% w/v)
Compound of formula (I) – (III)	0.0001-0.1
Polyoxyl 40 Stearate	0.1?
Boric Acid	0.25
Sodium Chloride	0.75
Disodium Edetate	0.01
Polyquaternium-1	0.001
NaOH/HCl	q.s., pH = 7.4
Purified Water	q.s. 100%

The above composition is prepared by the following method. The batch quantities of boric acid, sodium chloride, disodium edetate, and polyquaternium-1 are weighed and dissolved by stirring in 90% of the batch

quantity of purified water. The pH is adjusted to 7.4 ± 0.1 with NaOH and/or HCl. Under yellow light or reduced lighting, the batch quantity of the compound of formulas (I) – (III) as a stock solution is measured and added. Purified water is added to q.s. to 100%. The mixture is stirred for five minutes
5 to homogenize and then filtered through a sterilizing filter membrane into a sterile recipient.

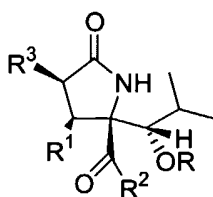
This invention has been described by reference to certain preferred embodiments; however, it should be understood that it may be embodied in
10 other specific forms or variations thereof without departing from its special or essential characteristics. The embodiments described above are therefore considered to be illustrative in all respects and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description.

WHAT IS CLAIMED IS:

1. A method for the treatment of dry eye and other disorders requiring the wetting of the eye which comprises administering to a mammal a composition comprising a pharmaceutically acceptable carrier and a pharmaceutically effective amount of one or more proteasome inhibitors.

5

2. The method of Claim 1 wherein the proteasome inhibitor is a compound of the formula



10

wherein:

R is H, alkyl, or acyl;

15

R¹ is OR⁴, where R⁴ is H or acyl, and R² is SR⁵, where R⁵ is alkyl, cycloalkyl, aryl, alkenyl, alkynyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl, all optionally containing one or more alkyl, halo, amino, acylamino, aminoacyl, hydroxyl, acyloxy, alkoxy, aryloxy, carboxyl, alkoxycarbonyl, or acyl groups; or

20

R¹ is O and R² is bonded to R¹ to form a β-lactone ring; and

R³ is CH₃, C₂H₅, *n*- or *i*-C₃H₇, or *n*- or *i*-C₄H₉.

25

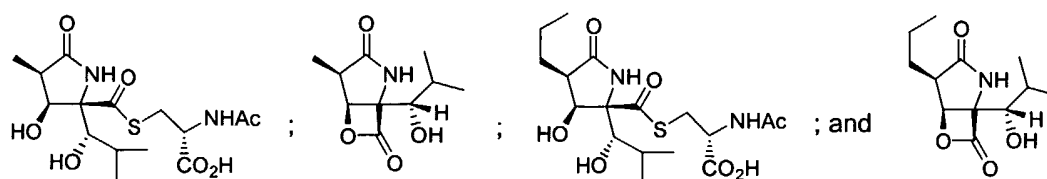
3. The method of claim 2, wherein for the compound of formula I:

R is H, CH₃CO, C₂H₅CO, Me₃CCO, or PhCO; and

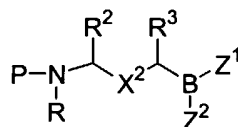
5 R⁴ is H, CH₃CO, C₂H₅CO, Me₃CCO, or PhCO.

4. The method of claim 3, wherein the compound of formula I is selected from the group consisting of:

10



5. The method of Claim 1 wherein the proteasome inhibitor is a
15 compound of the formula



wherein:

20

P is morpholinylcarbonyl or 2- or 8-quinolinyl-, 2-quinoxaliny-, 2- or 3-pyridyl-, piperazinyl-, 3-furanyl-, or 3-pyrrolylcarbonyl or -sulfonyl;

X² is CONH, CH₂NH, CH(OH)CH₂, CH(OH)CH(OH), CH(OH)CH₂NH, CH:CH,
25 COCH₂, SO₂NH, SO₂CH₂, or CH(OH)CH₂CONH;

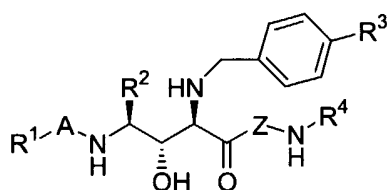
R is H or alkyl;

R^2 and R^3 are the same or different and are H, alkyl, cycloalkyl, aryl, heterocyclyl, CH_2R^5 , or alkyl-chalcogen;

5 R^5 is aryl, aralkyl, alkaryl, cycloalkyl, or heterocyclyl; and

Z^1 and Z^2 are the same or different and are alkyl, hydroxy, alkoxy, or aryloxy.

6. The method of Claim 1 wherein the proteasome inhibitor is a
10 compound of the formula



wherein:

A and Z represent a bond or an optionally substituted amino acid moiety;

15

R^1 is H, an amino protecting group (such as *t*-butoxycarbonyl or 9-fluorenylmethylcarbonyl), or a group R^5Y , where R^5 is H or a heterocyclylalkyl group and Y is CO, NHCO, NHCS, SO_2 , OCO, or OCS;

20

R^2 represents the side chain of a natural amino acid or is alkyl, arylalkyl, heteroarylalkyl, cycloalkylalkyl, trimethylsilylmethyl, 2-thienylmethyl, or styrylmethyl;

R^3 is halo, alkyl, alkoxy, or hydroxyalkoxy; and

25

R^4 is (2*R*)-hydroxyindan-(1*S*)-yl, (*S*)-2-hydroxy-1-phenylethyl, or 2-hydroxybenzyl, the phenyl rings of each being optionally substituted in the 4-position with a methoxy group.

7. The method of any of claims 1-6, wherein the composition is topically ophthalmically administered to the mammal.
8. The method of Claim 7 wherein the pharmaceutically effective amount
s of the proteasome inhibitor is 0.0001-1% w/v.
9. The method of Claim 1 wherein the dry eye and other disorders requiring the wetting of the eye is symptoms of dry eye associated with refractive surgery.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 02/15775

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/55 A61K31/4015 A61K31/407 A61P27/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

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

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

MEDLINE, EPO-Internal, PAJ, WPI Data, BIOSIS, CANCERLIT, SCISEARCH, PASCAL, EMBAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>DATABASE MEDLINE 'Online! December 2000 (2000-12) LEMP M A: "Evaluation and differential diagnosis of keratoconjunctivitis sicca." Database accession no. NLM11128698 XP002215303 abstract & THE JOURNAL OF RHEUMATOLOGY. SUPPLEMENT. CANADA DEC 2000, vol. 61, December 2000 (2000-12), pages 11-14, ISSN: 0380-0903</p> <p style="text-align: center;">--- -/--</p>	1-4,6-9

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

1 October 2002

Date of mailing of the international search report

18/10/2002

Name and mailing address of the ISA

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 02/15775

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>DATABASE MEDLINE 'Online! May 2000 (2000-05) BRIGNOLE F ET AL: "Flow cytometric analysis of inflammatory markers in conjunctival epithelial cells of patients with dry eyes." Database accession no. NLM10798650 XP002215304 abstract & INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE. UNITED STATES MAY 2000, vol. 41, no. 6, May 2000 (2000-05), pages 1356-1363, ISSN: 0146-0404</p>	1-4,6-9
Y	<p>ELLIOTT P J ET AL: "Proteasome inhibition: A novel mechanism to combat asthma." THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY. UNITED STATES AUG 1999, vol. 104, no. 2 Pt 1, August 1999 (1999-08), pages 294-300, XP001095887 ISSN: 0091-6749 abstract page 298, column 2, paragraph 2 page 299, column 1, paragraph 3</p>	1-4,6-9
Y	<p>WANG X C ET AL: "Suppression of NF-kappaB-dependent proinflammatory gene expression in human RPE cells by a proteasome inhibitor." INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE. UNITED STATES FEB 1999, vol. 40, no. 2, February 1999 (1999-02), pages 477-486, XP001106154 ISSN: 0146-0404 abstract</p>	1-4,6-9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 02/15775

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: 5
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 5

Claim 5 does not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The variable B of the Markush formula is not defined in the claim nor in the description. Hence, the subject matter of claim 5 is not clear.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.