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Dacryology Update

Recent developments on dry eye disease treatment compounds

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Abstract

Dry eye syndrome is a common tears and ocular surface multifactorial disease, described by changes in the ocular surface epithelia related to reduced tears quantity and ocular surface sensitivity, leading to inflammatory reaction. Managing the eye inflammation proved helpful to patients with dry eye disease and current treatment is based on the use of topically applied artificial tear products/lubricants, tear retention management, stimulation of tear secretion and using anti-inflammatory drugs. In this article we revise the corresponding literature and patents assembling the new treatment approaches of novel and future pharmaceutical compounds destined for the dry eye disease treatment. The most frequent categories of compounds presented are secretagogues and anti-inflammatory drugs. These compounds are the research outcome of novel therapeutic strategies designed to reduce key inflammatory pathways and restore healthy tear film.

Keywords: Dry eye, Anti-inflammatory, Keratoconjunctivitis sicca, Keratitis sicca, Xerophthalmia, Mucin secretion, Tear secretion, NSAID

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Introduction

Dry eye is a disease which starts with unpleasant levels of symptoms of dryness of eyes and an uncomfortable feeling, greatly preventing people from performing daily activities when the disease worsens. The number of dry eye patients is increasing yearly in association with the aging of the society and the increase in VDT (video display terminal) works with personal computers and devices. Dry eye prevalence in 2009 in Spain was 11.0% among adults. Dry eye was found to be more frequent in women (11.9%) than in men (9.0%), and was significantly associated with aging.[1] Although exact etiopathogenesis of dry eye is not known, it is believed that decrease in the tear volume on the corneal and conjunctival surface caused by either a decreased tear secretion or accelerated evaporation plays the main role. The clinical features of dry eyes include ocular discomfort, feeling of dryness, feeling of eye fatigue, hyperemia, keratoconjunctival epithelial disorders and abnormalities of vision. If these symptoms and observations progress, eventually abnormality occurs in vision. Therefore, it is quite important to treat dry eye properly at an early stage. Wetness of the ocular surface and other exposed mucosae is maintained by a continuous aqueous fluid secretion produced by exocrine glands.[2–4] In the eye, basal tear flow is adjusted to variations in environmental conditions and blinking rate. Tear flow occurring in the absence of emotional or exogenous irritant stimuli ('basal' tear secretion) is adjusted to variations in environmental conditions and blinking rate.[5] Tearing also increases markedly upon ocular surface irritation.[6] Irritating stimuli are detected by mechano-nociceptor and polymodal-nociceptor trigeminal nerve endings sensitive to injurious mechanical forces, noxious heat and irritant chemicals, that evoke pain and irritation-induced tearing.[7] However, the neural structures responsible for sensing ocular surface dryness to regulate basal tearing rate remain undefined. Dry eye syndrome is a disease

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characterized by persistent dryness of the conjunctiva and opacity of the cornea. Multiple causes can lead to dry eye, which is more common in elderly people. Among diseases causing dry eye are: vitamin A deficit, Sjögren's syndrome, rheumatoid arthritis and other rheumatologic diseases, chemical or thermal burns, drugs such as atenolol, chlorpheniramine, hydrochlorothiazide, isotretinoin, ketorolac, ketotifen, levocabastin, levofloxacin, oxybutynin, and tolterodine.

Current dry eye treatment

The symptomatic relief of dry eye includes tear supplements called ''artificial tears'' which are artificial lubricants, characterized by hypotonic or isotonic buffered solutions containing electrolytes, surfactants and various types of viscosity agents.[8] Another treatment option is the application of tear retention devices. Implants are developed to permanently occlude the lacrimal puncta. These kinds of implants also known as punctal plugs, and could be absorbables and non-absorbables.[9] Additional treatment is by using moisture chamber spectacles. It has been reported that increases in periocular humidity can cause a growth of the lipid layer tear film thickness and also that spectacle wearers with dry eye have a longer inter-blink interval than those who do not wear spectacles.[10] On the pharmacology side current treatment is mainly focusing on addressing inflammation and tear restoration.[11] Dry eye disease is the outcome of many factors resulting in inflammation of the cornea and conjunctiva. The dysfunction of the tear secretory glands leads to changes in tear composition such as hyper-osmolarity which stimulates the production of inflammatory mediators on the ocular surface. This inflammation can be initiated either by chronic irritative stress like contact lens wearing or a systemic inflammatory autoimmune disease like rheumatoid arthritis.[12,13] Anti-inflammatory drugs are widely used for the treatment of the inflammation produced by the disease with the topical corticosteroid drops being the most common therapy. Corticosteroids can rapidly and effectively relieve the symptoms and signs of moderate or severe dry eye.[14] Steroids on the other hand produce severe side effects after prolonged use. The effects include risk of bacterial or fungal infection, elevated intraocular pressure and cataract formation, therefore steroids are typically used only for one to two weeks in dry eye patients.[15] As a consequence, non-steroidal anti-inflammatory drugs (NSAID) are increasingly used as dry eye treatment instead of steroids because of their non-severe side effects and because steroids locally suppress the immune response in patients with an already compromised ocular surface. The NSAIDs acutely decrease the eye discomfort due to its analgesic effect and furthermore is reducing the inflammation. In 2002 U.S. Food and Drug Administration approved the drug RESTASIS® of the company Allergan as the first prescription medicine helping to increase tear production reduced by inflammation due to chronic dry eye disease. Topical RESTASIS® diquafosol tetrasodium is an ophthalmic emulsion containing cyclosporine 0.05%.[16] Other type of drug used is the antibiotics including oral doxycycline, azithromycin, and tetracycline. There is some research on the use of serum tears and intense pulse light treatment.[17]

In this review we are presenting the trends on the oncoming treatments, analyzing the patents filled from the research institutions. The data are represented on a table of drugs and properties (Table 1).

Recent developments

Cytokine receptor inhibitors

Cytokines are a group of hormones incapable to penetrate through the cell membrane and functioning by binding to cognate receptor proteins.[18] Cytokines use multiple signaling pathways with JAK-STAT pathway being the most important. Janus kinases (JAKs) are components of the cytokine receptor signaling pathway and signal transducers and activators of transcription (STATs). After the discovery of JAK's it was identified as the STAT family of transcription factors (STAT1-5a, 5b and 6).[19] The activation of the cytokine receptor JAK signaling complex leads to the stimulation of JAK kinases resulting in the phosphorylation of receptor chains, creating docking sites for STAT transcription factors.[20] Generally, cytokine receptors do not have intrinsic tyrosine kinase activity, and thus require receptor-associated kinases to propagate a phosphorylation cascade. Cytokines bind to their receptors, causing receptor dimerization, and this enables JAKs to phosphorylate each other as well as specific tyrosine motifs within the cytokine receptors. STATs that recognize these phosphortyrosine motifs are recruited to the receptor, and after this they are activated by a JAK-dependent tyrosine phosphorylation event. Upon activation, STATs dissociate from the receptors, dimerize, and translocate to the nucleus to bind to specific DNA sites and alter transcription.[21] Small molecules acting as JAK inhibitors are already accepted as pharmaceutical solutions for a variety of autoimmune diseases.[22] Among the cytokines the most prominent family is the group of interleukins from IL-1 to IL-36. They function in immune response in charge of the defense against extracellular infections and contributing to the pathogenesis of some autoimmune inflammatory diseases among them is the dry eye disease.[23–25] Cytokines, given their central role in the pathogenesis of dry eye, are attractive targets for treatment and a significant part of pharmacology research is based on their use as anti-inflammatory agents. Eleven Biotherapeutics, Inc., is using IL-17 family cytokine compositions as antagonists. The company is evaluating the proteins of interest in a mouse model for dry eye disease. The dry eye can be induced in mice by subcutaneous injection of scopolamine and afterward the mice are placed in controlled-environment chambers.[26] This method concerns binding proteins, including non-naturally occurring and recombinantly modified proteins that bind to an IL-17R and including proteins having a mutated IL-17 cytokine sequence (patent WO/2011/044563).[27] Additionally the company is studying methods of administering an IL-1 or IL-17 cytokine for treating dry eye disorder. The antagonists can be administered topically using an ophthalmic composition to the eye prior to sleep or nocturnal rest (patent WO/2011/163452).[28] As a result of this research Eleven Biotherapeutics, designed and engineered a novel and differentiated protein-based bio-therapeutic for dry eye disease, called EBI-005, which is the first IL-1 signaling inhibitor and is designed for topical ocular administration. The company commenced Phase 1b/2a clinical trials (ClinicalTrials.gov Identifier: NCT01748578) and presented the results at the 7th international conference

Table 1. Drugs and properties.

Patent	Company	Properties
WO/2011/044563	Eleven Biotherapeutics, Inc.	Recombinantly modified proteins that bind to an interleukin receptor IL-17R.
WO/2011/163452	Eleven Biotherapeutics, Inc.	Administration of an IL-1 or IL-17 cytokine.
WO/2010/047500	Benebiosis Co. Ltd.	Sialic acid and independent monosaccharide residues.
WO/2009/064983	Alcon Research, Ltd.	Protease, inhibiting peptide substrates, a borate salt and a galactomannan.
WO/2009/114512 WO/2010/039939 US20128158616	Incyte Corporation	Azetidine and cyclobutane derivatives as JAK inhibitors.
WO/2010/085684 WO/2011/017178 WO/2012/015972	Rigel Pharmaceuticals Inc.	2,4-Substituted pyrimidinediamine compounds as JAK inhibitors.
WO/2009/089036	Schepens Eye Research Institute	Physiological acceptable salt, poleaxes analogs with carpool, carpool/hydroxypropyl methycellulose (HPMC), carpool-methyl cellulose, a mucolytic agent, carboxymethylcellulose (CMC), hyaluronic acid, cyclodextrin, and petroleum.
WO/2010/124259 WO/2010/124262	Allostera Pharma Inc.	Allosteramers™ – short peptides that can effectively modulate TNF receptor activities.
WO/2009/048929	Lux Biosciences, Inc.	Voclosporin a calcineurin inhibitor.
US20107772433 US20118080682 US20118110562	University of Tennessee	Selective androgen receptor modulators (SARM) (a substituted acylanilide).
US20128158828	Gtx Inc.	Selective estrogen receptor modulators (SERMs)
WO/2009/082437	Ligand Pharmaceuticals Inc.	Selective androgen receptor modulators (SARMS).
WO/2010/092546	Consiglio Nazionale Delle Ricerche	Selective androgen receptor modulator (non-steroidal propionanilide and hydantoine structured compounds).
WO/2011/068786	Bridge Pharma, Inc.	R-salbutamol – increasing meibomian gland secretion.
US20097585877	Acadia Pharmaceuticals, Inc.	Selective androgen receptor modulators-aminophenyl derivatives.
WO/2008/153746	Aciex Inc.	Acular® (ketorolac tromethamine 0.5% ophthalmic solution) with a carboxymethyl cellulose (CMC)-based artificial tear.
WO/2009/039461 WO/2010/111353	Acadia Pharmaceuticals, Inc.	Serotonin receptor agents-Serotonin or 5-hydroxytryptamine (5-HT).
WO/2009/051439	AmorePacific Corporation	Modulator of the activity of calcium activated chloride channel.
WO/2011/106697	Schepens Eye Research Institute	Anti-lymphangiogenic agent.
WO/2010/047681 WO/2010/059894	Bridge Pharma, Inc.	Norketotifen, a cycloheptathiophene compound.
WO/2010/146132	Cellzome Limited.	ZAP-70 inhibitors, pyrimidine derivatives or sulfonamides and sulfamides or heterocyclylaminopyrimidines.
WO/2011/034207	Senju Pharmaceutical Co., Ltd.	A polypeptide having a partial sequence of lacritin
WO/2012/006083	University of Florida	siRNAs delivery to the cell cytoplasm by endocytosis
WO/2013/096226	Sylentis S.A.	SYL1001 siRNA to target Transient Receptor Potential Vanilloid 1 (TRPV1).

on tear film and ocular surface (TFOS) in 2013. The data demonstrated positive clinical results and the dual action EBI-005 was capable to treat both the signs and symptoms of dry eye disease. Based on these results, Eleven Biotherapeutics will advance EBI-005 into a new pivotal efficacy and safety, multi-center clinical trial. Additionally the Korean company Benebiosis Co., Ltd., and the Korean University of Sungkyunkwan are developing a compound comprised of sialic acid and independent monosaccharide residues (patent WO/2010/047500).[29] The compound suppresses the expression of the enzyme matrix metallopeptidase 9 (MMP-9), encoded by the MMP9 gene[30] and the inflammation causing cytokines IL-1β and tumor necrosis factor-alpha (TNF-α) on corneal epithelial cells. The compound suppresses as well the activity of the vascular endothelial growth factor VEGF-mediated VEGFR-2 on human retinal endothelial cells, possibly is involved in enhanced angiogenesis of the Sjögren's syndrome.[31] It is possible that this compound may also have a potential role in other ocular disorders with neovascularization. Another MMP-9 inhibitor is invented by Alcon Research, Ltd. The compound under development is a gelatin ophthalmic composition containing protease, inhibiting peptide substrates, a borate salt and a galactomannan (polysaccharides derived from natural gums) (patent WO/2009/064983).[32] The inhibiting peptide substrate is normally consisting of gelatin, alpha-2-macroglobulin, ovomacroglobulin, collagen and casein and after a topical administration is inhibiting the protease MMP-9. Nagelhout and colleagues suggested that elevated levels of MMP-9 and cytokines IL-1beta, IL-8, and TGF-beta1 were detected in the lacrimal gland and cornea of dry eye model rabbits.[33] Tear composition in dry eyes, or dysfunctional tear syndrome, may destabilize the tear film and cause ocular surface epithelial disease. Increased activity of matrix metalloproteinases (MMPs), especially MMP-9, plays a critical role in wound healing and inflammation and is primarily responsible for the pathologic alterations to the ocular surface that leads to a dysfunctional tear state.[34] Furthermore Incyte Corporation is developing azetidine and cyclobutane derivatives as JAK inhibitors for the treatment of dry eye (patents WO/2009/114512,[35] WO/2010/039939[36] and US20128158616).[37] The inhibitors under development are heteroaryl substituted pyrrolo 2,3-b pyridines and heteroaryl substituted pyrrolo 2,3-b pyrimidines (Fig. 1a) and (Fig. 1b) modulating the JAK kinase activity, able to bind and increase

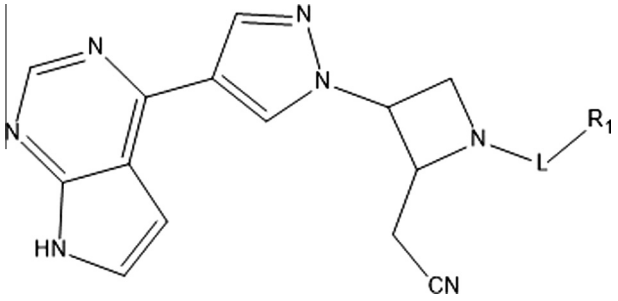

Figure 1a. Heteroaryl substituted pyrrolo 2,3-b pyridine.

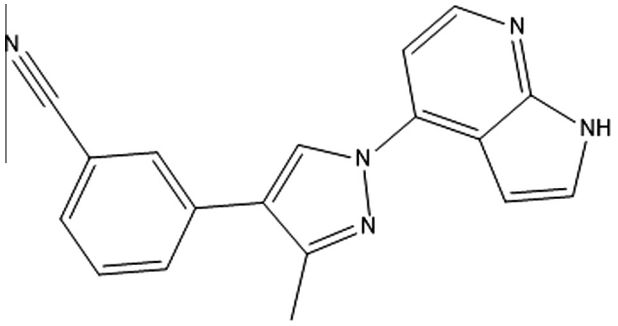

Figure 1b. Heteroaryl substituted pyrrolo 2,3-b pyrimidine.

or decrease the activity of one or more members of the JAK family of kinases by influencing IL-6 and STAT3 signaling.[38] The JAKs modulated could be of the type JAK1, JAK2, JAK3 or non-receptor tyrosine-protein kinase 2 (TYK2). The administration of the inhibitor may be systemic or topical. The dosage form is containing from about 5 to about 1000 mg, of the active ingredient. The compound was tested to animal preclinical models including the rabbit Concanavalin A (ConA) lacrimal gland model, the scopolamine mouse model (subcutaneous or transdermal) and the Botulinum mouse lacrimal gland model. Similarly Rigel Pharmaceuticals Inc. is developing 2,4-substituted pyrimidinediamine compounds (Fig. 1c) as JAK inhibitors for the treatment of dry eye disease (patents WO/2010/085684,[39] WO/2011/017178[40] and WO/2012/015972.[41] These compounds are potent and particularly selective inhibitors for cytokine signaling pathways containing JAK2 and JAK3 and it may be used to regulate or inhibit JAK kinase activity in the signaling cascades in which JAK kinases play a role and the biological responses are affected by such signaling cascades. It is considered that the compounds can be used to inhibit JAK kinase, either *in vitro* or *in vivo*, in a vast variety of cell types expressing the JAK kinase. They may also be used to regulate signal transduction cascades in which JAK kinases, particularly JAK3, play a role. The JAK-dependent signal transduction cascades are including the signaling cascades of cytokine receptors involved in the common gamma chain, such as, IL-4, IL-7, IL-5, IL-9, IL-15 and IL-21, or IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 receptor signaling cascades. According to the company the inhibitor could achieve beneficial clinical results by preventing the disease from occurring in a patient that is predisposed or does not yet display symptoms of the disease. The compound is relatively potent compared to other similar inhibitors and can be administered at low doses locally, thus minimizing systemic adverse effects. The administration of one or more of the class of 2,4-pyrimidinediamine compounds once daily to most twice a day, is effective to increase tear production volume as compared to untreated tear production volume and diminish the symptoms of dry eye syndrome. The tear production volume is increased within two to five days at about 25% to 50% over initial tear production. The increase in tear production upon administration results, in some instances, is comparable to normal tear production. In the same direction Schepens Eye Research Institute (patent WO/2009/089036)[42] is developing a composition which is inhibiting the function of the inflammatory cytokine IL-17 that acts as a potent mediator to inflammatory processes.[43,44] The composition was designed to treat IL-17-mediated ocular inflammatory disorders and comprises of a compound containing a physiologically acceptable salt, poleaxes analogs with carpool, carpool/hydroxypropyl methycellulose (HPMC), carpool-methyl cellulose, a mucolytic agent, carboxymethylcellulose (CMC), hyaluronic acid, cyclodextrin, and petroleum. The compound is locally administered to the patient eye, inhibiting the activity of the inflammatory IL-17 cytokine thus reducing the severity of the dry eye syndrome. The IL-17 receptor expression on ocular surfaces was analyzed using immunofluorescence microscopy. According to Schepens Institute, during the evaluation of the compound a significant decrease in the intensity of clinical signs of dry eye which was measured by corneal fluorescein staining (CFS) scoring, during the induction as well as the progression phases of the disease in the anti-IL-17 antibody-treated group was noticed. Finally Allostera Pharma Inc. is working on the discovery that short peptides based on amino acid sequences of extracellular or transmembrane regions of a tumor necrosis factor (TNF) receptor are effective in modulating TNF receptor activities (patent WO/2010/124259[45] and WO/2010/124262).[46] A tumor necrosis factor receptor, or death receptor, is a trimeric cytokine receptor that binds to TNF. The company is developing new and more effective therapeutic compositions called Allosteramers™ for treating inflammatory diseases associated with TNF or TNF receptor dysfunction. The Allosteramers™ are short peptides that can effectively modulate TNF receptor activities based on amino acid sequences of extracellular or transmembrane regions of a TNF receptor. The short peptide is composed of 5–25 amino acids and is derived from body's own proteins that can interact with the

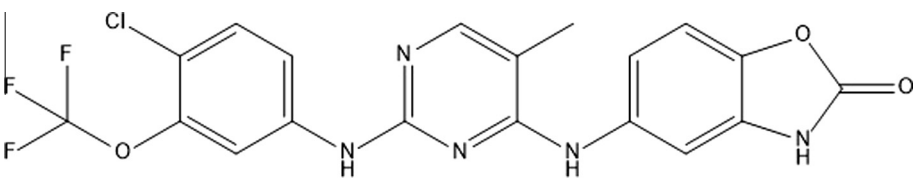

Figure 1c. 2,4-substituted pyrimidinediamine.

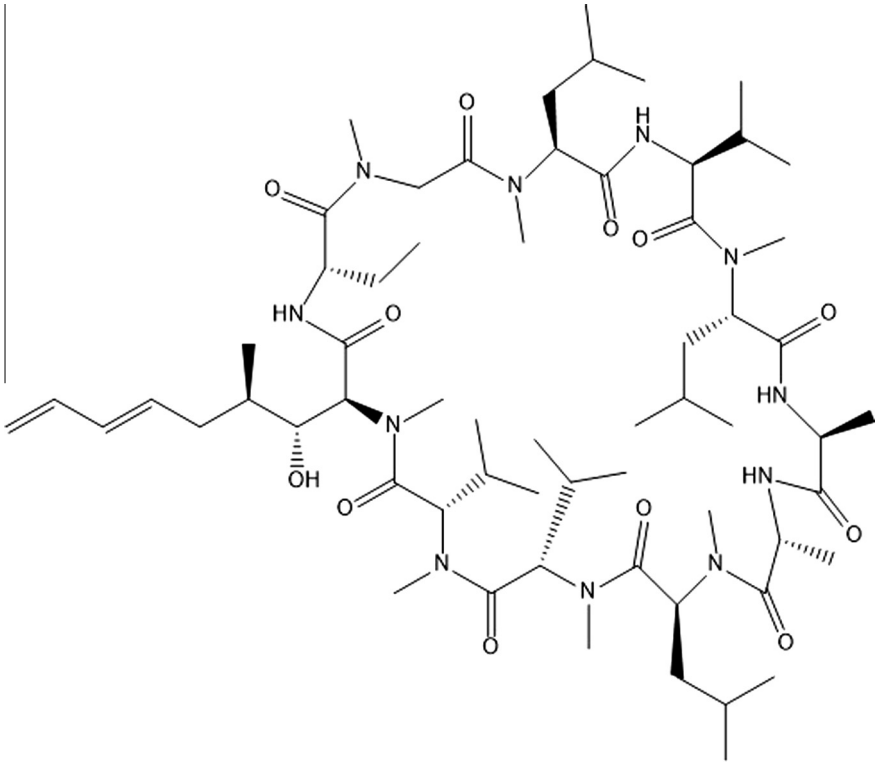

Figure 2a. Voclosporin.

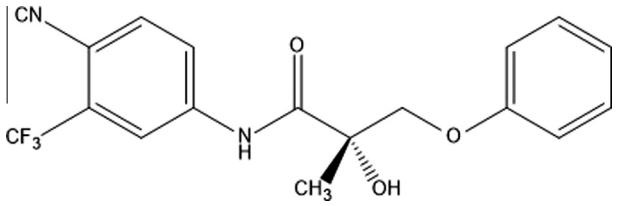

Figure 2b. Substituted acylanilide.

protein from which it was derived, or with its associated proteins, without competing with a natural ligand. Such effective modulators can be designed simply based on linear amino acid sequences of a biological target without first characterizing any secondary or tertiary structure of the target.[47]

Calcineurin and mTOR inhibitors

Tzu and colleagues demonstrated that adequate topical immunomodulation using topical calcineurin inhibitors may eliminate the need for steroids and favorably alter the long-term prognosis of patients with inflammatory ocular surface diseases.[48] Calcineurin inhibitors are potent immunosuppressants that reversibly inhibit T-cell proliferation and prevent the release of pro-inflammatory cytokines by blocking the activity of calcineurin, a ubiquitous enzyme that is found in cell cytoplasm. Calcineurin inhibitors can be highly effective in immune-mediated ophthalmic diseases such as uveitis, dry eye syndrome and inflammatory blepharitis.[49] Lux Biosciences, Inc. is developing an ophthalmic composition called Voclosporin (VCS, ISA247) (patent WO/2009/048929)[50] (Fig. 2a). Voclosporin is a novel calcineurin inhibitor, developed using a pharmacodynamic approach for use in solid organ transplantation and autoimmune disease. The compound is useful for inflammatory ocular surface diseases selected from the group consisting of dry eye syndrome, Sjögren's syndrome, uveitis, conjunctivitis, keratitis, keratoconjunctivitis, vernal keratoconjunctivitis, atopic keratoconjunctivitis, and autoimmune disorders of the ocular surface, including cicatrizing conjunctivitis, blepharitis, and scleritis.

Androgen and estrogen receptor inhibitors

Since some years there is growing evidence that sex hormones play a key role in the formation and course of dry eye disease and thus provide potentially promising approaches for therapy. There is growing evidence on the

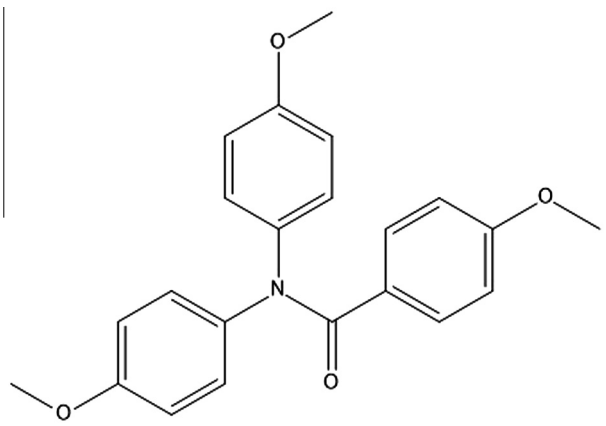

Figure 3a. 4-Methoxy-N,N-bis-(4-methoxyphenyl)-benzamide.

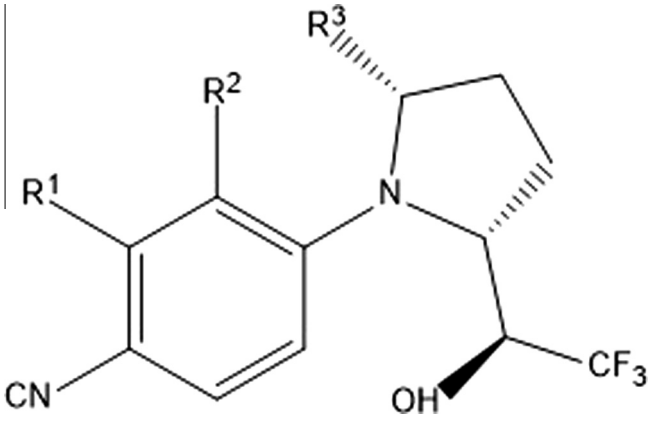

Figure 3b. 4-(2(jR)-(l(5)-hydroxyl-2,2J2-trifluoroethyl)pyrrolidinyl)-2-trifiuoromethyl-benzonitrile.

relationship between androgens, estrogens, and progesterones on one hand and the lacrimal and Meibomian glands on the other.[51] Most women who suffer from dry eye syndrome are post-menopausal however, not all post-menopausal women develop dry eye, suggesting that other factors, in addition to the decrease in ovarian hormones, are necessary for the development of the disease. Mostafa and colleagues demonstrated after *in vivo* experiments that lymphocytic infiltration preceded lacrimal gland apoptosis after ovariectomy and removal of ovarian sex hormones accelerated these effects in the genetically predisposed animals. These effects were more severe and persistent compared to control animals. In addition, sex hormone replacement at physiological levels prevented these symptoms. Still we are not clear about the mechanisms by which decreased levels of sex hormones caused lymphocytic infiltration and apoptosis and the interaction of lack of sex hormones with the genetic elements.[52] In this sense the University of Tennessee is developing a series of selective androgen receptor modulators (SARM) (a substituted acylanilide) (Fig. 2b) as potential agents for treatment of dry eye disease (patents US20107772433,[53] US20118080682,[54] US20118110562).[55] The compounds are non-steroidal ligands for the androgen receptor and exhibit androgenic and/or anabolic activity. They are partial agonists or partial antagonists in a tissue selective manner, which allows for tissue-selective androgenic and/or anabolic effects. These agents may be active alone or in combination with progestins or estrogens, or other agents. Furthermore Gtx Inc. is working with a novel class of selective estrogen receptor modulators (SERMs) for the treatment of dry eye syndrome containing 4-methoxy-N,N-bis-(4-methoxyphenyl)-benzamide (patent US20128158828)[56] (Fig. 3a). The nuclear hormone receptor superfamily of ligand activated transcription factors is present in various tissues, and responsible for a multitude of effects in these tissues. The nuclear receptor (NR) superfamily presently comprises approximately 48 different proteins, most of which are believed to function as ligand activated transcription factors, exerting widely different biological responses by regulating gene expression. Members of this family include receptors for endogenous small, lipophilic molecules, such as steroid hormones, retinoids, vitamin D and thyroid hormone. Members of the steroid nuclear receptor sub-family exhibit significant homology to each other and possess closely related DNA and ligand binding domains. Given the close similarity in ligand binding domains of the steroid nuclear receptors, it is not surprising that many naturally occurring and synthetic molecules possess the ability to modulate the activity of more than one steroid nuclear receptor. Similarly Ligand Pharmaceuticals Inc. is developing a series of selective androgen receptor modulators (SARMS) (patent WO/2009/082437)[57] (Fig. 3b). The compound 4-(2(jR)-(l(5)-hydroxyl-2,2J2-trifluoroethyl)pyrrolidinyl)-2-trifiuoromethyl-benzonitrile is binding to androgen receptors and modulate the activity of androgen receptors and could be agonists, antagonists, partial antagonists or receptor modulators. Equally the Institute Consiglio Nazionale Delle Ricerche is developing androgen receptor modulating nuclear hormone receptor binding compounds useful in the treatment of nuclear receptor, especially the steroid receptor, and in particular the androgen receptor (AR). The agents are novel non-steroidal propionanilide with hydantoine (Fig. 4a) and bicalutamide (Fig. 4b) structured compounds having utility as tissue-selective androgen receptor modulators (SARM) (patent WO/2010/092546[58] and WO/2010/116342).[59] These agents are a new class of androgen receptor compounds demonstrating anti-androgenic and androgenic activity of a non-steroidal ligand of the androgen receptor. Similarly Bridge Pharma, Inc. is developing R-salbutamol (Fig. 4c) which is increasing meibomian gland secretion (patent WO/2011/068786).[60] R-salbutamol is administered by instillation to the eye or into the conjunctival sac. may also be instilled into the nose via nose drops, nasal sprays, or nasal insufflation of dry powder containing R-salbutamol. Alternatively, R-salbutamol may be administered systemically, such as by the oral, intravenous or transdermal routes or by inhalation. Upon systemic administration, the active compound will reach the ocular tissues after systemic absorption and distribution. Although a beta-2 agonist, such as R-salbutamol, may have ocular therapeutic activity after systemic administration, it is a preferred method to administer drug formulations topically to the eye, for example as solutions, gels, ointments, emulsions, sprays, washes or as topical liposome formulations or as implantable devices that are releasing the beta-2 agonist in a controlled manner.

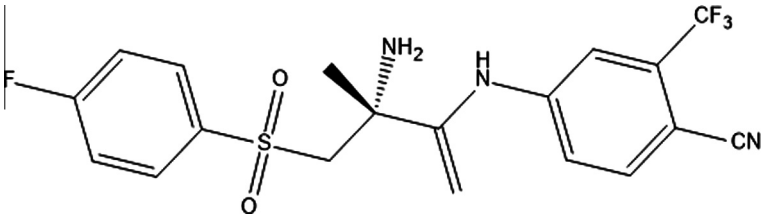

Figure 4a. Propionanilide.

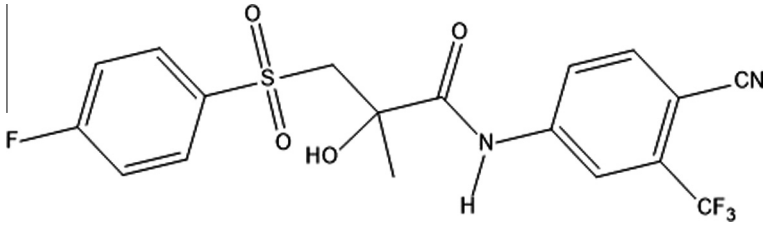

Figure 4b. Bicalutamide.

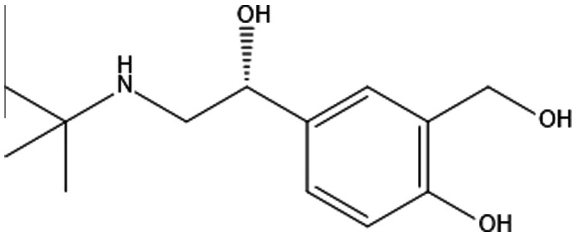

Figure 4c. R-salbutamol.

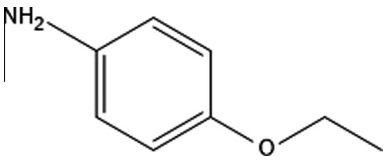

Figure 4d. Aminophenyl.

R-salbutamol may also be administered to the eye(s) via devices, such as for example pump-catheter systems, continuous ocular release devices or via contact lenses or ocular mini-tablets or gel-forming ocular mini-tablets that contain the active medication. Furthermore, Acadia Pharmaceuticals, Inc. is developing a novel class of aminophenyl (Amino phenyl) (Fig. 4d) derivatives as selective androgen receptor modulators (patent US20097585877).[61] The method of treating xerophthalmia comprises of administering this compound or a prodrug, stereoisomer, or pharmaceutically acceptable salt.

Non-steroidal anti-inflammatory drugs

The dry eye syndrome tends to be accompanied by ocular surface inflammation and the use of anti-inflammatory agents is beneficial for the patients. As no diagnostic tests are available to assess ocular surface inflammation severity, the right timing to launch an anti-inflammatory agent is difficult to determine. Patients with mild intermittent symptoms which can be alleviated with ophthalmic lubricants do not typically require anti-inflammatory therapy and this type of therapy has to be considered as last resource. Anti-inflammatory treatment of dry eye syndrome may include short-term corticosteroids, Cyclosporine A emulsion, oral tetracycline therapy, oral omega-3 fatty acid supplements, and autologous serum eye drops. Alternatives to corticosteroids should be considered when long-term treatment is required for an ocular surface condition as severe side effects were noticed after prolonged use of corticosteroids. A typical solution is the use of Non-steroidal anti-inflammatory drugs (NSAIDs). In this sense the company Aciex Inc. is developing ophthalmic formulations comprised of one or more components of a tear substitute and a low dose amount of non-steroidal anti-inflammatory drugs (NSAID) effective to increase tear film break up time (TFBUT) and the ocular protection index (OPI), as well as decrease ocular discomfort, treating and preventing signs and symptoms associated with dry eye disease (patent WO/2008/153746).[62] The compound under development is comprised of Acular® (ketorolac tromethamine (Fig. 5a) 0.5% ophthalmic solution) with a carboxymethyl cellulose (Fig. 5b) (CMC)-based artificial tear.

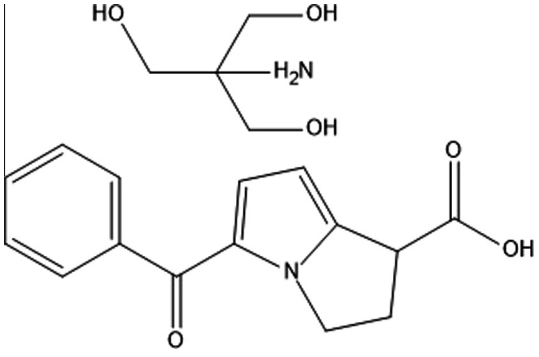

Figure 5a. Ketorolac tromethamine.

Serotonin receptor inhibitors

Acadia Pharmaceuticals, Inc. is developing N-substituted piperidine derivatives (Fig. 6a) as serotonin receptor agents (patent WO/2009/039461[63] and WO/2010/111353).[64] Serotonin or 5-hydroxytryptamine (5-HT) plays a significant role in the functioning of the mammalian body. In the central nervous system, 5 -HT is an important neurotransmitter and neuromodulator that is implicated in such diverse behavior and responses as sleeping, eating, locomotion, perceiving pain, learning and memory, controlling body temperature and blood pressure. In the spinal column, serotonin plays an important role in the control systems of the afferent peripheral nociceptors.[65] Peripheral functions in the cardiovascular, hematological and gastrointestinal systems have also been ascribed to 5-HT. 5-HT has been found to mediate a variety of contractile, secretory, and electrophysiological effects including vascular and nonvascular smooth muscle contraction, and platelet aggregation.[66] The 5-HT2 A receptor subtype (also referred to as subclass) is widely yet discretely expressed in the human brain.

Calcium activated chloride channel modulators

AmorePacific Corporation is developing (patent WO/2009/051439[67] a method of identifying agents which modulate the activity of calcium activated chloride channel.

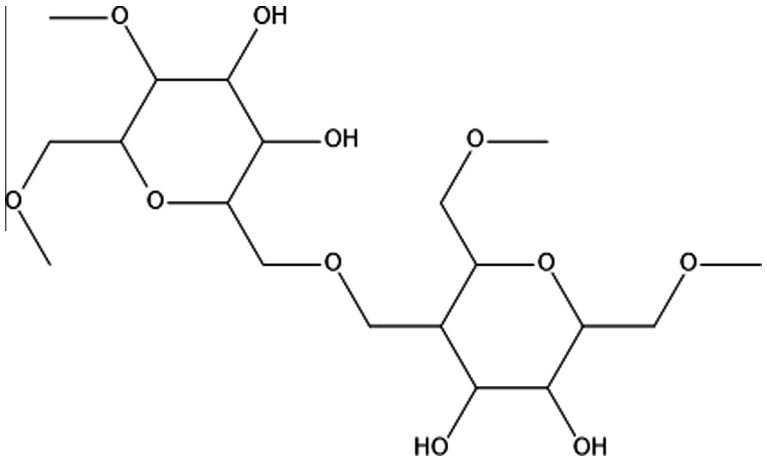

Figure 5b. Methylcellulose.

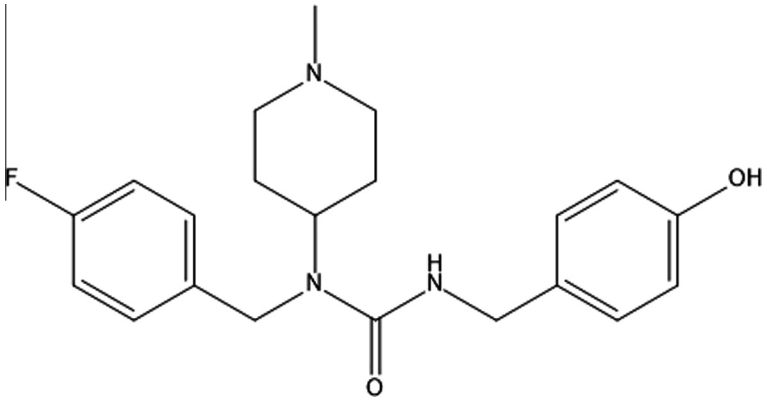

Figure 6a. N-substituted piperidine.

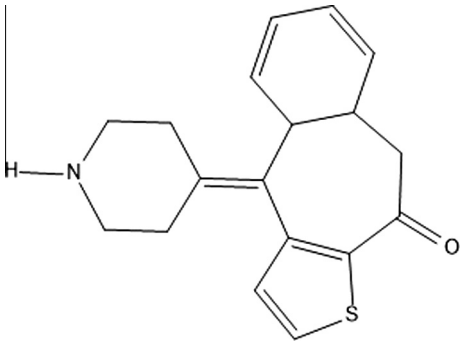

Figure 6b. Norketifen.

The method consists of identifying an agent which modulates an activity of a protein, wherein the protein is represented by the amino acid sequence or has at least 90% amino acid sequence identity and transports chloride ions across a cell membrane, comprising: (a) exposing cells which express the protein to the agent; and (b) measuring degree of chloride ion transport in the exposed cells, wherein a change in the degree of chloride ion transport is compared to control cells, which express the protein but not exposed to the agent, is indicative of an agent capable of modulating an activity of the protein. The agents identified by this method can be used for the prevention or treatment of xerophthalmia caused by the dysfunction of calcium activated chloride channel.

Anti-lymphangiogenic agent

There is growing evidence of corneal lymphangiogenesis in dry eye disease. Sunali et al. demonstrated that there is evidence of selective growth of lymphatic (but not blood) vessels in dry eye, providing new insights into the pathogenesis of the disease. These findings suggest that these newly formed corneal lymphatic vessels may serve as potential conduits for migration of corneal APCs to lymphoid tissues, where they generate auto-reactive TH17 and TH1 cells in dry eye. This study not only provides a link between ocular surface inflammation and the generation of T-cell-mediated immunity in the lymphoid compartment but also offers an example of how lymphangiogenesis and hemangiogenesis can be naturally dissociated in a pathologic state. The severing of the ''eye-lymphatic axis'' in other immune-mediated conditions, such as transplant rejection, has been shown to hold promise as a strategy for suppressing alloimmunity without inhibiting needed innate host defense mechanisms. Similarly, a strategy targeting prolymphangiogenic factors, such as VEGF-C and VEGF-D, may prove effective in improving dry eye.[68] Schepens Eye Research Institute developed a

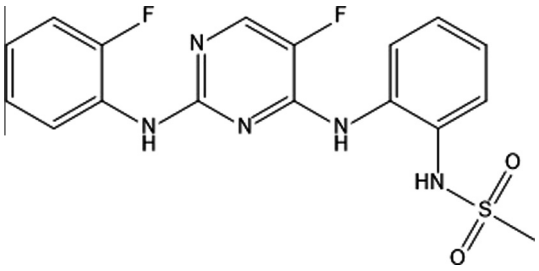

Figure 7a. Pyrimidine derivative.

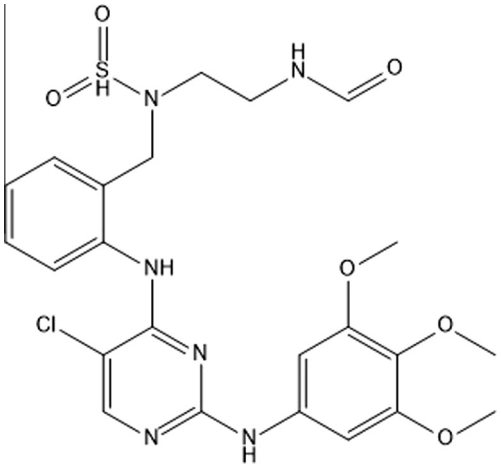

Figure 7b. Sulfonamides and sulfamides.

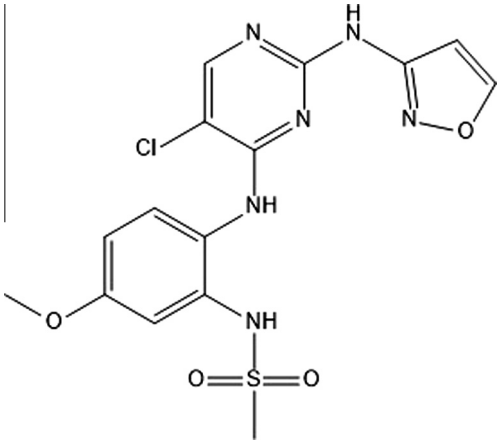

Figure 7c. Heterocyclylaminopyrimidines.

novel method for the treatment of dry eye disease in humans comprising administration of an anti-lymphangiogenic agent to the human patient (patent WO/2011/106697).[69] Preferably, the amount of the anti-lymphangiogenic agent employed is effective to inhibit the binding of VEGF-C and/or VEGF-D ligand to VEGFR-3 or the stimulatory effect of VEGF-C and/or VEGF-D on VEGFR-3. Targeting prolymphangiogenic growth factors or their receptors could inhibit the trafficking of antigen-presenting cells to the draining lymph nodes and hence prove to be a potential therapeutic target for dry eye disease.[70]

Cycloheptathiophene

Bridge Pharma, Inc. is developing Norketotifen a cycloheptathiophene compound for the treatment of xerophthalmia (patent WO/2010/047681[71] and WO/2010/059894)[72] (Fig. 6b). Norketotifen, is a de-methylated metabolite of ketotifen, which is a potent antimalarial drug.[73] Ketotifen blocks histamine H1 receptors, stabilizes mast cells, and prevents eosinophil accumulation. While ketotifen was found not to increase tear flow after topical administration to the eye, norketotifen was found to increase tear flow after topical administration to the eyes of laboratory animals. Topical ocular formulations of norketotifen preferably contain norketotifen in concentrations between 0.01% and 0.50% and preferred osmolality between 150 and 450 mOsm. The ocular formulation could contain combinations of norketotifen and cyclosporine in concentrations between 0.01% and 0.1%. The drug could be administered as ocular solution, ocular hydrophilic ointment and gel, ocular hydrophobic ointments ocular emulsion and ocular liposome formulation, all containing norketotifen. The formulation could allow for once-daily ocular administration and for repeated ocular administrations from two to five times daily to patients.

ZAP-70 inhibitors

The company Cellzome Limited is developing a series of ZAP-70 inhibitors. These inhibitors could be pyrimidine derivatives (patent WO/2010/142766)[74] (Fig. 7a), sulfonamides and sulfamides (patent WO/2010/146132)[75] (Fig. 7b) or heterocyclylaminopyrimidines WO/2010/146133[76] (Fig. 7c). ZAP-70 is a protein tyrosine kinase and its lack of expression is associated with an immunodeficiency. ZAP-70 is situated at the crossroad of several signaling pathways that control T lymphocyte development and function. Recent studies are associating abnormal ZAP-70 expression with pathological conditions. Mouse models also revealed that partial defects in ZAP-70 activity can be associated with autoimmunity and that ZAP-70 is involved between immunity and tolerance.[77] Several reports provided genetic evidence that ZAP-70 plays an important role in T cell activation. Mutations in ZAP-70 have been shown to be responsible for an autosomal recessive form of severe combined immunodeficiency syndrome (SCID) in humans.[78] This SCID syndrome is characterized by the absence of peripheral CD8+ T cells and by the presence of circulating CD4+ T cells that do not respond to TCR-mediated stimuli *in vitro*. Targeted disruption of the ZAP-70 gene in mice leads to defects in thymic development and T cell activation.[79] Inhibitors of ZAP-70 may therefore represent drugs useful for the treatment of diseases of the immune system such as dry eye disease.

Partial peptide of lacritin

Senju Pharmaceutical Co., Ltd. is developing a polypeptide having a partial sequence of lacritin, which promotes adhesion between a cell and extracellular matrix, claimed to be superior in stability in aqueous solution (patent WO/2011/034207).[80] The polypeptide is containing an amino acid sequence which is a particular partial sequence of lacritin and characterized by pyro-modification of N-terminal glutamine. Since the polypeptide promotes adhesion between a cell

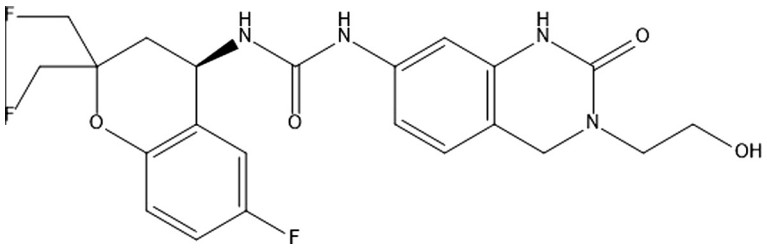

Figure 8. TRPVl antagonists.

and extracellular matrix, and is stable in aqueous solution, it can provide good preservation stability. Eye drops containing lacritin are likely to be useful in the treatment of ocular diseases such as dry eye syndrome, Sjögren's syndrome, and corneal epithelial wounds. The polypeptide is particularly useful for the treatment of a corneal epithelial disorder associated with these diseases. In addition, it has a promoting action of tear fluid secretion from lacrimal gland acinar cells. Only a handful of tear proteins appear to be selectively down-regulated in dry eye. Lacritin and lipocalin-1 are two tear proteins selectively deficient in dry eye. Both proteins influence ocular surface health. Lacritin is a pro-secretory mitogen that promotes basal tearing when applied topically. Levels of active monomeric lacritin are negatively regulated by tear tissue transglutaminase, whose expression is elevated in dry eye with ocular surface inflammation. Lipocalin-1 is the master lipid sponge of the ocular surface, without which residual lipids could interfere with epithelial wetting. It also is a carrier for vitamins and steroid hormones, and is a key endonuclease. Accumulation of DNA in tears is thought to be pro-inflammatory. Functions of these and other tear proteins may be influenced by protein–protein interactions. Here we discuss new advances in lacritin biology and provide an overview on lipocalin-1, and newly identified members of the tear proteome.[81]

RNA interference

RNAi consists of highly efficient selective and specific inhibition of gene expression. RNAi is mediated by small fragments of double-stranded RNA, consisting of 19–23 nucleotides, which promote degradation of messenger RNA (mRNA), thus inhibiting synthesis of the proteins for which they code and which are responsible for the pathology. RNAi therapy has great potential as this mechanism is used naturally by cells to regulate gene expression in a way that is both non-toxic and highly effective. The University of Florida is developing compositions and methods for the delivery of siRNAs to the cell cytoplasm by endocytosis (patent WO/2012/006083).[82] The compositions comprise molecular conjugates between a receptor ligand, most suitably a small molecule ligand and an siRNA that when administered to a patient, can modulate or relieve symptoms of dry eye disease as well as inhibiting or reducing such cellular processes as apoptosis of glandular acinar cells that trigger or sustain Sjögren's Syndrome. The siRNA moiety can reduce caspase-3 levels when delivered to a salivary or lacrimal cell. The method for delivery of a molecular conjugate to a cell, comprises the steps of contacting with a cell with a molecular conjugate comprising a ligand characterized as having affinity for a surface receptor of the cell, and an siRNA moiety linked to the ligand; and maintaining the cell, or population of said cells, under conditions whereby the ligand specifically binds to a surface receptor of the cell or cells, whereupon the molecular conjugate enters the cell or cells by endocytosis, thereby delivering the siRNA moiety to the cytoplasm of the cell or cells. Similarly the Spanish company Sylentis S.A. is developing SYL1001 a new chemical specifically designed to target the Transient Receptor Potential Vanilloid 1 (TRPV1) (patent WO/2013/096226).[83] (Fig. 8). This nociceptor is involved in pain stimuli transmission. The company started on November 2012 Phase I/II clinical trials for its compound named SYL1001 (ClinicalTrials.gov Identifier: NCT01776658) with ocular topical administration of SYL1001 for 10 consecutive days on 60 patients were performed. SYL1001 DP is administered in the form of eye drops for treating or preventing eye discomfort associated with dry eye and with corneal injuries, with a view to minimizing pain and improving patients' quality of life. SYL1001 eye drops is a sterile, isotonic saline solution presented in single-dose vials, eliminating the need for antimicrobial preservatives.[84] According to results published for Phase I clinical trials (ClinicalTrials.gov Identifier: NCT01438281) local tolerance was excellent with no serious adverse events reported and no modifications of the ocular surface or iris detected. The analytical results at final examination did not show differences from those observed during selection. Pharmacokinetic results indicated that no levels of siRNAs were detected above LOOQ (40 ng/mL) in any of the subjects. These results were expected as SYL1001 which is a non-modified siRNA, with a low stability in plasma (half-life is less than 5 min) (ARVO 2012).[85]

Concluding remarks

Current topical treatments for dry eye patients include the use of lubricants and anti-inflammatory drugs. However, lubricants only suppress negative symptoms temporarily, and chronic use of topical steroids is related to severe ocular side effects such as cataract and glaucoma. Further research is necessary toward the action mechanisms of new mucin and tear secretagogues compounds possibly possessing anti-inflammatory properties. Additionally further research is needed on understanding the immunomodulatory and inflammatory mechanisms of the conjunctiva.

The deeper understanding of the cellular mechanisms implicated in dry eye is necessary not only to improve symptoms but also to restore the homeostasis of the ocular surface. The pursuit of effective dry eye treatment strategies is delayed by the absence of an accepted set of definitive criteria for evaluating disease severity. There is a lack of an objective test for the diagnosis of the severity of the disease, because symptoms' evaluation is insufficient as a

measurement factor. There are several proposals for a novel objective testing such as molecular markers[86,87] or evaluation of the tear film osmolarity[88,89] but for the moment none of them advanced after the pre-clinical test.

Conflict of Interest

The authors declared that there is no conflict of interest.

Acknowledgments

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