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PTERYGIUM PROTEIN INVOLVEMENT: S100A8 AND S100A9 INHIBITORS

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ABSTRACT: The term "surfer's eye" refers to a pinkish, roughly triangular growth of conjunctival tissue that covers the eye's cornea, called a pterygium. The cornea next to the nasal cavity is often where the illness first appears. Although the development may occur gradually, it rarely gets to the point where it blocks the pupil and impairs vision. Pterygium is a severe condition. The calprotectin, mainly the S100A9/8 proteins system, is involved. This computational study aims to develop a calprotectin inhibitor to create an effective therapeutic alternative to this discussed condition. A computation study involved the calprotectin heteromer and monomer, which was docked with Ca+ and a series of organic molecules. Results show that calprotectin can form stable complexes (best docking energy of 162.788kcal/mol) with organic molecules. In conclusion, an effective calprotectin molecule can be further developed.

KEYWORDS: pterygium, molecular descriptors, docking, calprotectin.

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1. INTRODUCTION

A pterygium of the eye, commonly known as a surfer's eye, is a pinkish, roughly triangular growth of conjunctival tissue that extends over the eye's cornea. The condition usually originates in the cornea adjacent to the nasal area. The development may progress gradually, but it seldom reaches a size that completely obstructs the pupil and causes visual impairment. Frequently, both eyes are affected. The cause is ambiguous. There seems to be a partial correlation between prolonged exposure to ultraviolet (UV) radiation and dust. Genetic factors are also believed to play a role. The growth is non-malignant. Additional disorders that may exhibit comparable symptoms include pinguecula, malignancy, or Terrien's marginal corneal degeneration. Precautionary measures may consist of donning sunglasses and a hat when exposed to high levels of sunlight. An ocular lubricant can alleviate symptoms for those afflicted with the illness. Surgical extraction is often advised solely in cases when visual acuity is compromised. After undergoing surgery, there is a possibility of pterygium recurrence in approximately 50% of cases [1,2,3]. Pterygium in the conjunctiva is marked by the deterioration of collagen (actinic elastosis) and the growth of fibrovascular tissue. The pterygium consists of a head and a body, joined by a neck. Occasionally, a line of iron accumulation can be observed next to the head of the pterygium, referred to as Stocker's line. The positioning of the line can provide insight into the growth trend. The preponderance of pterygia on the nasal side may be attributed to peripheral light focusing. This occurs when sunlight passes horizontally through the cornea, undergoes refraction, and converges on the limbic area. The sunlight enters the eye from the side and travels without any obstacles, eventually centering on the inner edge of the cornea. On the other (medial) side, the nose's shadow lessens the strength of sunlight directed towards the outer side of the eye. Research reveals that there may be a genetic susceptibility to a condition related to the expression of vimentin. Vimentin is a protein involved in the movement of cells during the development of the cornea. Providing evidence for this statement is the occurrence of congenital pterygium when pterygium is observed in newborns. These cells also show elevated P53 expression, presumably caused by a deficiency in the tumor suppressor gene. These signs suggest that the pterygium originates from the limbal epithelium, giving the impression of a migrating limbus [4,5,6,7]. The development of pterygium is mainly linked to exposure to UV light; however, this connection is still debatable. The precise process of pterygium has yet to be fully elucidated. Possible causes of the condition include inflammation, viral infection, oxidative stress, DNA methylation, inflammatory mediators, extracellular matrix modulators, apoptotic and oncogenic proteins, loss of heterozygosity, microsatellite instability, lymphangiogenesis, epithelial-mesenchymal cell transition, and alterations in cholesterol metabolism. Multiple research has sought to elucidate the molecular mechanisms that govern the growth and proliferation of pterygium. Gaining insight into its molecular foundation offers novel opportunities for preventing and treating this condition. A thorough search of MedLine, EMBASE, and LILACS databases used the keywords

pterygium, epidemiology, pathogenesis, biomarkers, and review. This article provides an overview of the occurrence, symptoms, and current research on the biological substances that have a role in developing pterygium [8,9,10]. S100 calcium-binding protein A8 (S100A8) is a human protein produced from the S100A8 gene. Calgranulin A is an alternative name for it. Calprotectin is a heterodimer formed by the proteins S100A8 and S100A9. This gene encodes a protein that belongs to the S100 family of proteins, which have 2 EF-hand calcium-binding motifs. S100 proteins are distributed inside the cytoplasm and nucleus of various cells and govern multiple biological processes, including advancing the cell cycle and differentiation. The S100 genes comprise a minimum of 13 components in a cluster on chromosome 1q21. This protein can act as a cytokine and hinder the activity of casein kinase. The modified expression of this protein is linked to cystic fibrosis and post-COVID-19 disorders [11,12,13,14]. The human S100A9 gene produces S100 calcium-binding protein A9 (S100A9), calgranulin B, or migration inhibitory factor-related protein 14 (MRP14). Representing the S100 protein family, S100A9 possesses two EF-hand calcium-binding domains. The cytoplasm and nucleus of different cell types contain S100 proteins, which regulate multiple biological processes such as cell cycle progression and differentiation. The S100 genes are a group of at least 13 genes located on chromosome 1q21. It is believed that this protein aids in suppressing casein kinase activity. Complexes comprise MRP-14 and MRP-8 (S100A8), two more S100 family proteins that are calcium-regulated. To control the activity of myeloid cells, MRP8 and MRP14 bind to the receptor for advanced glycation end products and Toll-like receptor 4 (TLR4). Neutrophils' mitochondrial balance is disturbed by intracellular S100A9[15,16]. Consequently, neutrophils that do not have S100A9 create increased amounts of mitochondrial superoxide and experience higher levels of suicidal NETosis when exposed to bacterial infections. Moreover, mice lacking S100A9 are shielded from widespread Staphylococcus aureus infections, exhibiting reduced bacterial loads in the heart. This indicates that S100A9 may have a specific role in the functioning of this organ [17,18,19]. The calprotectin heteromer is represented in Figure 1.

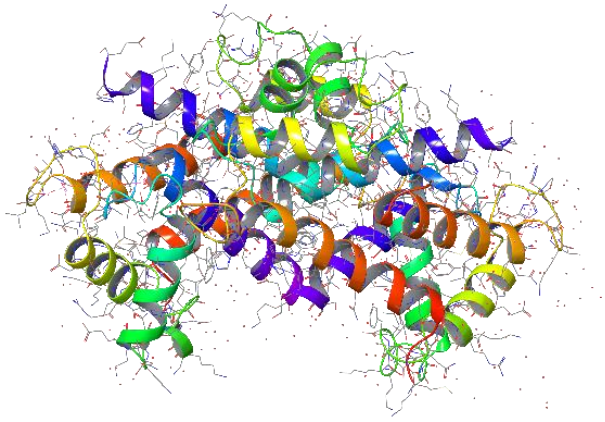


Figure 1. Calprotectin heteromer is represented as ribbons together with water molecules.

2. MATERIALS AND METHODS

S100A8 and S100A9 structures have been computed. The PDB structure was retrieved from the PDB data bank server. Charges have been corrected; the energy was minimized using the AMBER99 force field [20,21]. A protein-protein docking methodology was used to generate and search for the most energetically favorable conformation (kcal/mol). By obtaining these complexes, a virtual screening was performed to find molecules that bind to the S100A8 and S100A9 heterodimer to inhibit it. The molecules used for virtual screening were retrieved from the Chembl database [22]. For the virtual screening, the heterodimer PDb structure 1xk4 (calprotectin) was used [23]. Also, the monomer's ability to bind Ca+ was studied by docking the Calprotectin monomer with a Ca+. Docking and virtual screening studies were performed by using AutoDock Vina software [24,25].

3. RESULTS AND DISCUSSION

In Figure 2. Calprotectin monomer is represented as ribbons.

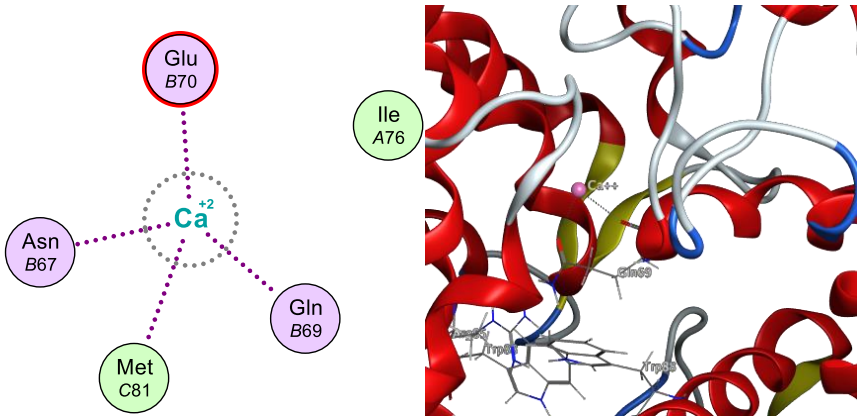


Figure 2. Calprotectin in complex with Ca+. a. Metal bonds are formed with Met C81, Asn B67, Glu B70 and GlnB69; b. Ca+(pink) in the binding pocket of calprotectin.

Ca+ has total energy (E total) of -6.9025, an electron pair energy (Epair) of 19.4615, and an electron energy (Eelec) of -0.425867 at a cut-off of r > 4.5 and -25.9381 at a cut off of r < 4.5. Two electrostatic interactions are of interest (see table below) Table 1.

Table 1. Binding energies of the complex Calprotectin -Ca+(kcal/mol).

Ligand Ca+	Donor	Energy (Electrostatic)	Length
1		-12.8307	2.54339
2		-13.1074	2.51641

Regarding the receptor (Calprotectin), the total complex energy is -9.853. The External Ligand interactions-6.903; Protein-Ligand interactions-6.903; Steric energy 19.461; Hydrogen bond s energy 0.0; Electrostatic (short range) -25.938; Electrostatic (long range) -0.426; Cofactor – Ligand energy 0.602; Water - Ligand interactions0.988; Torsional energy(sp2-sp2) 0.636. All energies are computed in kcal/mol.

The results of the virtual screening energy (using a random database, 176 molecules were selected) are represented below (Figure 3).

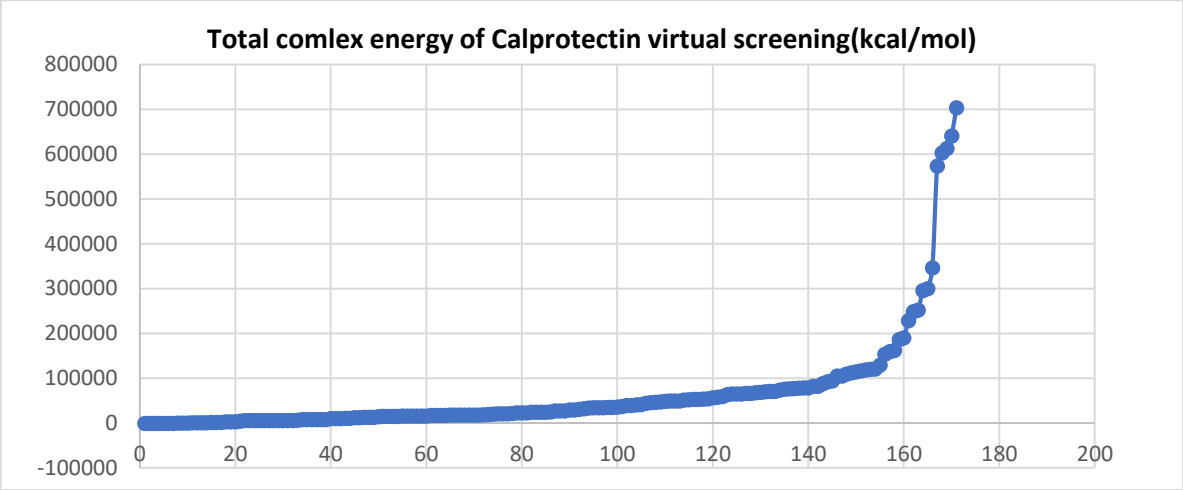
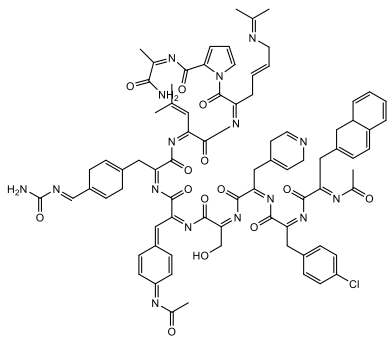
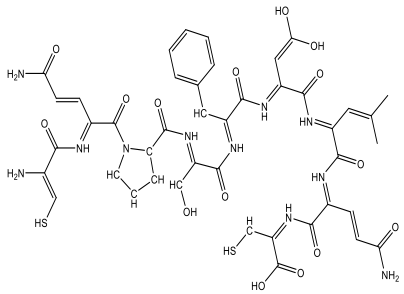
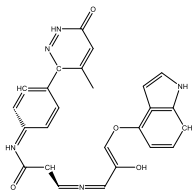


Figure 3. Docking energies of Calprotectin complexes resulted after virtual screening

The first three compounds with the best docking energy(kcal/mol) areas follows: -162.788-CHEMBL438018(1), -160.699-CHEMBL265653(2) -121.945CHEMBL1203155(3). Their structure is represented in the table below (Table 2).

Table 2: Molecular structures of the best three energetically favorable ligands resulted after the virtual screening.

1	2	3
		

As results show, Ca+ binds effectively to the calprotectin monomer with a complete energy of -6.9025. The binding of Ca+ includes the formation of four electrostatic interactions between Ca+ and the Aa in the binding pocket (Figure 2). Computationally, two are essential with relatively lower energies, as seen in Table 1.

The virtual screening results show that the calprotectin heteromer can bind relatively big molecules with favorably complex energies. As represented in Figure 3, about 5% of the 176 compounds have favorable energies when docked against calprotectin. Table 2 illustrates the structures of the best compounds (in terms of complex docking energies). S100A8 and S100A9 (MRP8 and MRP14, respectively) are Ca2+ binding proteins that are members of the S100 family, as stated in the

literature. Due to their stability, they rarely occur as homodimers, although they frequently exist in heterodimer form. As a Ca2+ sensor, S100A8/A9 is constitutively expressed in neutrophils and monocytes and is involved in the metabolism of arachidonic acid and cytoskeleton rearrangement. S100A8/A9 is actively produced during inflammation and plays a crucial role in regulating the inflammatory response by promoting leukocyte recruitment and triggering the release of cytokines. S100A8/A9 is a promising biomarker for follow-up and diagnosis and a therapy response predictor for disorders linked to inflammation. The heterodimer has potential as a therapeutic target since it can alleviate pathological conditions in murine models when antibodies or small-molecule inhibitors block S100A8/A9 function [26,27]. Moreover, additional ocular diseases are associated with calprotectin (S100A9). Infiltrating granulocytes and monocytes express S100A9, a pro-inflammatory protein globally. S100A9's function in endotoxin (LPS)-induced keratitis and uveitis (EIU) in Wistar rats was identified. Between 18 and 36 hours, the anti-S100A9 antibody somewhat reduced the LPS group's clinical scores, protein, and cell counts in the aqueous humor. The iris-ciliary body (ICB) and cornea exhibited S100A9-positive cells between 24 and 48 hours. From 18 to 48 hours after LPS injection, S100A9, and activated caspase-3, linked to apoptosis, were co-expressed in ICB. ED2-positive cells in the ICB did not express S100A9. Following an injection of lipopolysaccharide (LPS), dexamethasone (DEX) decreased S100A9 mRNA and protein levels in ICB but raised them in circulating blood leukocytes. After LPS injection, leukocytes (43.5%) and ICB (68.5%) showed decreased levels of S100A9 mRNA due to the inhibitor of I-kappaB phosphorylation, BAY 11-7085. S100A9-positive granulocytes and monocytes may influence the late phase of EIU and keratitis. DEX may prevent these cells from migrating from the blood into extravascular tissues, and S100A9 expression may be mediated by the nuclear factor (NF)-kappaB pathway. When inflammatory cells are eliminated in the latter stages of EIU, S100A9 may be involved [28,29]. In addition, calprotectin—a heterodimer of the EF-hand calcium-binding proteins S100A8 and S100A9—is a crucial component of the innate immune response. Toll-like receptor 4 (TLR4), cluster of differentiation 33 (CD33), and receptor for advanced glycation end products (RAGE) are only a few of the pattern recognition cell surface receptors that calprotectin (CP) binds to. The receptors use the NF-kB pathway to trigger kinase signaling cascades that incite inflammation. Certain chronic inflammatory disorders are linked to a positive feedback loop in which receptor activation by CP causes overexpression of both receptor and ligand. As a result, some chronic inflammatory diseases have been thought to be susceptible to suppression by CP and its two constituent homodimers. Over three decades, numerous inhibitors of CP and other S100 proteins have been studied; nevertheless, not a single candidate has made substantial progress toward clinical trials[30,31]. Overall, this study shows that developing a calprotectin inhibitor is crucial in treating pterygium and other ophthalmic diseases. Also, in creating such inhibitors, the ADME properties of the hit molecule must be further computed. Furthermore, complexing the molecules with some bio

nanostructure will presumably increase their therapeutic potential.

4. CONCLUSION

Calprotectin heteromer can form stable complexes with organic molecules. The best-fit complex has a total energy of - 162.788 kcal/mol. The interaction of calprotectin with Ca+ is also energetically favorable -resulting in a stable complex. Further studies are needed to develop an efficient calprotectin inhibitor that will presumably be an effective therapy against pterygium and other S100A9 involvement-related diseases.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals or humans were used for the studies that are based on this research.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

There are no conflicts of interest.

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