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STRUCTURE PREDICTION AND MOLECULAR DOCKING STUDIES OF "PROSTAGLANDIN D SYNTHASE": INHIBITOR OF HAIR GROWTH IN ANDROGENETIC ALOPECIA

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ABSTRACT

Testosterone is necessary for the development of male pattern baldness, known as androgenetic alopecia (AGA); yet, the mechanisms for decreased hair growth in this disorder are unclear. It has been found that prostaglandin D synthase (PTGDS) is elevated at the mRNA and protein levels in bald scalp compared to haired scalp of men with AGA. The sequence of PTGDS is available in NCBI (accession no: AAK07679) however no report has been found about its 3D protein structure. In this work, we have taken six templates by similarity search of homologous sequences through BLAST. The models were generated by using Modeler 9v10 software. Furthermore, the best model with dope score (-17862.80664) was validated through PROCHECK with Ramachandran plot analysis. The protein-ligand docking was done by using Autodock 4.0 software. The novel drugs designed through chemsketch software shows much better binding energy (binding energy: -26.0 kcal/mol, Ligand 5) in comparison with the synthetic drugs (binding energy: -24.9 kcal/mol, DB01926, Carboxymycobactin S) available in market. This study employs that our novel drugs may show better anti-baldness activity and efficient inhibitor to treat baldness.

Keywords: Testosterone, baldness, androgenetic alopecia, prostaglandin D synthase, Modeler, Autodock.

INTRODUCTION

Many men experience thinning of hair and baldness but till now the cause behind it was unknown. The male pattern baldness (MPB) form of androgenetic alopecia (there is also a female pattern baldness) accounts for more than 95% of hair loss in men. About 25% of men

who suffer from male pattern baldness begin the painful process before they reach 21. Contrary to societal belief, most men who suffer from male pattern baldness are extremely unhappy with their situation and would do anything to change it. Hair loss affects every aspect of their life. It affects interpersonal relationships as well as their professional life. It is not uncommon for men to change their career paths because of hair loss [1]. Researchers have identified the protein responsible for male pattern baldness, raising expectations that an effective treatment for the most common cause of hair loss in men is on the horizon. Male pattern baldness affects 8 out of 10 men and causes the hair follicles to shrink and produce microscopic hairs, which grow for a shorter duration of time than normal hairs [2].

It has been found that the baldness is due to the thinning of hair. According to the researchers, the male sex hormone testosterone plays vital role in the thinning of hair. Genetic factors also said to cause baldness among men. All these factors led to the shrinking of the hair follicles which are not visible through naked eyes. During a study, the researchers found that a key protein called prostaglandin D synthase on the scalp of men who have experienced baldness or thinning of hair. This particular protein was absent from the scalp of men with natural density of hair [3]. The typical pattern of male baldness begins at the hairline. The hairline gradually moves backward (recedes) and forms an "M" shape. Eventually the hair becomes finer, shorter, and thinner, and creates a U-shaped (or horseshoe) pattern of hair around the sides of the head. Male pattern baldness does not indicate a medical disorder, but it may affect self-esteem or cause anxiety. The hair loss is usually permanent. Human cerebrospinal fluid (CSF) is the richest source of prostaglandin (PG) D synthase, a key enzyme in sleep regulation [4]. The hair loss occurs in an atypical pattern, including rapid hair loss, widespread shedding, hair loss in patches, or hair breakage. Hair loss may occurs with itching, skin irritation, redness, scaling, pain, or other symptoms. It may also begins after starting a medication.

Prostaglandin (PG) D2 is actively formed in a variety of tissues [5] and is involved in many physiological events. Prostaglandin (PG) D synthase catalyzes the isomerization of PGH2, a common precursor of various prostanoids, to produce PGD2 in the presence of sulfhydryl compounds. PGD2 induces sleep, regulates nociception, inhibits platelet aggregation, acts as an allergic mediator, and is further converted to 9α, 11β-PGF2 or the J series of prostanoids, such as PGJ2, Δ12-PGJ2, and 15-deoxy-Δ12,14-PGJ2 [6]. Prostaglandins are lipid autacoids derived from arachidonic acid. They both sustain homeostatic functions and mediate

pathogenic mechanisms, including the inflammatory response. They are generated from arachidonate by the action of cyclooxygenase isoenzymes, and their biosynthesis is blocked by nonsteroidal antiinflammatory drugs, including those selective for inhibition of cyclooxygenase-2. Despite the clinical efficacy of nonsteroidal antiinflammatory drugs, prostaglandins may function in both the promotion and resolution of inflammation [7]. So a drug can be designed that can block the receptor that allowed PGD2 to work, to cure from Baldness.

MATERIALS AND METHOD

Homology Modelling

Homology modelling is based on the observation that proteins with similar sequences tend to adopt similar three-dimensional conformations. In this step we use Easy Modeller, a front end graphical user interface to Modeller developed [8]. The aim of the tool is to perform modelling, assessment, visualization and optimization of protein models in a simple way. Six templates (PDB ID: 2CZT, 2HZR, 1X71, 1EW3, 1EPA and 1LF7) were downloaded from PDB on the basis of similarity and E-value. All these templates were submitted by X-ray Crystallography method in PDB. The resolution of the templates is < or = 3.00 A^o and the R-value is, < or = 0.5. Finally five models were generated and their dope scores were obtained (shown in Figure 1).

Validation of Predicted Structure

Validation of Model was done by using Procheck program [9] available on SAVES server [10]. The model with minimum percentage of generous and disallowed region was identified. This model will be then subjected to loop building using Modloop software [11].

Active Side Prediction

The Active side of the validated protein was found by using CastP [12]. Figure 2. shows binding sites of Model 5 (having best binding energy).

Computer Aided Drug Designing

Computer –Aided Drug Design (CADD) is a specialized discipline that uses computational methods to simulate drug – receptor interactions. CADD methods are heavily dependent on bioinformatics tools, applications and databases [13]. Auto Dock is an automatic docking tool. It is designed to predict how small molecules, such as substrates, bind to a receptor of known 3D structures. A graphical user interface called Auto Dock Tools or ADT was utilized

to generate grids, calculate the dock score and evaluate the conformers [14]. A comparative protein-ligand dock analysis was performed using ligands taken from drugbank [15] and pubchem [16]. To perform the task, the powerful genetic algorithm method implemented in the program Auto Dock 4.0.1 was employed [17]. All water molecules were removed from the original Protein Data Bank file. Polar hydrogen atoms and Kollman charges 18 were added. Grid maps were generated by Auto Grid program. The grid dimensions were 42 Aº X 56 Aº X 52 Aº with points separated by 1.000 Aº. Each ligand was docked by the protein one by one. The ligand having best binding energy was the used to obtain ten new molecules from Chemsketch [18] following the basic rules of chemistry.

RESULT AND DISCUSSION

In this work, we describe homology modeling of prostaglandin D Synthase based on the X-ray structure of templates downloaded from PDB. The model with the lowest objective function (-17862.80664), shown in figure 3, which was considered as the best one, was selected and subjected to quality evaluation. The PROCHECK Ramachandran plot analysis shows that the mainchain conformations for 92.8% of amino acid residues are within the most favored or allowed regions (Figure 4). Loop modeling using MODLOOP analyzed by PROCHECK 3.0 showed 97% residues in the most favorable region and 0.0% in both generously allowed and disallowed regions of a Ramachandran plot (Figure 5). The active site of prostaglandin D synthase protein shows structural pocket of area 61.6 with volume 11001 and area 3486. The synthesized drugs retrieved from drugbank and Pubchem were docked with the protein which shows Carboxymycobactin S having the lowest binding energy (-24.9 kcal/mol) shown in Table 1. As Carboxymycobactin S is having the best binding energy it was further used for designing new drugs that will we our novel drugs. Eight drugs were designed by using chemsketch in which compound no. 4 shows better binding energy (-26.0 kcal/mol) than Carboxymycobactin S available in market (Table 2).

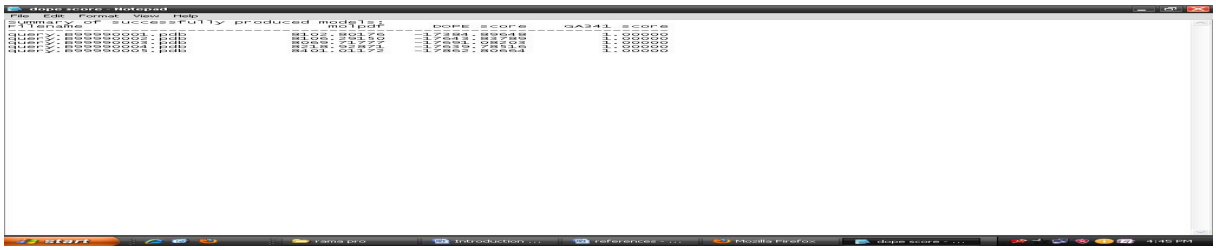


Figure 1: Dope Score predicted models.

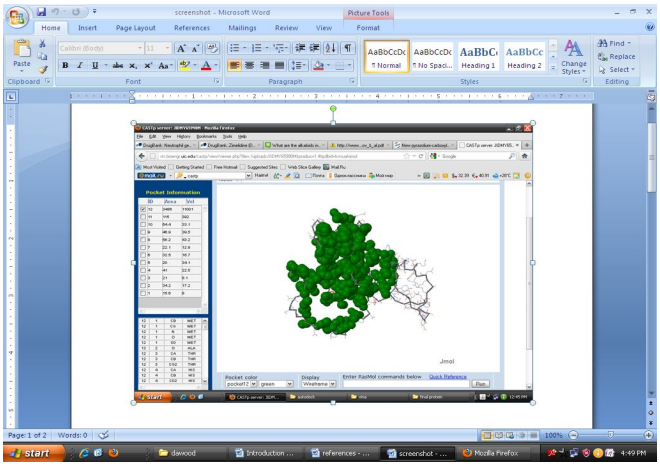


Figure 2: Active site prediction by using Cast P.

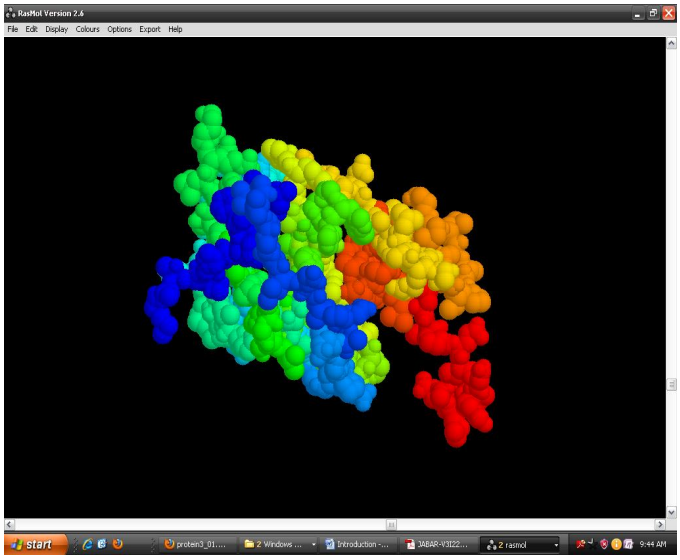


Figure 3: Modelled structure of Prostaglandin D Synthase

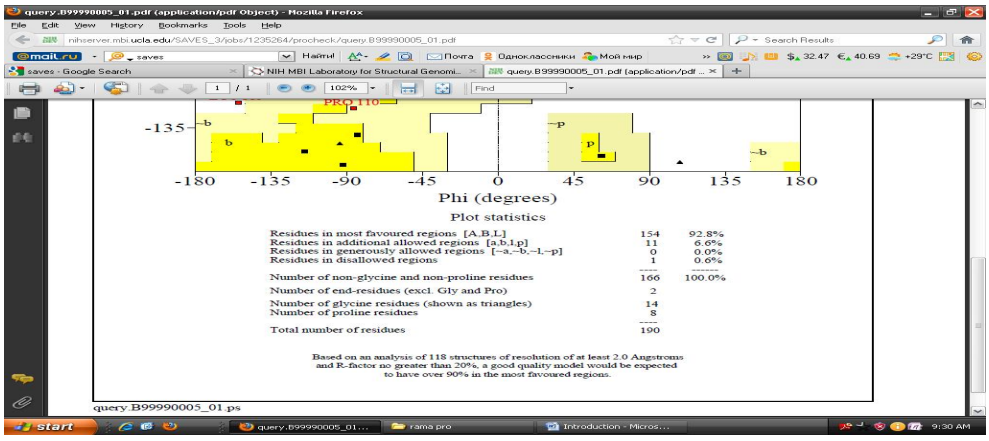


Figure 4: PROCHECK Ramachandran Plot Analysing 92.8% of amino acid residues are within the most favored or allowed regions (Model 5)

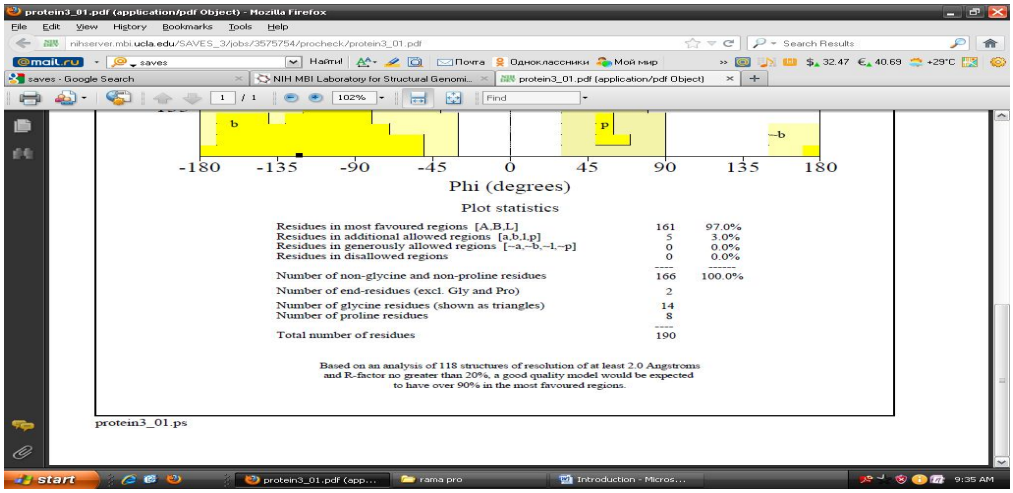
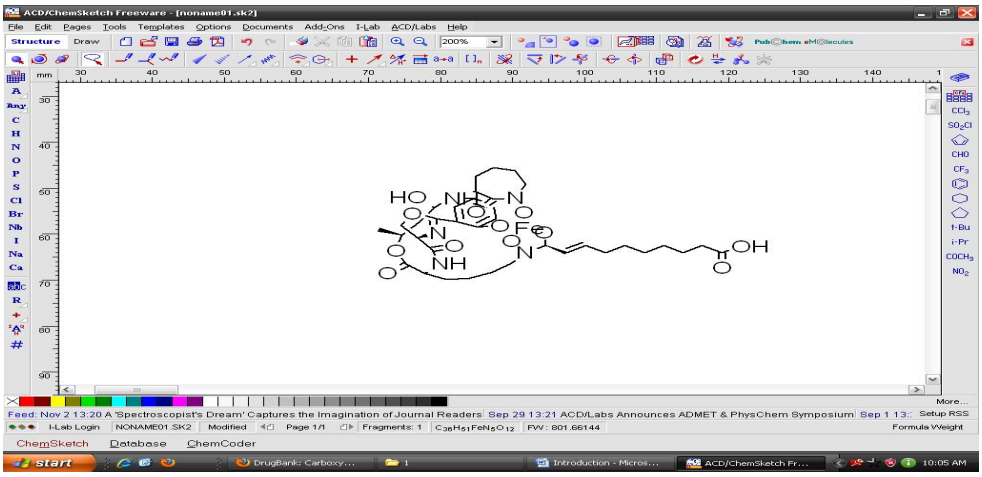


Figure 5: Procheck showing 97.0% residues in favoured region after loop modeling.

Table 1: Docked score of approved compounds retrieved from Drugbank and pubchem.

Source Id	Compound name	Binding Energy (kcal/mol)
DB04272	Citric Acid	-7.8
DB01672	2,3-Dihydroxy-Benzoic Acid	-7.0
DB02710	2,3-Dihydroxybenzoyl serine	-10.0
DB02944	Alpha D-Mannose	-6.9
DB01926	Carboxymycobactin S	-24.9
DB04043	Carboxymycobactin T	-24.8
DB01631	Methyl Nonanoate (Ester)	-8.2
DB04476	Trencam-3,2-Hopo	-7.0
CID_21368	Nigelline	-6.7



Structure of Carboxymycobactin S

Table 2: Docked Score of Newly Designed experimental compounds by using chemsketch

Compound	Binding Energy (kcal/mol)
1	-24.9
2	-25.0
3	-24.9
4	-26.0
5	-25.0
6	-24.9
7	-25.0
8	-24.8

CONCLUSION

On the basis of the results obtained by both the methods of docking, it was found that the experimental compound no. 5 was the one which binds to the protein with least binding energy. On comparison of the binding affinity of this ligand with the approved ligands obtained from Drugbank, it was found that our modified ligand was better. This drug may used in future for the cure of baldness.

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