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Computer-aided Screening of Therapeutic Ligands against KLF8 Protein (*Homo sapiens*)

Renukesh Verma, Mayank Agarwal and Vinod Kumar Jatav*

Department of Biotechnology Madhav Institute of Technology & Science Gwalior-474005, Madhya Pradesh, India.

*Corresponding author: Vinod Kumar Jatav; e-mail: vinod.mits@gmail.com

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ABSTRACT

Kruppel like factors (KLFs) are highly related zinc-finger proteins that are important components of the eukaryotic cellular transcriptional machinery. It is expressed in the nucleus of many cell types and its expression is elevated in several human cancers. KLF8 expression is increased in several types of human cancer cells and tissues, and ectopic expression of KLF8 induces transformation. The encoded protein is thought to play an important role in the regulation of epithelial to mesenchymal transition, a process which occurs normally during development but also during metastasis. In this study previously modelled structure was used for Docking and finding best binding ligand in the protein and that can alter the factions of KLF8 protein. For this purpose, a virtually screened 3D model of KLF8 was explored. The unliganded KLF8 was docked and best five docking solutions complex were selected and analyzed by Ligplot (http://www.ebi.ac.uk/thrnto-srv/software/LIGPLOT/). The analysis showed that Dasatinib, Doxorubicin, Pazopanib, Sorafenib & SU 6656 has maximum potential against unliganded protein. The analysis was done on the basis of scoring and binding ability and Dasatinib indicated minimum energy score and highest number of interactions with active site residue and could be a promising inhibitor for KLF8 as Drug target.

Keywords: KLF8, Metastasis, NIH3T3, Docking, Ligplot

INTRODUCTION

Kruppel-like factor 8 (KLF8) is a young member of the KLF transcription factor family proteins. It is highly overexpressed in several types of human cancers and regulates various cellular processes important for tumour progression. Increasing evidence has made KLF8 a new focus in cancer research and a potential target for cancer therapy [1].

17 members in the KLF protein family are reported [1]. Many of them play a crucial role in cancer [2]. Some of these transcription factors act as transcriptional activators, some act as transcriptional repressors and other members have a dual role in both activating and repressing gene expression [1]. All of the KLF proteins share three highly conserved Cys2His2 zinc finger motifs in DNA (CACCC GT-box or GC rich element) binding domain at their C-terminus with diverse N-terminal regulatory elements [3].

Several reports have been followed to demonstrate the aberrant overexpression of KLF8 in various types of human cancer including breast [4], ovarian [5], renal [6], liver [7], gastric [8], and brain [9], cancer. Various signalling pathways regulating KLF8 and its target genes associated with cancer have been identified. Initial effort began with a study of the role of KLF8 in oncogenic transformation of NIH3T3 cells [10].

KLFs are named after homology with Kruppel protein of *Drosophila melanogaster*. KLF8 is present in most of the species [1]. In human KLF8 is located in the chromosome X [11]. Human KLF8 protein consists of 359 amino acid residues [1]. It was first isolated from the K562 leukemia cell line [12] [13]. A typical transcription factor contains three essential domains for its activity, 1) a nuclear localization signal to transport the protein into the nucleus, 2) a DNA binding domain to interact with promoter DNA and 3) a transcriptionally regulatory domain to regulate gene

expression [1]. The DNA binding domain is highly conserved in all KLF proteins [1]. In some cases the Zinc finger domains also mediate protein-protein interaction [1].

Kruppel like factor 8 (KLF8) is a GT-box (CACCC) binding dual-transcription factor that has a critical role in the regulation of cell cycle progression [12] [8]. Recent investigation revealed that KLF8 induces tumour cell epithelial to mesenchymal transition (EMT) and maintains the invasive potential of cancer, which seemingly plays a crucial role in metastatic progression of human carcinoma [10]. In this study ligands are screened against the KLF8 by using the AutoDock4.2.1 according to their binding energy, inhibition constant and other parameters to prevent cancer in humans.

MATERIALS AND METHODS

Retrieval of protein structure

Modelled protein structure was collected from Department of Biotechnology, MITS, Gwalior (M.P.).

Binding site Prediction

Binding sites were characterized by CASTp [13-14] (http://sts-fw.bioengr.uic.edu/castp). Q-Site finder and compared by extensive literature search. By comparing prediction of CASTp algorithm and Q-Site Finder, best active sites were selected. CASTp method was used to identify and measure the binding sites, active sites, surface structural pockets (accessible), interior cavities (inaccessible), shape (alpha complex and triangulation), area and volume (solvent and molecular accessible surface) of each pockets and cavities of proteins. CASTp could be used to measure the number, area, circumference of mouth openings of each pocket in solvent and molecular accessible surface [13-14].

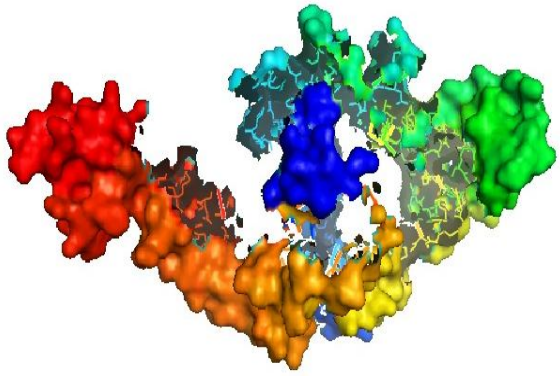

Figure 1. Active site of protein as visulaized by CASTp

Compounds selection and Preparation

A total of 90 compounds were screened against the cancer protein by using AutoDock 4.2.1. The 3D structure of ligand was collected from PubChem (http://pubchem.ncbi.nlm.nih.gov). Ligand preparation includes addition of hydrogen atoms, neutralization of the charge groups and removal of any miscellaneous structures from the ligand. Prepared and optimized structures of ligand and protein were ultimately used for molecular docking.

Drug likeliness prediction

Lipinski rule of five helps in distinguishing drug-like and non-drug-like properties and predicts high probability of success or failure due to drug likeliness for molecules. The Lipinski filter helps in early preclinical assessment and thereby avoiding costly late-stage preclinical and clinical failures. All value for that purpose was taken using PubChem (http://pubchem.ncbi.nlm.nih.gov).

Protein ligand interaction using Autodock 4.2.1

Virtual screening of the Ligand-protein interaction for their binding affinity was carried out using AutoDock 4.2.1 [15] and the results that include the understanding of association that involves H-bonding and hydrophobic interactions were analyzed using Ligplot1.4.5 (http://www.ebi.ac.uk/thrntosrv/ software /LIGPLOT/, Developed Wallace AC., et al. on linux/unix), a programme to generate schematic diagrams of protein ligand interactions.

Analyzing the Docking Results

The search for the best ways is to fit ligand molecules into structure, using Autodock 4.2.1 resulted in docking files that contained detailed records of docking. The obtained log files were read in ADT (Auto Dock Tool) to analyze the results of docking. The similarity of docked structures was measured by computing the root mean square deviation (RMSD) between the coordinates of the atoms and creating clustering of the conformations based on the RMSD values [16]. The lowest binding energy conformation in all cluster were considered as the most favourable docking pose [16]. Binding energies that are reported represent the sum of the total intermolecular energy, total internal energy and torsional free energy minus the energy of the unbound system [16]. The top five ligands were selected based on the energy score after virtual screening Table 1.

ADMET Analysis

The various properties of the best ligand i.e. Dasatinib were predicted by using Online ADMET prediction tool (preadmet.bmdrc.org) and are shown in Table 2.

RESULTS AND DISCUSSION

From the inspection of ligand molecules, it was found that the RMSD value of the selected five ligands were Zero and the five ligands which showed best value are given in Table 1.

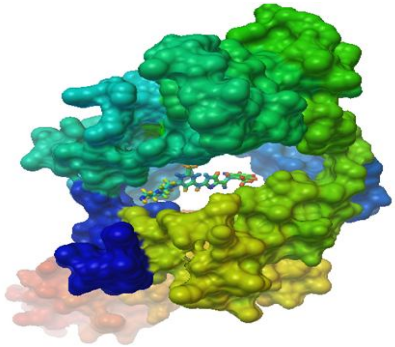

Figure 2. Showing the ligand (Dasatanib) in the cavity of protein.

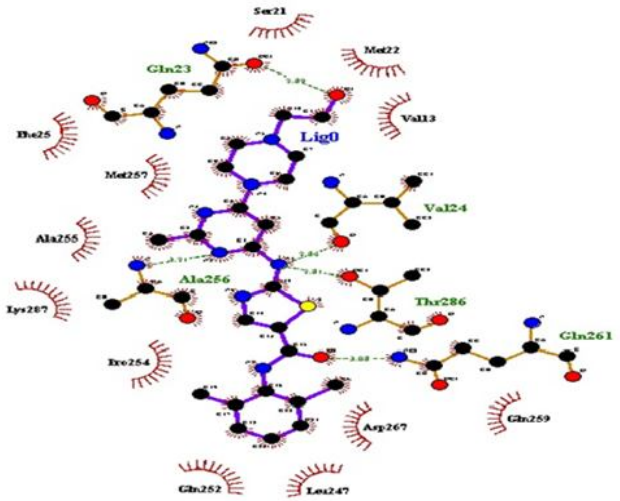

Figure 3. Ligplot showing the hydrogen bonds at Gln23, Val24, Thr286, Gln261 and Ala256 (shown in green) between protein and Dasatinib

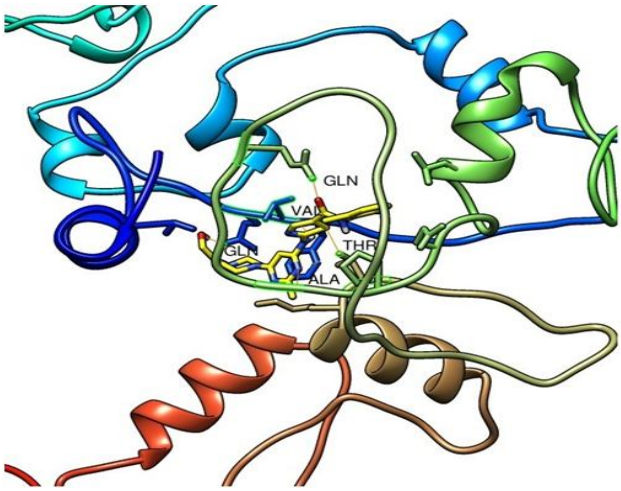

Figure 4. Molecular visualization of interaction between ligand and the target protein

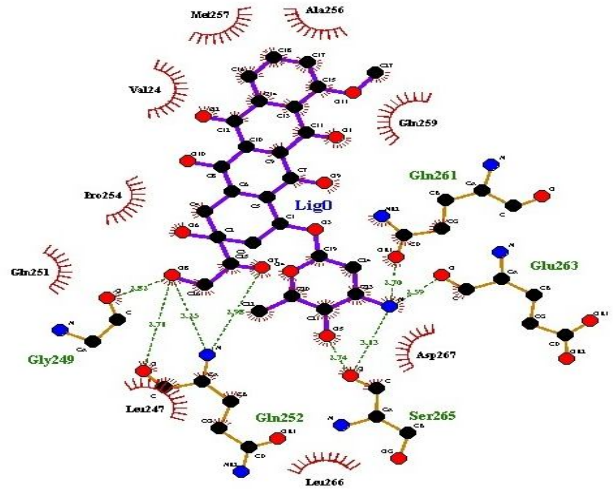

Figure 5. Ligplot showing the hydrogen bonds at Gln261, Glu263, Ser265, Gln252 and Gly249 (shown in green) between protein and Doxorubicin.

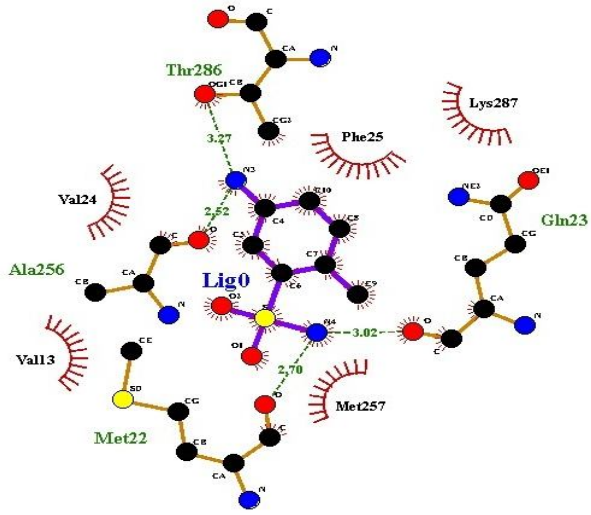

Figure 6. Ligplot showing the hydrogen bonds at Gln23, Met22, Ala256 and Thr286 (shown in green) between protein and Pazopanib.

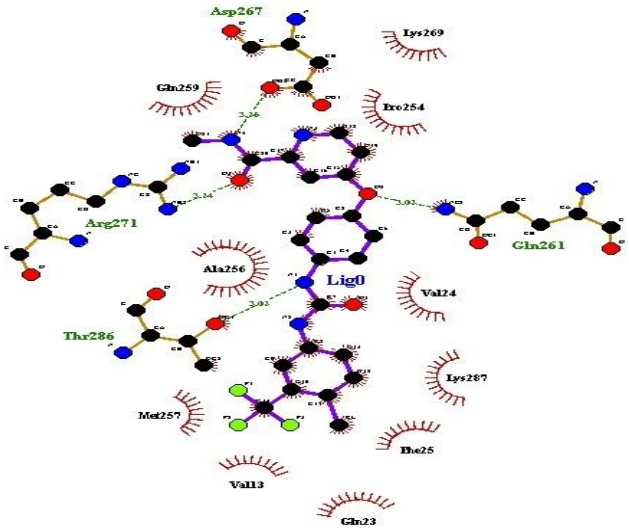

Figure 7. Ligplot showing the hydrogen bonds at Gln261, Thr286, Arg271 and Asp267 (shown in green) between protein and Sorafenib.

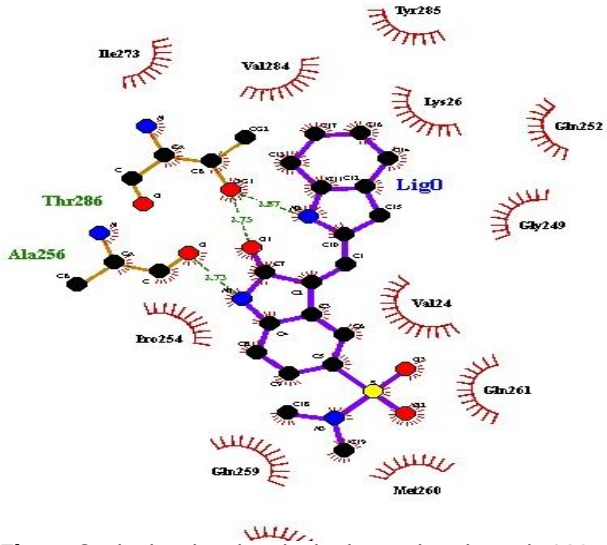

Figure 8. Ligplot showing the hydrogen bonds at Thr286 and Ala256 (shown in green) between protein and ligand (Su6656).

Table 1. Binding energy and other parameters of the ligands

Ligand	Binding energy	K_i	H Bond	MW	ClogP
Dasatinib	-8.86	320.48	5	488.0054	3.6
Doxorubicin	-8.97	264.58	8	543.5193	1.3
Pazopanib	-9.96	50.29	4	437.518	3.1
Sorafenib	-9.27	159.31	4	464.825	4.1
SU 6656	-8.96	271.2	3	371.4533	2.1

Table 2. Ames test and other parameters of ligand by Pre ADMET

Distribution				Metabolism		Tox
Intestinal absorption	***In-vitro* CaCO2**	**Skin perme-abilty**	**MDCK**	***In-vitro* BBB**	***In-vitro* plasma protein binding**	**Ames test**
93.591	32.0059	-4.20687	0.26952	-0.0350358	70.291918	Negative

CONCLUSION

Molecular docking is a key tool in structural molecular biology and computer assisted drug design. The goal of ligand-protein docking is to predict the predominant binding mode (s) of a ligand with a protein of known three dimensional structures [17]. Screening studies of these 90 ligands obtained from Pubchem database are docked against protein (KLF8) whose expression is elevated in several human cancers using Autodock 4.2.1 resulted in 5 ligands mentioned in table 1 obtained as best compounds. The present study concludes that the Dasatinib was found to be most active against KLF8 protein and it could be used as drug for preventing cancer.

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