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Research Article

Homology Modeling of Mus Musculus CDK5 and Molecular Docking Studies with Flavonoids

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ABSTRACT

A 3D model of Cyclin-dependent kinase 5 (CDK5) (Accession Number: Q543f6) is generated based on crystal structure of *P. falciparum* PFPK5-indirubin-5-sulphonate ligand complex (PDB ID: 1V0O) at 2.30 Å resolution was used as template. Protein-ligand interaction studies were performed with flavonoids to explore structural features and binding mechanism of flavonoids as CDK5 (Cyclin-dependent kinase 5) inhibitors. The modelled structure was selected on the basis of least modeler objective function. The model was validated by PROCHECK. The predicted 3D model is reliable with 93.0% of amino acid residues in core region of the Ramachandran plot. Molecular docking studies with flavonoids viz., Diosmetin, Eriodictyol, Fortuneletin, Apigenin, Ayanin, Baicalein, Chrysoeriol and Chrysosplenol-D with modelled protein indicate that Diosmetin is the best inhibitor containing docking score of -8.23 kcal/mol. Cys83, Lys89, Asp84. The compound Diosmetin shows interactions with Cys83, Lys89, and Asp84.

Keywords: CDK-5, Flavonoids, Molecular modeling, Modeller9.17, Autodock4.2.

INTRODUCTION

The cyclin-dependent kinases (CDKs) belong to the serine/threonine protein kinases subfamily. CDK5 involved in brain development and neurodegeneration[1]. It also paly very important role in good control of cell cycle progression[2]. Cyclin-dependent kinase 5 (CDK5), a member of the cyclin-dependent kinase family, which is expressed predominately in mature neurons. Cell cycle progression is controlled by the activity of CDKs. Cdks are cell cycle control proteins activated by cyclins[3]. CDKs are inactive as monomers, and activation requires binding to cyclins, a diverse family of proteins whose levels fluctuate during the cell cycle, and phosphorylation by CDK-activating kinase on a specific threonine residue[4]. Cdk5 protein expression also associated with apoptosis in a number of nonneuronal model systems[5].

In the present study, MODELLER9.17 was used to generate 3-Dimensional model of CDK5 (Cyclin-dependent kinase 5, isoform CRA_c) (uniprot accession number: Q543F6)[6] protein from mus musculus. Structure of *P. falciparum* PFPK5-indirubin-5-sulphonate ligand complex (PDB ID: 1V0O) was used as a template for model build up. Developed model was validated by using PROCHECK program. Active site prediction was predicted by using SYBYL6.7[7] SITEID module and molecular docking studies were performed by using AutoDock4.2.

Experimental data

Sequence alignment and structure prediction

The amino acid sequence of query sequence CDK5 (Mus musculus Cyclin-dependent kinase 5, isoform CRA_c) was retrieved from the UniProtKB database (Accession number: Q543F6) (http://www.uniprot.org/). A Basic Local Alignment Search Tool[8] (BLAST) search was performed to select the template and resulted with the best match Structure of *P. falciparum* PFPK5-indirubin-5-sulphonate ligand complex (PDB ID: 1V0O) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) with 60% similarity having a resolution of 1.90 Å making it an excellent template. The 3D structure was generated by using Modeller 9.17[9]. The model was finally validated by using PROCHECK. The RMSD (root mean square deviation) was calculated by superimposing template (1V0O) over the generated model to access the accuracy and reliability of the generated model using SPDBV[10] by selecting the main chain atom (i.e. the backbone atoms of alpha carbon).

MODELLER 9.17 was then used to gain acceptable models; an automated approach to molecular modeling by satisfaction of spatial restrains[11]. Initially, alignment was performed to both the query and template by using clustalX. After manually modifying the alignment input file in MODELLER 9.17 to match the query and template sequence, 20 models were generated. The model was selected on the basis of least modeler objective function value, selected model was then validated by using PROCHECK[12] to explore the stereochemistry of the generated model. Ramachandran plot explains comprehensive residue by residue listing facilitates, the in depth assessment of Psi/Phi angles and the backbone conformation of the models.

Docking protocol

The eight flavonoid derivatives were sketched in sybyl6.7 and saved it into .mol2 format. Then the molecules were

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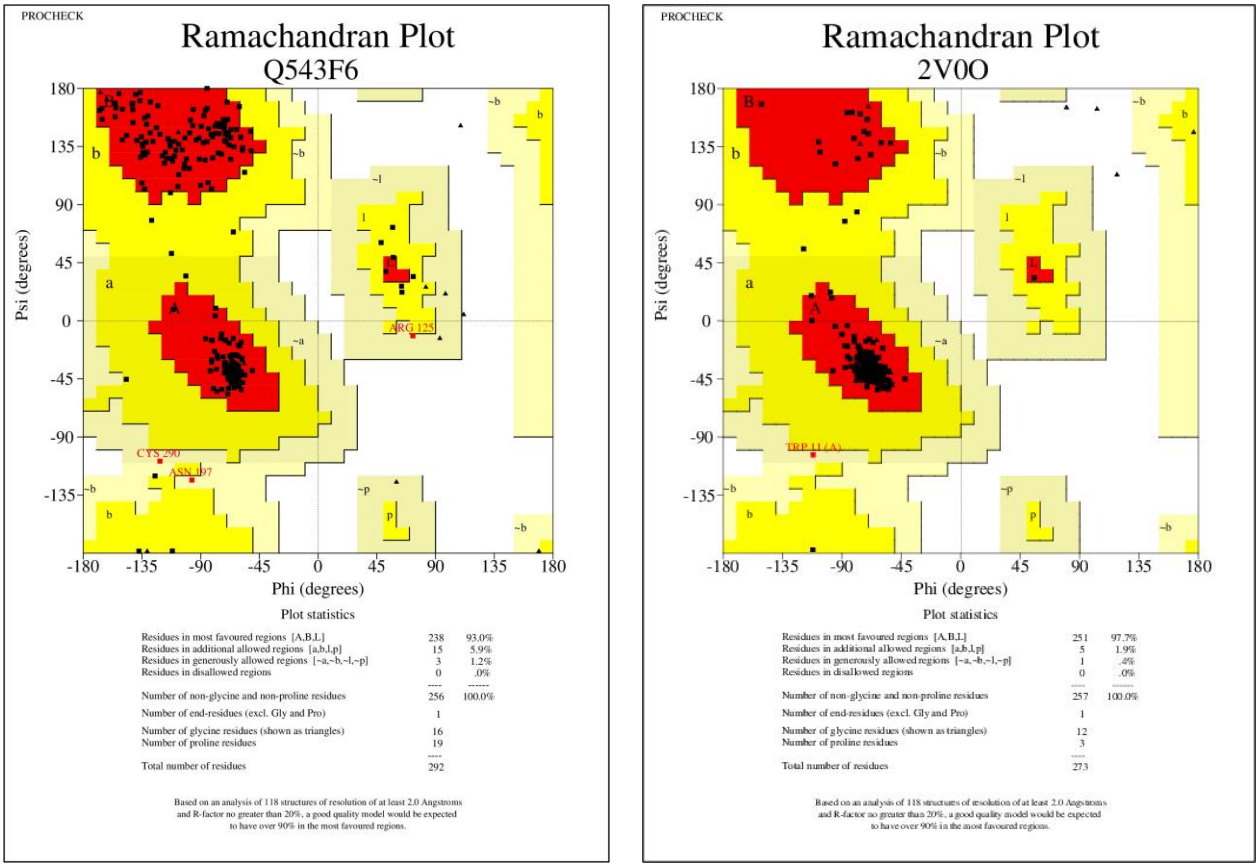


a b

Figure 1: Ramachandran plot analysis of the backbone dihedral angles PSI (Ψ) and PHI (ϕ) of (a) the generated model and (b) the template model 1V0O chain A.

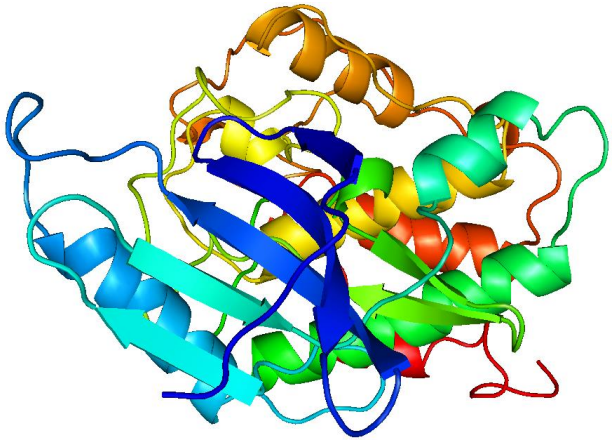


Figure 2: secondary structure of the predicted model.

minimized using Tripos force field, Gasteiger-Huckel charges were added and used convergence criterion of 0.005 kcal/mol Å. Molecular Docking study was performed to all the flavonoid molecules separately by using AutoDock4.2 program, using the Lamarckian Genetic Algorithm (LGA) and implemented empirical free energy function[13]. Initially, the modelled protein was loaded and polar hydrogen were added. The molecule was loaded and set conformations and saved it in PDBQT format and then saved generated PDB file to PDBQT format. The grid maps were selected and calculated using AutoGrid[14]. For all dockings, a grid map with 60×60×60 points and also used a grid-point spacing of 0.375 Å was applied. Coordinates of x, y, z was set as -25.996, -15.595 and 38.894 respectively. For all docking parameters, default values were used.

RESULTS AND DISCUSSION

Homology modelling and model evaluation

The present study reports that the template protein (PDB ID: 1V0O) having high degree of homology with Q543F6 protein was used as a template with good atomic resolution of its crystal structure. The target sequence of CDK5 (Cyclin-dependent kinase 5, isoform CRA_c) (Accession No. Q543F6) having 292aa residues was retrieved from the Uniprot protein sequence database. Template protein 1V0O was identified and selected as template using BLAST having 60% identity. The structure was then modelled using modeller9.17. The generated model was validated by PROCHECK. The model shows 93.0% of amino acid residues in core region, 5.9% of amino acid residues in additionally allowed region, 1.2% of amino acid residues in generously favored region. There is no amino acid present in disallowed region. Both target and template molecules show nearly same amino acid residues in most favored region that is query sequence shows 93.0% in most favored region and template molecule contains 97.5 % in most favored region. Ramachandran plot and secondary structure of the modelled protein is shown in

Table 1: Flavonoid derivatives used for molecular docking.

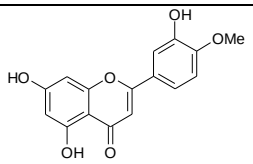
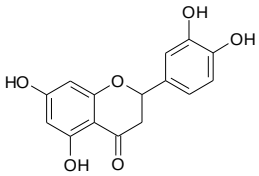
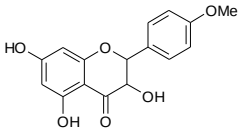
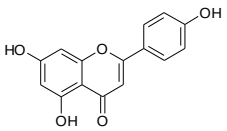
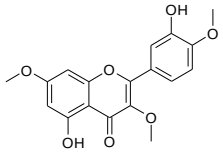
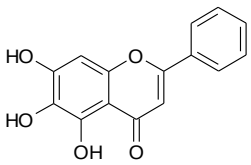
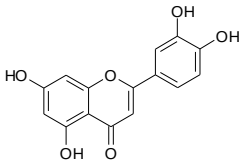
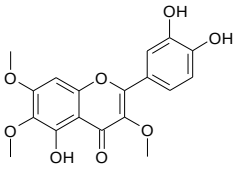
C.no	Compound Name	Structure	Binding energy	Ki	Protein-ligand interactions
1	Diosmetin		-8.23	931.23 (nm)	Cys83, Lys89, Asp84
2	Eriodictyol		-7.67	2.38 (uM)	Lys33, Ile10
3	Fortuneletin		-7.07	6.52 (uM)	Lys89, Asn131
4	Apigenin		-7.61	2.64 (uM)	Cys83, Glu12, Glu81
5	Ayanin		-7.63	2.56 (uM)	Glu8
6	Baicalein		-7.41	3.69 (uM)	Lys89, Asp84
7	Chrysoeriol		-7.51	3.12 (uM)	Cys83, Lys89, Glu81
8	Chrysosplenol-D		-7.37	3.94 (uM)	Lys89, Asp86, Glu81

fig.1 and fig.2 respectively

Molecular docking Results

Molecular docking is the most widely used method for the calculation of protein–ligand interactions. Docking is a most efficient technique to predict the potential ligand binding sites on the whole protein. To explore the predictability as well as the characteristics of the binding pocket of the modelled model and to make the rational design of novel and more selective antagonists of CDK5. Molecular docking was carried out on developed CDK5 binding pocket using a set of flavonoid antagonists shown in Table 1. The 8 docking conformations for each molecule were generated. Autodock4.2 also uses free energy binding assessment to assign the best binding conformation. Energies estimated by Autodock are described by intermolecular energy (including Vander Waals, hydrogen bonding, and electrostatic energies), internal energy, and torsional free energy.

The hydrogen bond interaction and electrostatic interaction between the receptor and ligand is the most important, because it can allocate the strength of binding and the exact position of the ligand in the active site. Structures of molecules are given in table 1.

Molecular docking studies were carried out for eight flavonoid derivatives against CDK5 protein of Mus musculus. The binding energy, inhibition constant, hydrogen bond forming residues and interacting residues of all the eight flavonoid derivatives when docked with CDK5 is as given in Table 1. The binding energy for all the molecules range from -8.23 to -

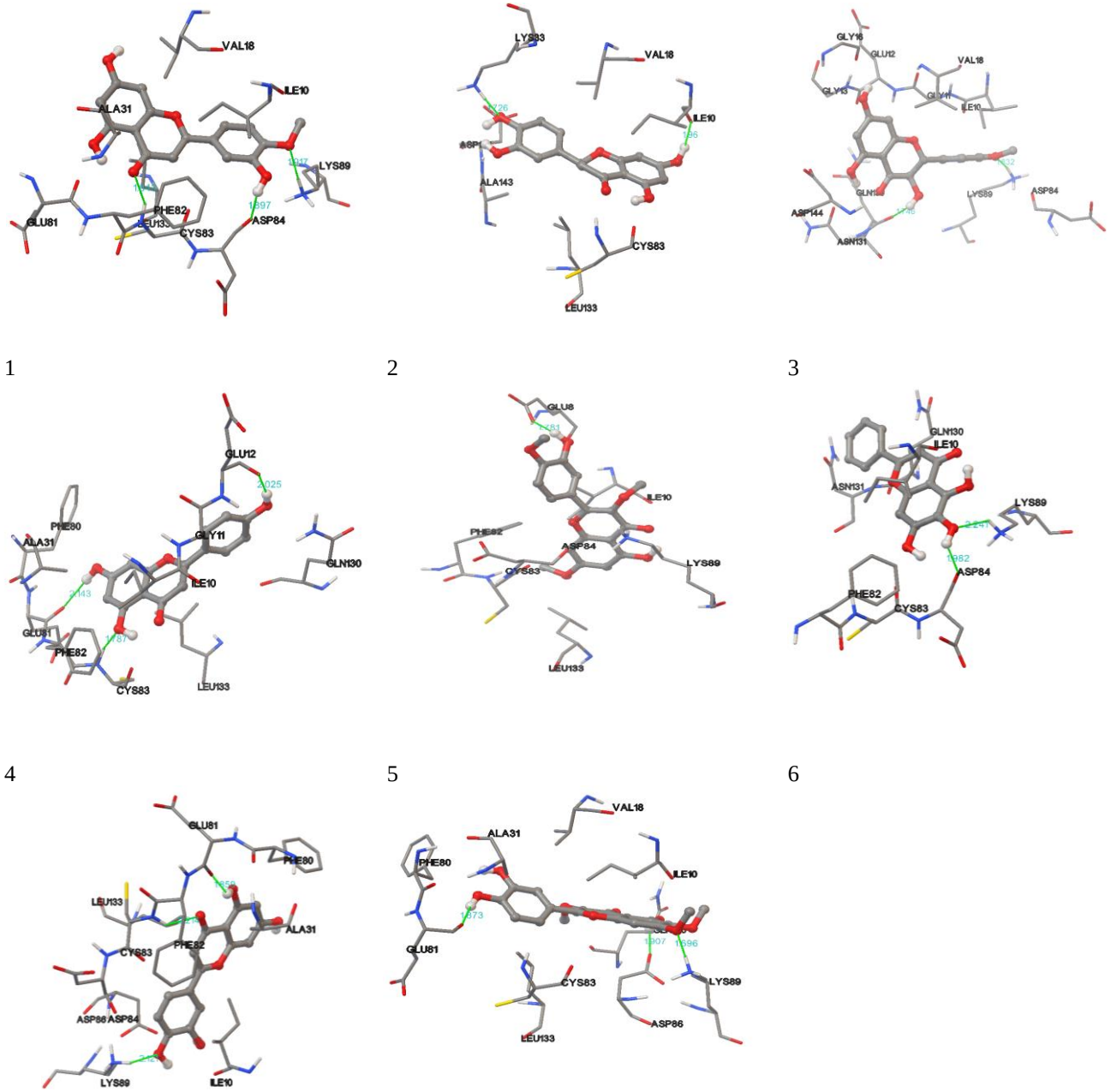


Figure 3: Docking poses of the compounds (1) Diosmetin (2) Eriodictyol (3) Fortuneletin (4) Apigenin (5) Ayanin (6) Baicalein (7) Chrysoeriol (8) Chrysosplenol-D in the active site of CDK5.

7.37kcal/mol. Compound one having highest binding energy of -8.23kcal/mol. This compound had shown three interactions with Cys83, Lys89, and Asp84 as shown in fig 3 thus indicating that CDK5 has lowest affinity towards compound one. Compound 4, 7, 8 are also shows three interactions.

CONCLUSION

The 3D structure of Q543F6 of Mus musculus was generated using Modeller 9.17. The generated model assessment was revealed that the model is reliable and a quality model with stable energies. Additionally, the molecular docking studies were performed to all the compounds into the binding cavity of Q543F6, which showed favorable interactions with all the compounds. All the eight flavonoid derivatives were docking against modelled protein. All compounds shows good binding energy. Compound one shows binding energy of -8.23kcal/mol. Hence, we conclude that all these flavonoid compounds could be a potential lead molecules for CDK5.

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