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Natural Flavonoids as CDK5 inhibitors: A Homology modeling and Molecular Docking Study

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Abstract : In this study, homology modeling and molecular docking studies were performed to explore structural features and binding mechanism of natural flavonoid derivatives as Cyclin-dependent kinase 5 (CDK5) inhibitors. A homology modeling procedure was employed to construct a 3D model of CDK5 of Mus musculus protein by using MODELLER9.15. For this procedure, the X-ray crystal structure of FCHO2 F-BAR DOMAIN (PDB ID: 2V0O) at 2.30 Å resolution was used as template. The predicted model was analyzed by PROCHECK. The 3D structure of predicted model shows 93.0% of amino acids in most favored region. The predicted model was used for molecular docking studies by using Autodock4.2. All the selected natural flavonoid derivatives show good binding energy and interactions with the model. Compound Gardenin-B shows two interactions with Lys89 and Glu81.
Key words: Natural Flavonoids, Homology modeling, Modeller9.15, Molecular docking.

Introduction

The cyclin-dependent kinases belong to the serine/threonine protein kinases subfamily. Cyclin-dependent kinase 5, 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.[1] 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.[2] Cdk5 protein expression also associated with apoptosis in a number of nonneuronal model systems.[3]

In the present study, MODELLER9.15 was used to generate 3D model of Cyclin-dependent kinase 5, isoform CRA_c (CDK5) (uniprot accession number: Q543F6) protein from mus musculus. FCHO2 F-BAR DOMAIN (PDB ID: 2V0O) is used as a template for model build up. Validation of model was performed by PROCHECK program. Active site prediction was performed by using 3D ligandsite, an online active site prediction tool and molecular docking study was performed using AutoDock4.2.

Experimental data

Sequence alignment and structure prediction

The amino acid sequence of mus musculus Cyclin-dependent kinase 5, isoform CRA_c (CDK5) (uniprot accession number: Q543F6) was retrieved from the UniProtKB database (http://www.uniprot.org/)[4]. A

BLAST[5] (Basic Local Alignment Search Tool) search was performed to select the template and resulted with the best match Crystal Structure of FCHO2 F-BAR DOMAIN (PDB ID: 2V0O) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) with 60% similarity having a resolution of 2.30 Å making it an excellent template. The three dimensional structure was generated using Modeller 9.15[6]. The final validation of the model was performed using PROCHECK for Ramachandran plot. The RMSD (root mean square deviation) was calculated by superimposing (2V0O) over the generated model to access the accuracy and reliability of the generated model using SPDBV[7] by selecting the main chain atom (i.e. the backbone atoms of alpha carbon).

MODELLER 9.15 was then used to gain satisfactory models; an automated approach to homology modeling by satisfaction of spatial restrains[8]. Initially, both the query and template were aligned by using clustalX. After manually modifying the alignment input file in MODELLER 9.15 to match the query and template sequence, 20 models were generated. After generating files least modeler objective function value containing PDB was selected to validate the model. These models were then checked in detail for the protein structure stereochemistry by using PROCHECK[9], which generates Ramachandran plot and comprehensive residue by residue listing facilitates, the in depth assessment of Psi/Phi angles and the backbone conformation of the models.

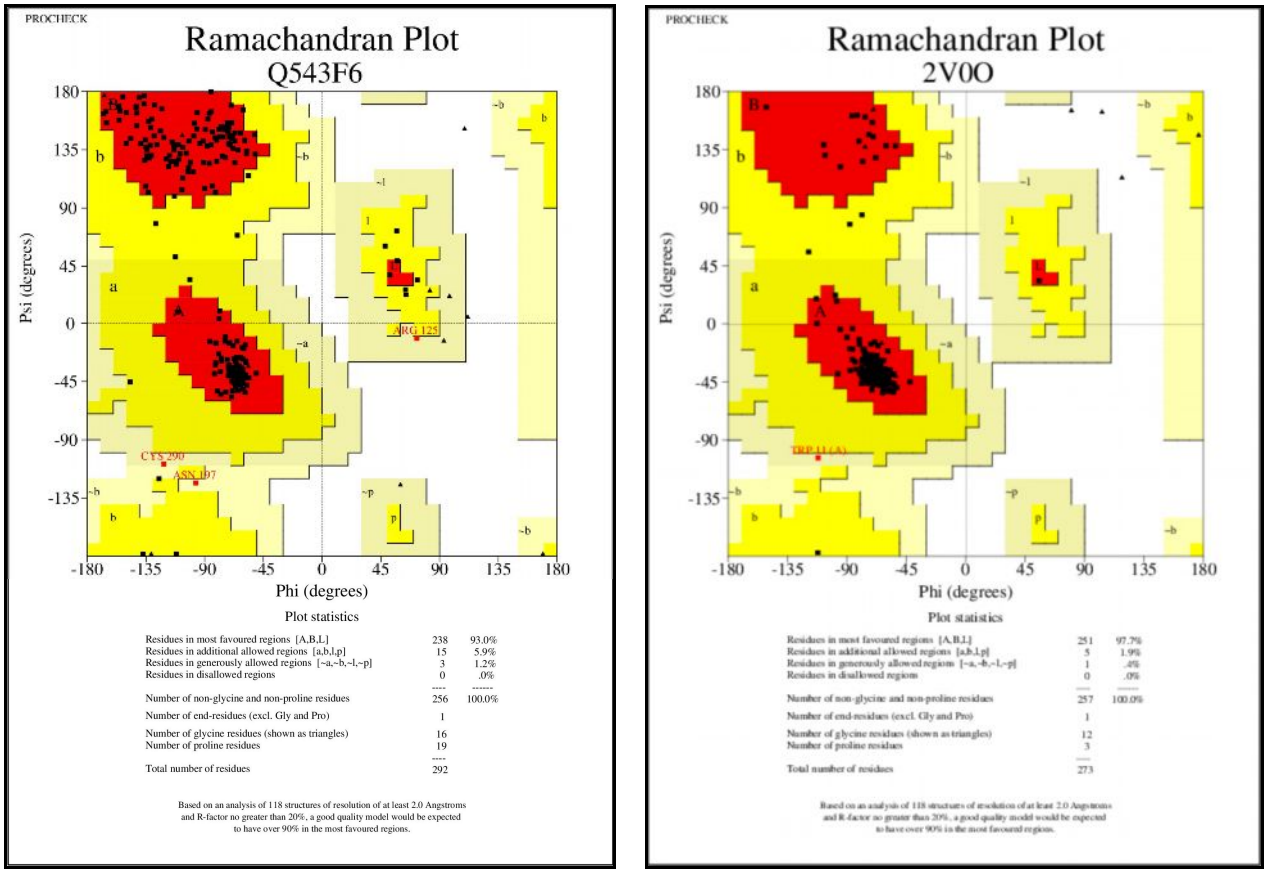
Docking protocol

The six natural flavonoid derivatives were sketched in sybyl6.7 and saved it into .mol2 format. Then the molecules were 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 natural flavonoid molecules separately by using AutoDock4.2 program, using the Lamarckian Genetic Algorithm (LGA) and implemented empirical free energy function.[10] 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.[11] 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: 2V0O) 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 Cyclin-dependent kinase 5, isoform CRA_c (CDK5) having 292 amino acid residues was retrieved from the uniprot protein sequence database with Accession No. Q543F6. PDB Id 2V0O was identified and selected as template using BLAST having 60% identity. The structure was modelled using modeller9.15. The generated structure was validated using Protein Structure and 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 fig.1 and fig. 2 respectively.



(a) (b)

Fig. 1: Ramachandran plot analysis of the backbone dihedral angles PSI (Ψ) and PHI (ϕ) of (a) the generated model and (b) the template model 2V0O chain A.

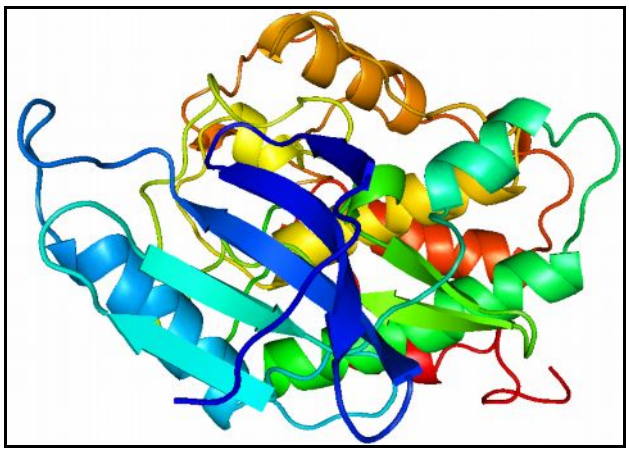


Fig. 2 secondary structure of the predicted model

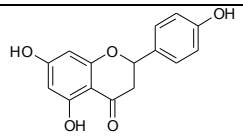
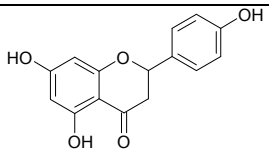
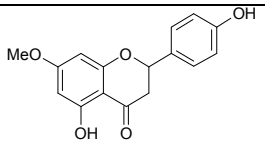
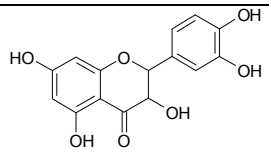
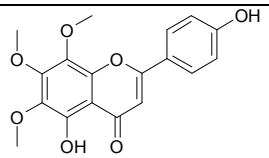
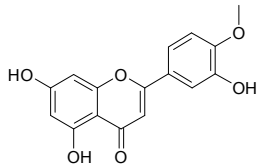
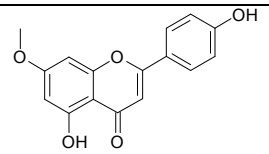
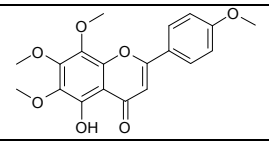
Molecular docking Results

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 natural flavonoid antagonists shown in Table 1. The 10 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.

Table 1: Flavonoid derivatives used for molecular docking

c.no	Compound Name	Structure	Binding Energy	Ki (um)	Protein-Ligand Interactions
1	Naringenin		-7.09	6.36 uM	Lys33, Asp86
2	Quercetagetin		-3.67	2.04 uM	Glu8
3	Sakuranetin		-7.48	3.30 uM	Cys83
4	Taxifolin		-7.16	5.69 uM	Lys20, Glu8, Asp86
5	Xanthomicriol		-8.19	1.0 uM	Lys33, Asp84
6	Hesperitin		-7.16	5.64 uM	Lys20, Ile10, Cys83
7	Genkwanin		-7.96	1.47 uM	Cys83, Gln130, PHE82
8	Gardenin-B		-8.22	938.52nM	Lys89, Glu81

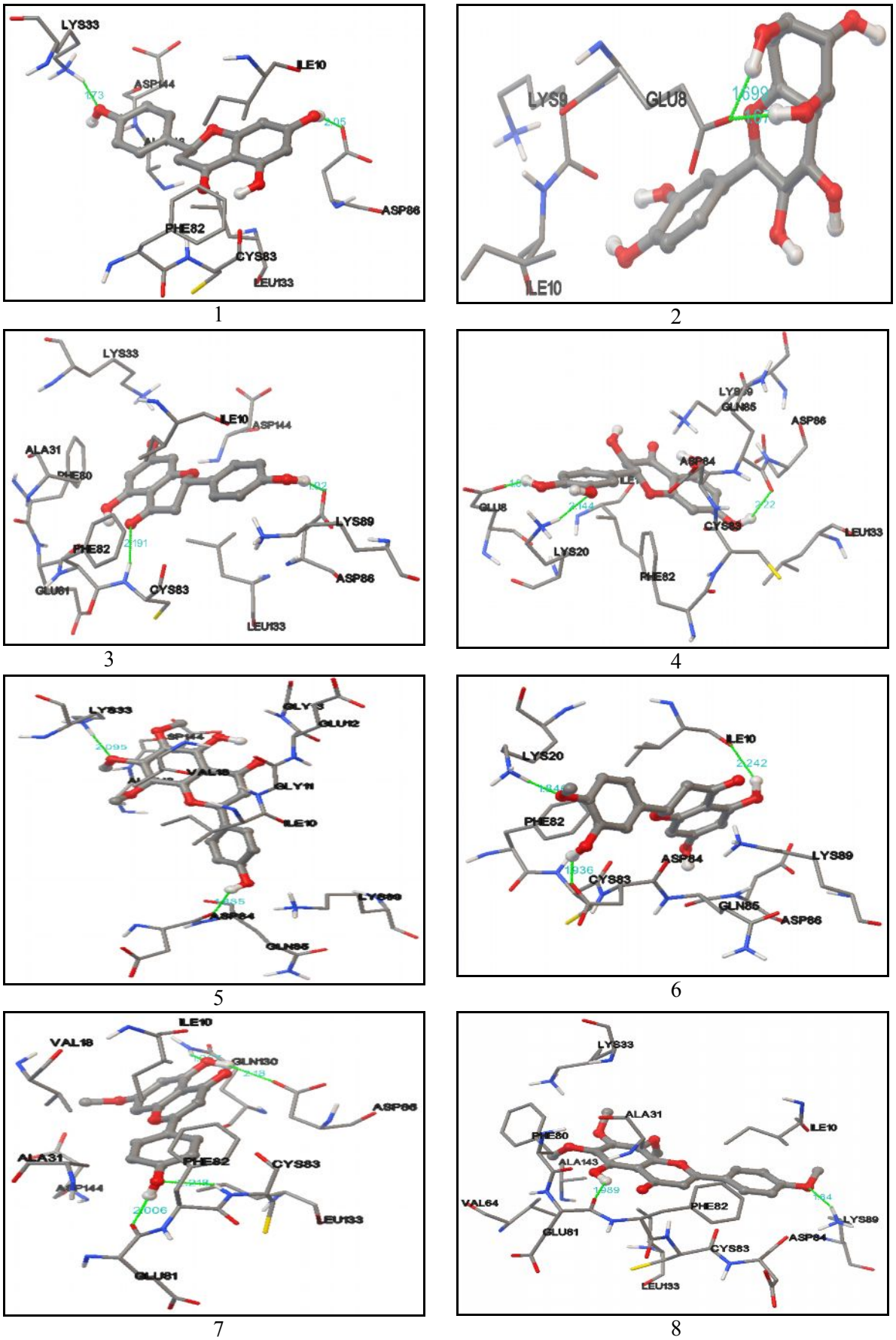


Fig. 3 Docking poses of the compounds (1) Naringenin (2) Quercetagetin (3) Sakuranetin (4) Taxifolin (5) Xanthomicriol (6) Hesperitin (7) Genkwanin (8) Gardenin-Bin the active site of CDK5 of Q543F6

Molecular docking studies were carried out for eight natural 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 -3.67 to -8.22kcal/mol. Compound Gardenin-B having highest binding energy of -8.22kcal/mol. This compound had shown two interactions with Lys89 and Glu81 as shown in fig 3 thus indicating that CDK5 has lowest affinity towards compound one. Compounds Taxifolin, Hesperitin, Genkwanin shows three interactions.

Conclusion:

The 3D structure of Q543F6 of Mus musculus was generated using Modeller 9.15. 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 natural flavonoid derivatives were docking against modelled protein. All compounds shows good binding energy. Compound one shows binding energy of -8.22kcal/mol. Hence we conclude that all these natural flavonoid compounds could be a potential lead molecules for CDK5.

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