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A computational study on binding affinity of Bio-flavonoids on the crystal structure of 3-hydroxy-3-methyl-glutaryl-CoA reductase – An *insilico* molecular docking approach

***Karthik Dhananjayan[1], Khairunnisa Kalathil[1], Arunachalam Sumathy[2], Palanisamy Sivanandy[3]**

** [1]Department of Pharmacology, Molecular Pharmacology and Drug screening division, Grace College of Pharmacy, Kondunthirapully post, Palakkad, Kerala, India*
[2]Department of Pharmaceutical Chemistry, Medicinal chemistry and Drug Design division Grace College of Pharmacy, Kondunthirapully post Palakkad, Kerala, India
[3]Department of Pharmacy Practice, International medical university, Malaysia

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ABSTRACT

Hyperlipidaemia is one of the etiological factor in the development of cardiac disoders. Many traditional plants have been reported to reduce the level of harmful cholesterol. We have studied the binding affinity of some selective bioflavonoids towards HMGCoA reducatse , an enzyme responsible for rate limiting step in mevalonate pathway. Insilico docking studies were performed using Autodock 4.2, to evaluate the binding affinity of bio-flavonoids like (-)-epiafzelechin,2',3,5,6',7 Pentahydroxyflavanone, 3-Dehydrokievitone,2'-Hydroxygenistein, 3,7-O-Diacetylpinobanksin, 3-O-Acetylpinobanksin, 4'-Hydroxywogonin,Acacetin, on crystal structure of 3-hydroxy-3-methyl-glutaryl-CoA reductase . Among them 2,3-Dehydrokievitone shows lowest binding energy (-8.29 kcal/mol). Atorvastatin was used as standard showing free energy of binding -6.14 kcal/mol. Binding site analysis shows interactions with amino acid residues like LYS691, ASP690, VAL805, ASN658, ILE762, ALA768, ASP767, GLY808, MET655, ASP767, GLY656. Analysing binding sites and free energy of binding produced, explains the importance of bioflavonoids in targeting 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibition in the treatment of hyperlipidemia next to statins and other drugs in future.

Keywords: Hyperlipidemia, Bio-flavonoids, 3-hydroxy-3-methyl-glutaryl-CoA reductase, Molecular docking, Computational drug discovery.

INTRODUCTION

Growing lifestyle modifications with increased consumption of fatty dietary substances leads to adverse cardiac disorders like hyperlipidemia,atherosclerosis. Obesity is causing a broad range of health problems that previously weren't seen until adulthood. These include high blood pressure, type 2 diabetes and elevated blood cholesterol levels. There are also psychological effects. Obese children are more prone to low self-esteem, negative body image and depression. Recent estimates suggest that the overall rates of obesity have plateaued or even declined, obesity is widespread and continues to be a leading public health problem in the U.S. **[1]**

3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) is the rate -controlling enzyme of the mevalonate pathway, the metabolic pathway that produces cholesterol and other isoprenoids. It is suppressed by

cholesterol derived from the internalization and degradation of low density lipoprotein (LDL) via the LDL receptor as well as oxidized species of cholesterol. Competitive inhibitors of the reductase induce the expression of LDL receptors in the liver, which in turn increases the catabolism of plasma LDL and lowers the plasma concentration of cholesterol, an important determinant of atherosclerosis.**[2]**

Statins targets HMG-CoA reductase anchored in the membrane of the endoplasmic reticulum in order to reduce the cholesterol level. Molecular structure of HMGCoA reductase shows having seven transmembrane domains, with the active site located in a long carboxyl terminal domain in the cytosol.**[3]**

Hmg CoA reductase inhibitors reduces the serum level of LDL and as well as they increase moderately the level of HDL in blood. **[4]**

Both citrus flavonoids and palm toco-trienols reduce cholesterol levels in laboratory animals**[5]**. Poly methoxylated flavones (PMFs) decrease blood serum levels of apoproteinB, the structural protein of low-density lipoprotein(LDL),which is the major cholesterol carrier in the blood. **[6]**

In this study, we have selected some bio-flavonoids to target 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCoA reductase) and to analyse their binding affinity through computational insilico docking studies. Molecules which possess lowest binding energy should have better binding affinity with a property of enzyme inhibition. Through this evaluation study, we can go for these molecules application in the relevant area of therapy in treating Hyperlipidemia.

Computational methods (*insilico*) are a supportive tool for *invitro and invivo* evaluation. In our study we have employed latest version of AutoDock 4.2. AutoDock 4.2 utilizes Monte Carlo simulated annealing and Lamarckian genetic algorithm (LGA) to create a set of possible conformations **[7]**. LGA is used as a global optimizer and energy minimization as a local search method. For the evaluation of possible orientations, AMBER force field model in conjunction with free energy scoring function is used. Coordinate files preparation, atomic affinities (Auto Grid) calculation. Semi empirical free energy force field is used to evaluate conformations during docking. The Ligand and protein stay in an unbound conformation. Then binding is evaluated in two steps by force field. Force field evaluates intramolecular energetics during the translation from their unbounded states to the conformation of both ligand and protein into the form of bound state. **[8]**

MATERIALS AND METHODS

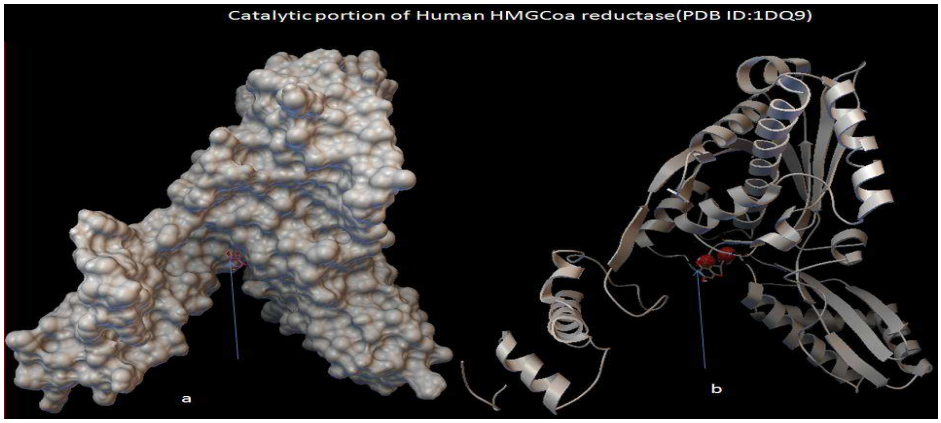


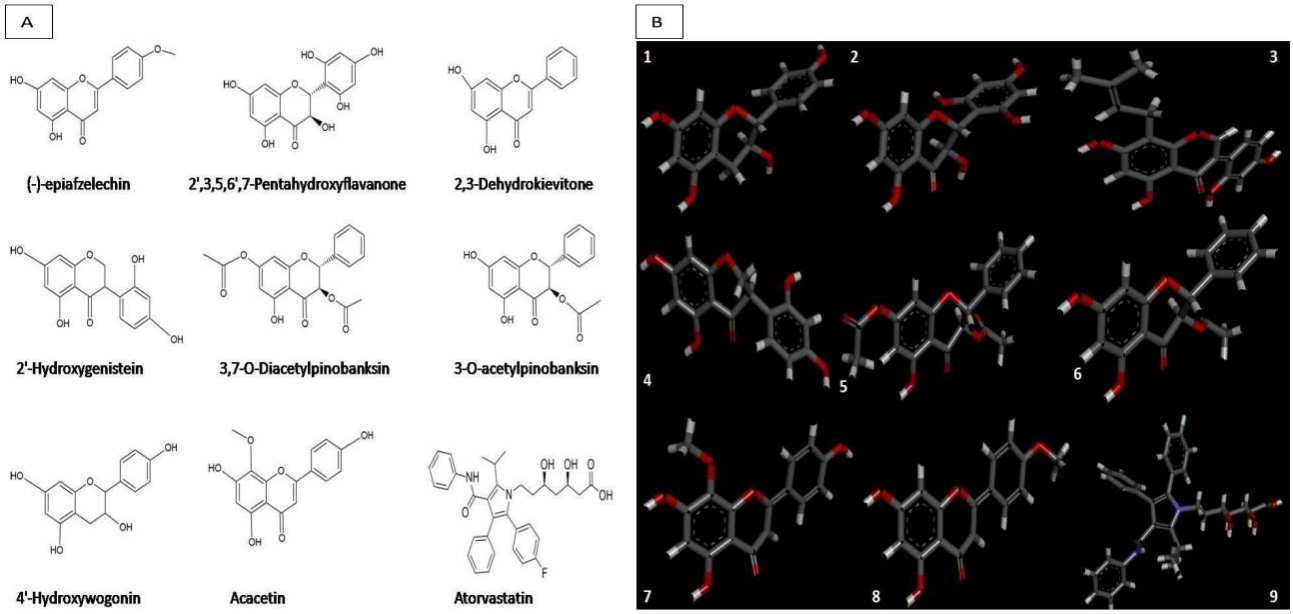
Fig I: Space filled and ribbon model images showing the catalytic portion of the 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCoA reductase) RCSB PDB ID:1DQ9. a) & b) space filled and ribbon model showing the ligand binding at active site

The crystal structure of the investigational enzyme HMG CoA reductase was downloaded from RCSB protein data bank bearing the PDB code - 1DQ9.Python 2.7 - language downloaded from www.python.com,cygwin- a data storage c:\program downloaded from www.cygwin.com, MGL (Molecular Graphics Laboratory) tools– AutoDock4.2 downloaded from www.scripps.edu, Chem sketch downloaded from www.acdlabs.com. Python 2.5

downloaded simultaneously during cygwin download, Accelry's Discovery studio visualizer 3.1–downloaded from www.accelerys.com, Chem Office package- Chem 3D ultra- from www.cambridgesoft.com.

TABLE I: List Of Molecular Formula, Molar Mass, Torsions Of Investigative Ligands

S.NO	LIGAND	MOLECULAR FORMULA	MOLECULAR WEIGHT	TORSIONS (No of rotatable bonds)
1.	Epiafzelchin	$C_{15}H_{14}O_5$	274.3	9
2.	2',3,5,6',7-Pentahydroxyflavanone	$C_{15}H_{12}O_7$	304.3	7
3.	2,3-Dehydrokievitone	$C_{20}H_{18}O_6$	354.4	7
4.	2'-Hydroxygenistein	$C_{15}H_{10}O_6$	286.2	5
5.	3,7-O-Diacetylpinobanksin	$C_{19}H_{16}O_7$	356.3	6
6.	3-O-Acetylpinobanksin	$C_{17}H_{14}O_6$	314.3	5
7.	4'-Hydroxywogonin	$C_{16}H_{12}O_6$	300.3	5
8.	Acacetin	$C_{16}H_{12}O_5$	284.3	4
9.	Atorvastatin	$C_{33}H_{35}FN_2O_5$	558.64	15



FigII: A)image showing 2D structure of the ligands generated using ChemSketch from ACD labs B) 3D optimized ligands after energy minimization using chemdraw 3D 9.0, viewed and photographed from accelry's discovery studio visualizer 3.1 client.1) (-)-epiafzelechin, 2)2',3,5,6',7-Pentahydroxyflavanone,3) 2,3-Dehydrokievitone,4) 2'-Hydroxygenistein,5) 3,7-O-Diacetylpinobanksin, 6) 3-O-Acetylpinobanksin, 7) 4'-Hydroxywogonin,8)Acacetin, 9) Atorvastatin.

Methods:

Docking[8] was performed in making enzyme molecule rigid and ligand to get flexible, in this way different conformation arises during each run and the best conformer fits with lowest binding energy (kcal/mol).

Using the latest version of AutoDock4.2, the enzyme molecule is loaded and stored as hmgcoa.pdb after assigning hydrogen bonds and kollman charges. The investigative ligand was loaded and their torsions along their rotatable bonds are assigned and their file is saved as ligand.pdbqt. Grid menu is toggled, after loading enzyme.pdbqt the map files are selected directly with setting up grid points with 110 X 100 X 98 dimensions for the searching of ligand within the active site of the enzyme molecule. This way the grid parameter files are created with setting up of map files directly. Followed by setting up docking parameter files with search parameter as genetic algorithm and docking parameter utilizing Lamarckian genetic algorithm. Then the docking process is carried out using cygwin interface and their results are are viewed after final Lamarckian genetic algorithm gets completed successfully

RESULTS

Most of the flavonoids possessed satisfactory lowest binding energy on comparing with standard (-6.14kcal/mol). Table II shows the overall binding energy during each conformation through Lamarckian genetic algorithm docked state. Among the flavonoids under our investigation, free energy binding of 2,3-Dehydrokievitone, 3-O-Acetylpinobanksin,Acacetin, 3,7-O-Diacetylpinobanksin, 2'-Hydroxygenistein was found to be -8.29 kcal/mol, -7.46 kcal/mol, -7.28 kcal/mol ,-7.18 kcal/mol, -7.57 kcal/mol respectively. Followed by Epiazelchin and 4' hydroxywoginin showed lowest binding energy of -5.95 and -6.99 kcal/mol respectively. From the graph it is easy to identify the clustering of energy levels with best conformation at the active site of enzyme. Fig.4 shows the conformations of the ligands with their lowest binding energy. The positioning of ligands at the active site shows their angle stretching towards the amino acid residue with hydrogen bond (Å) formation.

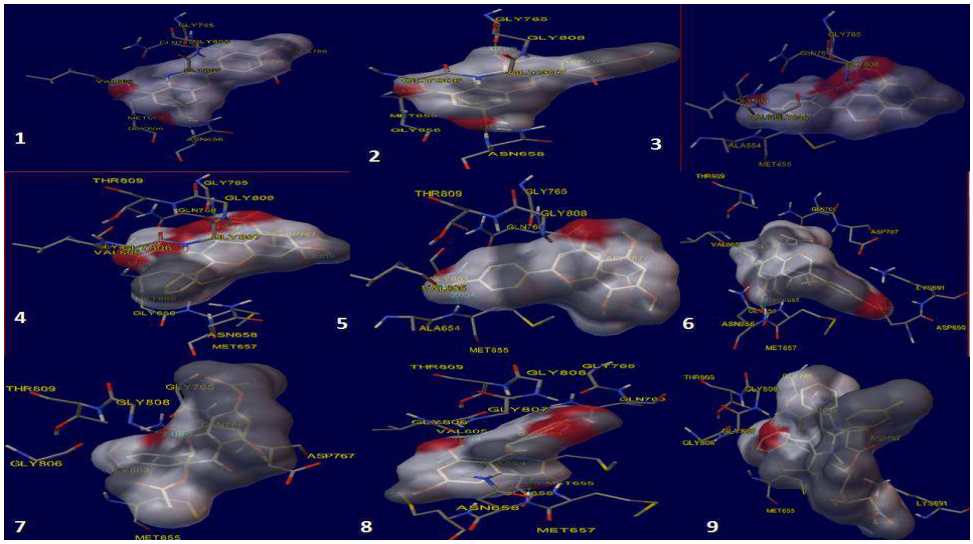
Table II: Final Lamarckian Genetic Algorithm Docked State – Binding Energy of Ligands With The Active Site of The Enzyme During Ten conformations

S.No		Ligands	Final Lamarckian Genetic Algorithm Docked State Over all Binding energy during each Conformation (kcal/mol)									
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1.	1	Acacetin	-7.28	-7.27	-7.27	-7.26	-7.25	-7.23	-7.18	-6.85	-6.58	-6.56
2.	4	-(-)Epiafzelchin	-5.95	-5.94	-5.94	-5.91	-5.83	-5.76	-5.7	-5.17	-5.15	-5.06
3.	5	2',3,5,6',7 Pentahydroxyflavanone	-6.54	-6.52	-6.26	-6.22	-6.21	-5.59	-5.58	-5.57	-5.34	-4.9
4.	6	2,3-Dehydrokievitone	-8.29	-8.27	-7.82	-7.13	-7.12	-7.11	-6.96	-6.63	-6.14	-6.6
5.	7	2'-Hydroxygenistein	-7.46	-7.39	-7.39	-7.32	-7.2	7.14	-7.3	-7.06	-7.02	-6.39
6.	8	3,7-O-Diacetylpinobanksin	-7.18	-7.02	-6.88	-6.82	-6.71	6.67	-6.64	-6.45	-6.41	-6.38
7.	9	3-O-Acetylpinobanksin	-7.57	-7.53	-7.51	-7.44	-7.42	-7.4	-7.33	-7.16	-6.93	6.46
8.	10	4'-Hydroxywogonin	-6.99	-6.82	-6.97	-6.26	-6.24	-6.24	-6.14	-6.11	-6.05	-5.93
9.	11	Atorvastatin	-6.14	-6.1	-5.5	-4.7	-4.1	-4.31	-4.16	4.04	-3.9	-2.46

Table III. shows the estimated inhibition constant (K_i) of those ligands with lowest binding energy in range of nM to µM. This shows the potential of the ligand affinity upon binding with the active site of the enzyme. While remaining ligands, have showed estimated inhibition constant in the range of millimole (mM). Intermolecular energy was found to be efficient in creating a definite pose prior to formation of hydrogen bond formation.

Table III: Parameters of best conformer at lowest binding energy (kcal/mol)

S.No	Ligand	Lowest Binding Energy (kcal/mol)	Estimated Inhibition Constant (kI)	Inter-molecular Energy (kcal/mol)	Internal Energy (kcal/mol)	Torsional Energy (kcal/mol)	Unbound Extended Energy (kcal/mol)	Cluster Rms	Ref Rms
1	Acacetin	-7.28	4.64 µM	-8.47	-0.97	1.19.	-0.97	0.0	15.87
2	Epiafzelchin	-5.95	43.39 µM	-7.44	-0.14	1.49	-0.14	0.0	12.11
3	2',3,5,6',Penta-hydroxyflavanone	-6.54	16.15 µM	-8.63	-1.14	2.09	-1.14	0.0	14.67
4	2,3-Dehydrokievitone	-8.29	843.61nM	-10.37	-1.55	2.09	-1.55	0.0	17.41
5	2'-Hydroxygenistein	-7.46	3.42 µM	-8.95	-0.56	1.49	-0.56	0.0	18.12
6	3,7-O-Diacetylpinobanksin	-7.18	5.41 µM	-8.97	-1.49	1.79	-1.49	0.0	15.11
7	3-O-Acetylpinobanksin	-7.57	2.55 µM	-9.06	-1.31	1.49	-1.31	0.0	15.17
8	4'-Hydroxywogonin	-6.99	7.57 µM	-8.48	-1.19	1.49	-1.19	0.0	15.29
9	Atorvastatin	-6.14	31.39 µM	-10.62	-1.17	4.47	-1.17	0.0	15.37



FigIII: Images showing the conformers of investigating ligands at the active site area of HMGCoA reductase after protein-ligand docking. Acacetin, epizelechin, 2hydroxygenestein, 30acetylpinobanksin, 4hydroxywogonin, 23dehydrokievitone, 370acetylpinobanksin,23567 pentahydroxyflavone, atorvastatin

The hydrogen bond distance (between the ligand and aminoacid residue of the enzyme) and binding site analysis with respect to ligands positioned at the active site will be dealt in discussion. Table IV shows the aminoacid residues interacted with the ligand's lowest binding energy. Cluster RMSD values of investigative ligands showed in table with reference cluster RMSD values explains the best conformation (fig.5) of the ligand at the active site.

Table IV. Amino acids involved in interaction with the ligands at the active site

S.No	Ligand	Lowest Binding Energy (kcal/mol)	Amino acid residue involed in Hbond formation
1.	Acacetin	-7.28	ASN658 ,MET655,GLY808
2.	Epiafzelchin	-5.95	ASN658 ,MET655,GLY808, ,GLY765
3.	2',3,5,6',7 Pentahydroxyflavanone	-6.54	ILE762,ALA768,GLY808,CYS526, VAL805,ASN658
4.	2,3-Dehydrokievitone	-8.29	LYS691,ASP690, VAL805, ASN658,ILE762, ALA768,ASP767, GLY808
5.	2'-Hydroxygenistein	-7.46	MET655,ASP767,GLY808,GLY656
6.	3,7-O-Diacetylpinobanksin	-7.18	GLY808;ALA768
7.	3-O-Acetylpinobanksin	-7.57	VAL757,VAL846,CYS843GLY765,GLY808,MET655
8.	4'-Hydroxywogonin	-6.99	MET655,ASN658,MET655,VAL805
9.	Atorvastatin	-6.14	GLU528,ARG590,LYS691,GLY808, MET659, ALA769,GLY808.

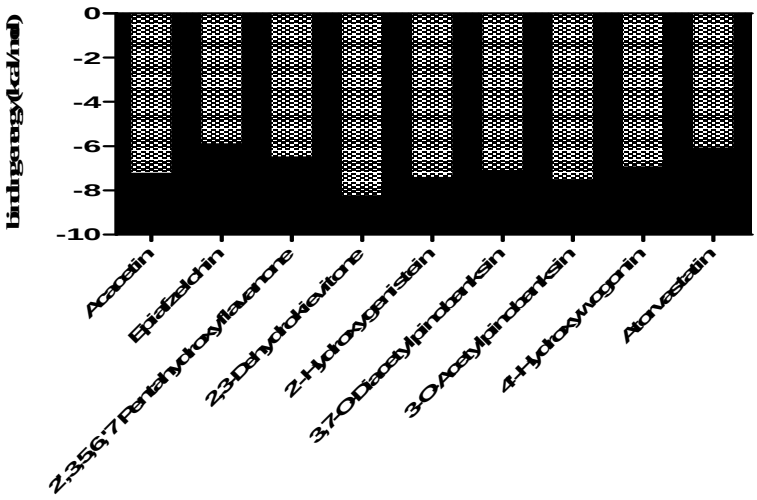


Fig IV. Graphical representation showing the binding energy (kcal/mol) values of ligands used in study.

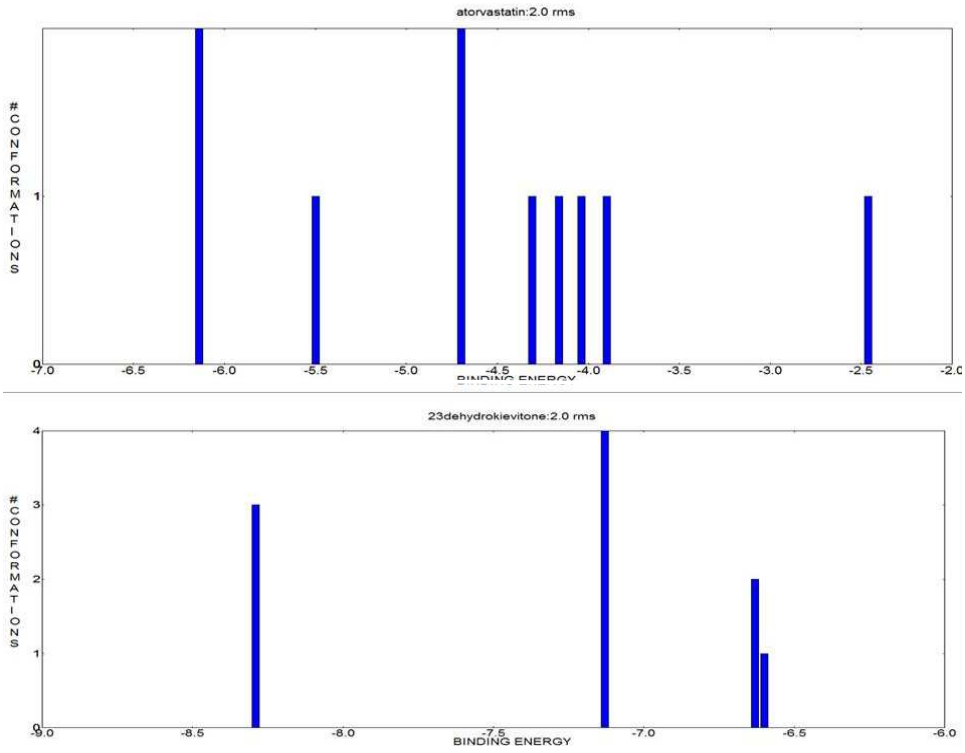


Fig V: cluster analysis image showing conformations of Atorvastatin and 2,3dehydrokievitone at their free energy of binding. (Source: Image generated using AutoDock 4.5)

DISCUSSION

Discussion is followed with special emphasis on analysis regarding interaction of ligands at the active site of the enzyme

At their lowest binding energy, the ligands are positioned at the active site as shown in the figure 4. Investigational ligands at their lowest binding energies occupied the active site with maximum affinity.

The Crystal structure of HMG-CoA reductase has shown in fig1.The potential drugs of synthetic importance like statins found to be accumulate at the active site residues like LEU 562 , VAL 683 , LEU 853 , ALA 856 , AND LEU 857, SER 565, ARG 590,LYS691.**[9]**

This substrate specificity and ligands developed with inter atomic distance measurements between ligands and aminoacid residue at the active site of the enzyme has its importance in acting as inhibitors. The catalytic portion (residues 426–888) of recombinant human HMGR was expressed and discussed in detail catalytic portion contain residues 477–863, while additional N- (439–476) and C–terminal residues (863–872).**[10]**

Atorvastatin was found to bind with amino acid residues LYS691, GLY808. Oxygen atom at -NH –C=O of 4-phenyl carbomyl attached to pyrrole ring Hbond with amino group of GLY808 (1.956Å) and terminal hydroxyl group of dihydroxy heptanoic acid extends it arm by making Hbond at terminal amino group of LYS 691 at its lowest binding energy (-6.14 kcal/mol). At different runs of their conformations atorvastatin has interaction with GLU528, ARG590, MET659, ALA769. Atorvastatin binds with residues such as ARG515, ASP516, TYR517 AND ASN518 are found to be conserved in HMGCR isoform 2 with various statins. **[11]**

Atorvastatin Several polar interactions are formed between the HMG-moieties and residues that are located in the cis loop (SER 684, ASP 690 , LYS 691 , LYS 692). LYS 691 also participates in a hydrogen-bonding network with GLU 559, ASP 767 and the O5-hydroxyl of the statins.**[10]**

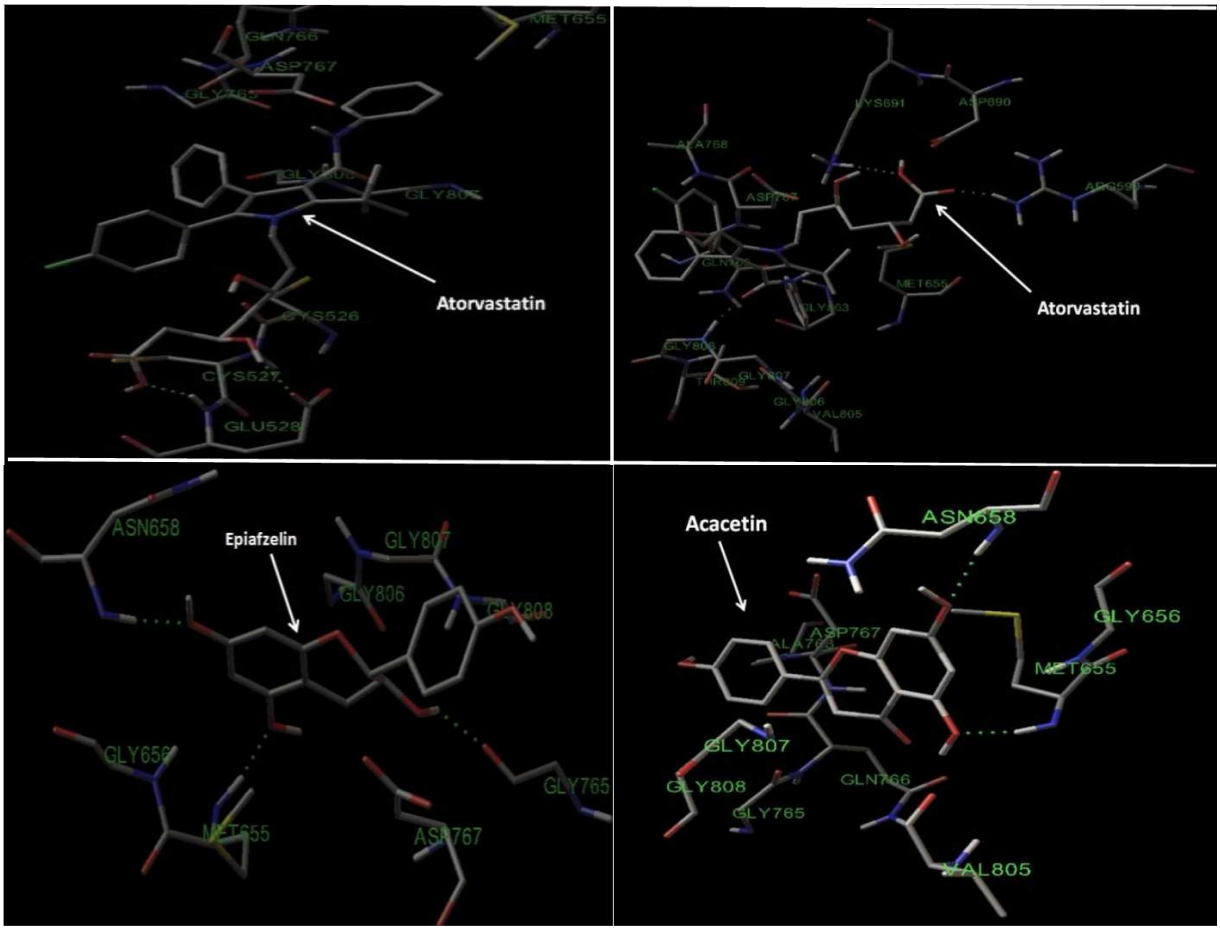


Fig VI: Binding site analysis :Showing the interaction between 3D structures of ligands (atorvastatin,epiafzelchin,acacetin) and the crystal structure of HMG CoA reductase inhibitors

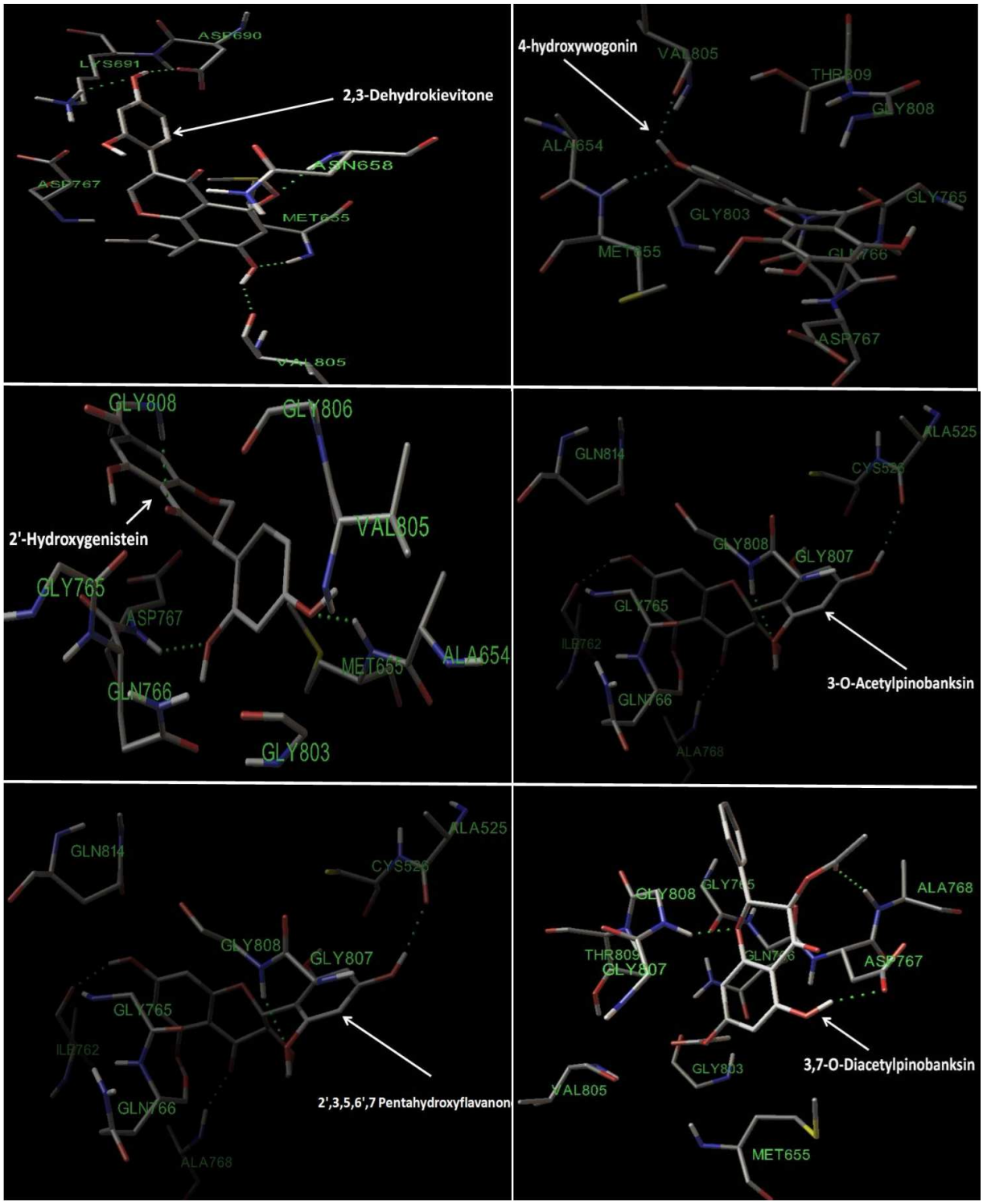


Fig VII: binding site analysis: Showing the interaction between 3D structures of ligands (2,3 Dehydrokievitone, 4-hydroxywogonin, 2'Hydroxygenin,3-O-Acetylpinobanksin, 2'3,5,6,7'-Penta- hydroxyflavone, and 3,7 O-diacetylpinobanksin) with the crystal structure of HMG CoA reductase inhibitors.

Acacetin was found to bind with ASN658, MET655; GLY808 at its free energy of binding (-7.28kcal/mol). O1 interacts with GLY808. C6 and C7 substituted hydroxyl groups makes an arm on both sides by

hinged on the flavone nucleus , in such a way Hbond forms between the continuous amino acid residue MET 655(1.752 Å) AND ASN 658(1.993Å) leaving out in between residues, this explains distance variation of the ligand positioning at the active site.

Epiafzelechin was found to interact with ASN658, MET655, ASN658,GLY765 at its lowest binding energy(-5.95 kcal/mol). As like in Acacetin , C6 and C7 substituted hydroxyl groups of epiafzelchin makes an arm on both sides by hinged on the flavone nucleus interacts with MET 655(1.831 Å) and ASN658(1.878 Å),whereas C3 hydroxyl group interacts at GLY765(1.799Å).

2',3,5,6',7Pentahydroxyflavanone interacts with ILE762,ALA768,GLY808, CYS526, VAL805. C2' and C6'substituted hydroxyl group of Trihydroxy phenyl attached at C2position of flavonone nucleus interacts with peptidyl oxygen (C=O)atom of VAL805(1.95 Å) and peptidyl –NH atom ASN658(2.0 Å) at its lowest binding energy (-6.54 kcal/mol)through Hydrogen bonding.

2,3-Dehydrokievitone pose itself at the vicinity of active site with a lowest binding energy of -8.29 kcal/mol . Interactions made with residues like LYS691, ASP690, VAL805, ASN658, ILE762, ALA768, ASP76. C4' hydroxyl group of Dihydroxy phenyl group at C3 of flavones interacts through Hbond with terminal C=O group of ASP 690 (1.917Å). C5 and C7 hydroxyl groups interacts with VAL805 (1.878 Å) and MET655 (2.02 Å). At the second most lowest binding energy (kcal/mol)has its different conformer of interaction with residues LYS691(1.75 Å), ASN658(2.14Å), ILE762(1.15 Å), ALA768 (1.12 Å)and ASP76 (0.68 Å).

2'-Hydroxygenistein 2hydroxygenestein at its free energy of binding (-7.46 kcal/mol) interacts with MET655, ASP767, GLY808. C2' and C4' hydroxyl group of Dihydroxy phenyl group at C3 of flavones interacts through hydrogen atom of peptidyl –NH of ASP 767 (2.18 Å)and fragmental Hbond with GLY806 & VAL805(1.854 Å) , hydrogen atom of peptidyl -NH group of MET655 through Hbond(2.087Å) respectively. Whereas the main nucleus containing C=O group at C4 position Hbond with GLY808 (1.9Å).

3, 7-O-Diacetylpinobanksin interacts with GLY808; ALA768 at its lowest binding energy (-7.18 kcal/mol). Oxygen atom (O1) of flavone nucleus Hbond with GLY808 (2.096Å). At its next lowest binding energy (kcal/mol) Oxygen atom (O1) of flavanone nucleus Hbond with GLY808 (1.98Å) and acetyl group (H_3C-C=O) at C3 and C7 has its significant role in interaction with amino acid ALA 768(1.54 Å) and LYS691 (2.65 Å).**[11]**

3-O-acetylpinobanksin conformer at its lowest binding energy (-7.57kcal/mol) makes a fragmental (GLY765 and GLY766) Hbond (1.955 Å); GLY808; MET655; ASP757; VAL846, CYS843. C3 ketonic oxygen accepts Hbond from peptidyl –NH atom of GLY808 (2.567 Å). C3 and C7 hydroxyl groups interacts with VAL 846(1.95 Å) AND CYS843(1.54 Å) .

4′hydroxywogonin with its conformer of lowest binding energy(-6.99kcal/mol)interacts with MET655, VAL805 .C2 substituted phenolic hydroxyl group containing oxygen and hydrogen atom interacts with peptidyl –NH group of MET655 and VAL805.

Overall binding site analysis reveals the flavone with hydroxyl groups at C5 and C7, oxygen atom at O1 and C3 (-C=O) were involved in majority of interactions with amino acids at the active site of 3-hydroxy-3-methyl-glutaryl-CoA reductase.

CONCLUSION

It has been concluded that bioflavonoids shows lowest binding energy than Atorvastatin. These ligands interacted with the amino acids with more efficiency than the standard. These flavone compounds can be analysed for *invitro* enzyme inhibition studies in order to be a useful lead in the HMGCoA reductase inhibiton and in the treatment of hyperlipidemia.

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