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Insilico docking analysis of Dipeptidylpeptidase-4 (DPP-IV or CD26) with some selective bioflavonoids using Genetic Lamarckian Algorithm

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ABSTRACT

Some selective polyphenolics was designed computationally and screened through insilico docking studies against crystal structure of Dipeptidylpeptidase-IV (DPP-IV) as a projected target for Type 2 Diabetes Mellitus. Insilico docking (rigid and flexible) methodology using Auto Dock 4.2 comprising a search method Genetic Lamarckian algorithm was used. Genetic Lamarckian algorithm performs an Automated Docking and has an advantage of empirical binding free energy force field that allows the prediction of binding free energies, and hence binding constants, for docked ligands. In-silico evaluation shows satisfactory docking results, when compared with standard using rigid and as well as flexible docking It is concluded that investigational ligands has the potential of inhibiting DPP-IV and there by further screening (invitro and invivo) studies can be carried out in order to find out optimized bioflavonoids for treating type2diabetes mellitus.

Key words: Bio-flavonoids, AutoDock4.2, Rigid docking, Flexible docking, Genetic Lamarckian Algorithm, DPPIV-inhibition.

INTRODUCTION

The investigational enzyme, Dipeptidylpeptidase-IV is a GIP and GLP-1 inhibitor. GIP and GLP-1 acts on GLP receptors and regulate the insulin release in pancreatic islets of langerhans. But this activity was found to inhibited by T-cell activation antigen CD26 (DPP-IV), and there by increases the serum glucose level upon decrease in release of insulin from the pancreatic islets of langerhans [1]. Furthermore, it appears to work as a suppressor in the development of neoplasia[2][3][4]. CD26/DPPIV plays an important role in tumor biology, and is useful as a marker for various cancers, with its levels either on the cell surface or in the serum increased in some neoplasm and decreased in others [5]. DPP-4 also binds the enzyme adenosine deaminase specifically and with high affinity. The significance of this interaction has yet to be established.

The crystal structure has been extensively discussed showing DPP-IV is a serine protease that specifically cleaves N-terminal dipeptides from polypeptides with Pro and Ala at the penultimate position. In DPP-IV, Each monomer consists of an N-terminal β-propeller domain (Lys56-Asn497) and a C-terminal catalytic domain (Gln508-Pro766, together with segment Leu45-Val55). Catalytic domain and propeller domain together embrace an egg-shaped cavity of approximate dimensions 40Å X 20Å X 20Å, which harbours the active centre[6].Most dipeptidylpeptidase-4inhibitors were designed according to the substrate P1 site structure (occupied by proline), namely the proline-like compounds[7]. The majorities of these are peptide-like compounds and contain cyanopyrrolidine moiety, which forms covalent bond to the catalytic residue Ser630 by the nitrile group. In addition to the proline-like compounds, a variety of non-peptide-like and reversible dipeptidylpeptidase-4inhibitors[8] were also discovered via high-throughput screens and offered new recognition motifs to dipeptidylpeptidase-4.

Dipeptidyl peptidase IV (DPIV, CD26,)is a multifunctional membrane-anchored serine ectopeptidase belonging to the α,β-hydrolases and sequentially related to the prolyl oligopeptidase (POP). Then human DPPIV cDNA encodes a 766 amino acid residue type II transmembrane glycoprotein consisting of six cytoplasmic residues, a 22 residue transmembranespanning region, and a 738 residue extracellular domain. [9]

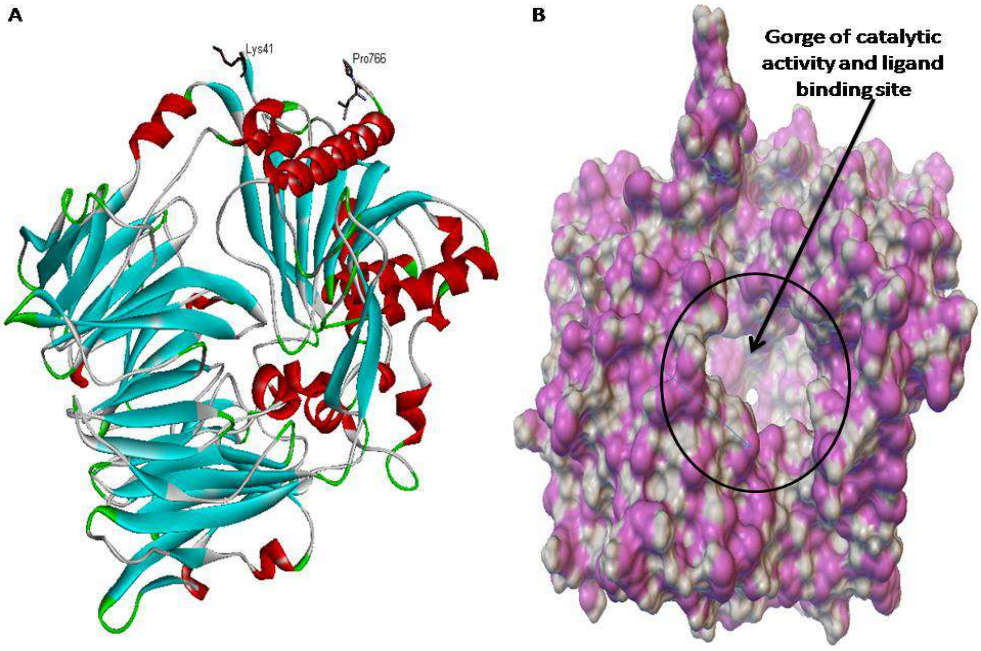

Fig 1: The crystal structure of DPP-IV bearing the RCSB PDB code: 4FFW visualized using Accelerys Discovery studio visualizer 3.1 client A. Ribbon diagram showing N-terminal β-propeller domain (Lys56-Asn497) and a C-terminal catalytic domain (Gln508-Pro766, together with segment Leu45-Val55). B.The surface diagram showing the gorge of catalytic activity and ligand binding site

Dipeptidylpeptidase-4 exists as a homodimer and each monomer consists of two domains; an alpha/beta hydrolase domain and an eight-blade beta-propeller domain [10] Dipeptidylpeptidase-4 binds to, but does not cleave, adenosine deaminase, kidney Na+/H+ ion ex-changer and fibronectin, which localizes these molecules to the cell surface. A soluble form of dipeptidylpeptidase-4 does also exists, although it is only known to function in relation to T-cell proliferation. The human gene encoding T-cell activation antigen CD26 is localized to chromosome 2q24.2 [11]

Citrus flavonoids have been evaluated for on enzyme targets related to Diabetes like GSK3β, DPP-IV, and PPARγ through molecular Docking Studies [12].

Then *in-vitro* DPP-IV inhibitory activity by extracts of *P.daemia* shows significant activity ($P<0.01$) towards the enzyme [13].

Computational methodologies and their tools made drug discovery process less time consuming and decreased the usage of animals in prior to preclinical studies. In silico studies were employed for the simulation of physiological systems including physiological macromolecules like receptors, enzymes were designed using modeling softwares and analysed for their simulating activities. In this advanced drug discovery process the simulated proteins can be targeted, if any underlying implications are because of all those enzymes. In this study, some selective bioflavonoids were computationally designed and potential binding affinity studies against crystal structure of DPP-IV was carried out [14]

Using Auto Dock 4.2 we can perform both rigid docking and as well as flexible docking. *Insilico* docking (rigid and flexible) methodology using Auto Dock 4.2 comprising a search method Genetic Lamarckian algorithm was used[15] Genetic Lamarckian algorithm performs an Automated Docking and has an advantage of empirical binding free energy force field that allows the prediction of binding free energies, and hence binding constants, for docked ligands [16]. The vast majority of genetic algorithms mimics the major characteristics of Darwinian evolution and applies Mendelian genetics. It is an one-way transfer of information from the genotype to the phenotype. However, in those cases where an inverse mapping function exists i.e., one which yields a genotype from a given phenotype, it is possible to finish a local search by replacing the individual with the result of the local search. This is called the Lamarckian genetic algorithm LGA , and is an allusion to Jean Batiste de Lamarck's

discredited assertion that phenotypic characteristics acquired during an individual's lifetime can become heritable traits[17]

MATERIALS AND METHODS

The crystal structure of DPP-IV with RCSB PDB code: 4FFW was downloaded from www.rcsbpdb.com.Python 2.7 - language downloaded from www.python.com, Latest version of MGL (Molecular Graphics Laboratory) tools– AutoDock 4.2.5.1 downloaded from www.scripps.edu, Chem sketch downloaded from www.acdlabs.com. Accelry's Discovery studio visualizer 3.1 was downloaded from www.accelerys.com, Chem Office package- Chem 3D ultra- from www.cambridgesoft.com.

Preparation of macromolecule

Macromolecule has to be prepared, prior to docking process. Preparation involves removal of water molecule and any unwanted hetero atoms. After refining enzyme macromolecule is saved as dpp4.pdb execution file.

Preparation of ligands

The ligands were designed using chemsketch and their 2d structure was converted to 3D structures using Chem3D ultra 6.1 and they were energy minimized using MM2.These energy optimized ligands were used for docking evaluation. Fig 2 shows the energy minimized ligands and Table 1 shows the ligands with molecular formula, molar mass, and number of torsions in the ligands.

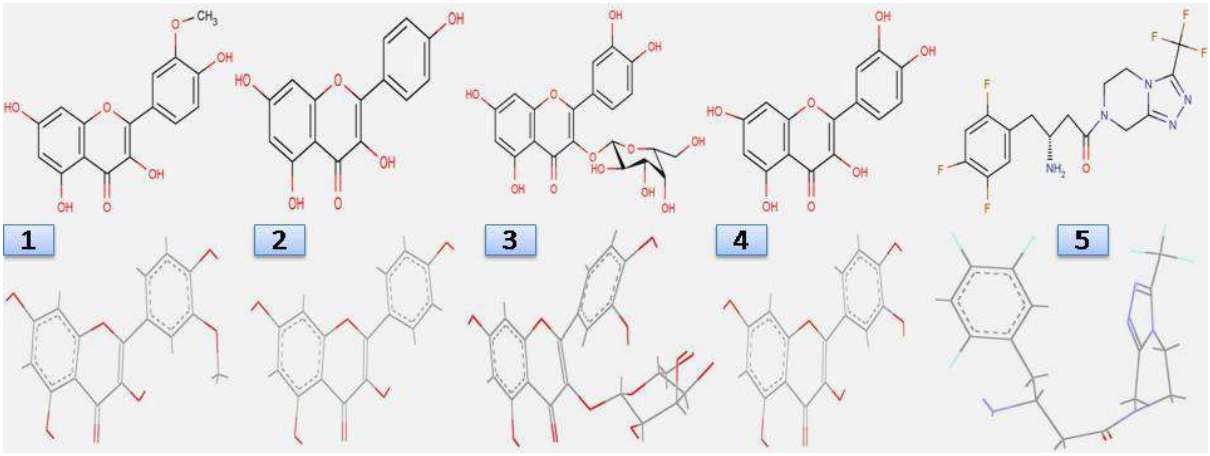

Fig2. Image showing the 2D (above the numeral) and 3D (below the numeral) optimized ligands (1.Isorhamnetin, 2. kaempferol 3. hyperoside, and 4. Quercetin 5.Sitagliptin) using Chem 3D Ultra 9.0

TABLE 1: The general Molecular Formula, Molar Mass, Number Of Torsions (No Of Rotatable Bonds) of the ligands

S.No	Ligands	Molecular Formula	Molar Mass* g mol−1	Log P	Hydrogen bond donors	Hydrogen bond acceptors	Rule Of 5 No of Violations	Torsions No Of Rotatable Bonds
01.	Hyperoside	$C_{21}H_{2O}O_{12}$	464.8	1.75	12	06	2	12
02.	Isorhamnetin	$C_{16}H_{12}O_7$	316.26	1.76	07	04	0	05
03.	Kaempferol	$C_{15}H_{10}O_6$	286.24	2.05	06	04	0	05
04.	Quercetin	$C_{15}H_{10}O_7$	302.24	2.08	07	05	1	06
05.	Sitagliptin**	C16H15F6N50	407.31	1.30	06	02	0	06

**The above mentioned values are at 25 °C 100 kPa*
** * standard or reference ligand used for docking evaluation*

Validation of molecular docking [18]

To know the accuracy of molecular docking, the methodology has to be validated prior to investigation of ligands. The co-crystallized ligand was extracted from the DPP-IV crystal structure and re-docked on to its active site one other than the other and table 2 shows the free energy of binding. The standard reference ligand (sitagliptin) was extracted from the pdb of structure and re-docked again onto active site to evaluate the docking power.

Docking methodology [19][20]

Auto dock 4.2.5.0 needed cygwin interface for running the AutoDock in windows platform but new version of AutoDock 4.2.5.1 (release date 30-05-2013) can be run without a cygwin interface

Rigid docking was performed after making enzyme molecule rigid and ligand to get flexible. By this way different conformation arises during each run and the best conformer fits with lowest binding energy (ΔG)

Using the latest version of AutoDock4.2.5.1, the enzyme molecule is loaded and stored as DPP-IV.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 X104X108 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 of docking parameter files with search parameter as genetic algorithm and docking parameter utilizing Lamarckian genetic algorithm was carried out. Then the docking process is carried out using command prompt and their results are viewed after final Lamarckian genetic algorithm gets completed successfully.

Flexible docking is performed by selecting the strings in order to make the flexibility of the aminoacids desired. The respective aminoacids has to be entered, the torsions in that aminoacids has to be chose with saving their rigid and flexible files. Grid parameter files are then created with setting up grid points 110 X104X108 without setting up map files directly. Docking parameter files are created by setting up rigid and flexible molecules then 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 command prompt and their results are viewed after final Lamarckian genetic algorithm gets completed successfully.

The lowest binding energy, binding site interactions, dissociation constant can be analysed for each run with best cluster.

RESULTS

Rigid docking against crystal structure of dipeptidylpeptidase-4

Table.2 Shows the overall final lamarckian genetic algorithm docked state i.e., binding energy of ligands with the active site of the enzyme during ten conformations generated as rigid docking against crystal structure of dipeptidylpeptidase-4 was performed. hyperoside (-5.04kcal/mol), isorhamnetin(-7.51kcal/mol), kaempferol (7.17kcal/mol), quercetin (-7.19kcal/mol), Estimated dissociation constant (Kd)values was found to be nano molar values. Table.3 shows varying estimated dissociation constant (k_d) depending upon the binding energies of the ligand, which supports the potential of the ligands.

Table 2: 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 of various runs Binding energy during each Conformation (kcal/mol)									
		1	2	3	4	5	6	7	8	9	10
01.	Hyperoside	-5.04	-5.02	-4.51	-4.41	-4.32	-3.97	-3.46	-3.31	-3.21	-2.35
02.	Isorhamnetin	-7.51	-6.6	-6.6	-6.58	-6.21	-6.14	-6.12	-5.3	-6.31	-5.48
03.	Kaempferol	-7.17	-6.49	-6.49	-6.45	-6.45	-6.44	-6.66	-6.14	-5.9	-5.72
04.	Quercetin	-6.71	-6.74	-6.71	-6.7	-6.17	-6.06	-6.0	-5.95	-5.96	-5.8
05.	Sitagliptin	-8.27	-7.85	-7.72	-7.05	-7.04	-6.99	-6.87	-6.49	-5.99	-5.67

Table 3: Rigid Docking – Parameters of best conformer at lowest binding energy (kcal/mol)

S.No	Ligand	Lowest Binding Energy (kcal/mol)	Estimated Dissociation constant (Kd)	Inter molecular Energy (kcal/mol)	Internal Energy (kcal/mol)	Torsional Energy (kcal/mol)	Unbound Extended Energy (kcal/mol)	Cluster Rms	Ref Rms
01.	Hyperoside	-5.04	202.48 uM	-8.62	-3.78	3.58	-3.78	0.0	62.05
02.	Isorhamnetin	-7.51	3.11uM	-9.01	-1.23	1.49	-1.23	0.0	62.39
03.	Kaempferol	7.17	5.55uM	-8.66	-0.29	1.49	-0.29	0.0	61.69
04.	Quercetin	-7.19	5.4uM	-8.98	-0.6	1.79	-0.6	0.0	61.64
05.	Sitagliptin	-8.27	870.32nM	-10.06	-0.22	1.79	-0.22	0.0	74.73

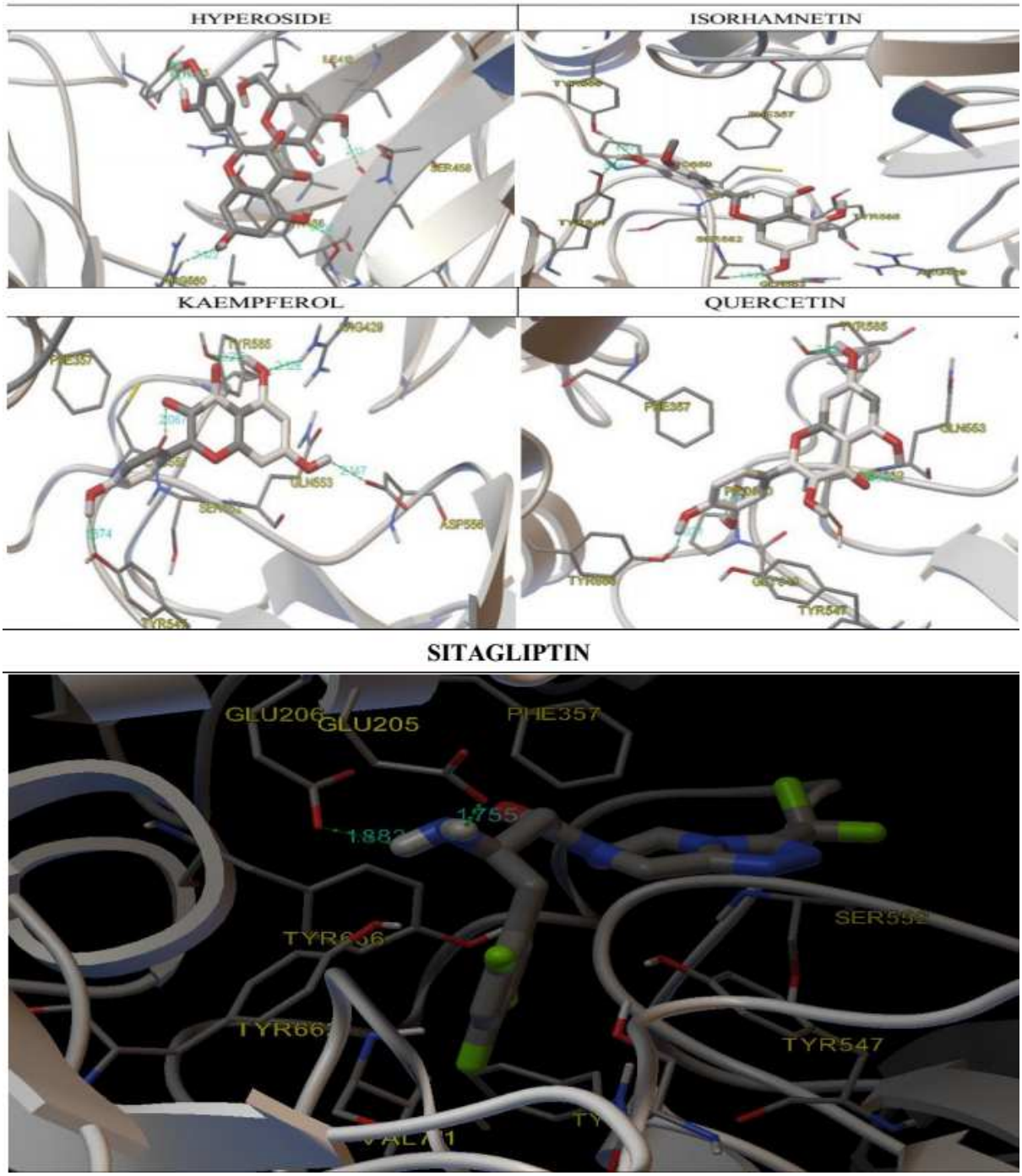

Figure: AutoDock 4.2 generated image shows the interaction of ligands with the aminoacid residues at their free energy of binding (ΔG)

Flexible docking against crystal structure of dipeptidylpeptidase-4

Table 4 Shows binding energy of ligands with the active site of the enzyme during flexible docking with ten conformations generated against crystal structure of dipeptidylpeptidase-4 was performed. Table 4 list out the amino acids selected for torsions and as well as the presence of torsions in those selected amino acid residues. Flexible docking results were different from rigid docking results in the view of observing binding energies and estimated dissociation constant.

Table 4: Table showing the hydrogen bond length between aminoacids involved in hydrogen bond interaction with the ligands

S.No	Ligand	Binding affinity (Kcal/Mol)	Amino Acids Involved In Hydrogen Bond Interaction	Hydrogen Bond Length(Å)
01.	Hyperoside	-5.04	DPPIV:A:SER473:HG 1 DPPIV:A:ARG560:HE 1	1.702 2.122
02.	Isorhamnetin	-7.51	ISORHAMNETIN::FRA 1:H1 ISORHAMNETIN::FRA 1:H1 DPPIV:A:TYR666:HE 1	1.827 2.181 1.970
03.	Kaempferol	7.17	Kaempferol: :FRA 1:H1 Kaempferol: :FRA 1:H1 Kaempferol: :FRA 1:H1 DPPIV:A:ARG429:HH 12 1	1.485 2.095 2.113 1.712
04.	Quercetin	-7.19	QUERCETIN: :FRA1:H1 QUERCETIN: :FRA1:H1 DPPIV:A:TYR666:HH 1 DPPIV:A:GLN553:HN 1	1.955 2.062 2.031 1.998
05.	Sitagliptin	-8.27	DPPIV:A:GLU205:HH 11 1 DPPIV:A:GLU206:HH 11 1	1.775 1.882

DISCUSSION

The selective polyphenolics was found to interact during rigid docking via., hydrogen bonds were Arg125, His 126, Ser158,Ser209, Gly355, Glu361,His363, Phe357, Arg356, Arg358, Arg 382, Gly 406 , Arg 429, Ile407,Ser473, Tyr547, Cys551 , Ser552 , Gln553,Asp556 , Tyr585,Ser630,Tyr666.

The following discussion explains the mode of binding interactions of the flavonoids on the active site of DPP-IV in relation to its crystal structure.

Moreover all the docked ligands have its interaction with expected amino acids of active centre shown in figure 6.

Table 4: Table shows the free energy of binding of computationally designed ligands with their estimated dissociation constant (kd) created during flexible docking on aminoacid residues selected for torsions. against crystal structure of Dipeptidylpeptidase-IV

S.NO	Rigid Ligand	Amino Acids Selected For Flexibility	Torsions Of Selected Amino Acids (No: Of Rotatable Bonds)	Binding Energy (Kcal/Mol)	Estimated dissociation constant (Kd)
01.	Hyperoside	SER158, TYR585, TYR547,SER630	5/32	-2.26	29.83 uM
02.	Isorhamnetin	TYR666, SER552, PHE35, LYS554	5/32	-7.50	3.18 uM
03.	Kaempferol	Tyr547, Tyr585	4/32	-6.43	19.31 uM
04.	Quercetin	ILE407, SER552, TYR666	7/32	-1.55	73.52 mM

Hyperoside contains galactoside at C3 position with with four hydroxyl groups involving in Hbond during their run at the lowest binding energy (-5.04 kcal/mol). In this state, the flexibility of galactoside in its conformation interacts with SER158 through one of its hydroxyl group at C3' and where as the parent quercetin structure in this ligand containing hydroxyl groups at C5 and C7 interacts with SER473 (HO-CH2-**CH**(NH2)-COOH) and terminal secondary amino group of ARG560 (HN=C(NH2)-**NH**-(CH2)3-CH(NH2)-COOH). On the perpendicular plane of this ligand attached is the dihydroxy phenolic group involving in Hbond with TYR 585 possessing oxygen atom of hydroxyl group. SER 630 has its Fragmental Hbond with quercetin nucleus.

Isorhamnetin has binding through Hbonds with GLN553,TYR666 at its lowest free energy of binding (-7.15kcal/mol). Isorhamnetin's fused ring structure branched with a phenolic group substituted with a methyl(-CH3) group upon methylation (in this case dehydrogenation) of kaempferol at C5' position. This alkoxy group stretched along their plan of axis perpendicular to the fused ring structure in this conformation and Hbond with TYR–CH-OH..... (OH)-CH-(C6H5OH)-C10H11O5. The flexibility towards TYR 666 is of significant in this interaction. The fused ring at its C4 position bears –C=O group change its conformation along with adjacent dihydroxyphenyl ring in such a way align parallel in order to bind with -NH group of SER552 and adjacent dihydroxyphenyl ring has binding with peptidyl linkage (SER-**HN**-CH-(C=**O**)-CH2-GLN) of GLN553 with SER552. But the conformer TYR 666 maintains its plane along fused ring in its hydrogen binding with TYR666. Upon its conformational change ,fused ring at its C5 position containing -HC-OH Of which '–H' of hydroxyl group orients towards GLN553 and 'O' atom of C-O bind Nitrogen atom (–NH group) of ARG429 and at C7 position containing substituted hydroxyl group containing "H" atom makes sulfhydryl linkage with CYS551.

Kaempferol with its flexibility in the active site of the enzyme has its better conformation at its lowest binding energy (-7.17 kcal/mol) made with TYR547, ASP556, ARG429, TYR585, CYS551 (SER 552). C2 position of the fused ring system substituted with a phenolic group has a hydroxylic group at C4' Hbond with –OH of TYR547. The fused ring at its position distantly makes C5 substituted –OH group to Hbond with TYR 585 and ARG429

.Whereas ASP556 containing –NH group binds with affinity as Hbond C7 hydroxyl group of kaempferol. The intervening aminoacid CYS551 and SER552 with –NH linkage between them shares the affinity of common Hbond with hydroxyl group at C3 position. As the conformation changes on flexibility of the ligand changes upon their orientation making over free energy of -6.49 kcal/mol towards -NH group of HIS363 binds with carbonyl (–C=O) of the fused ring at C4 position and at the same time the adjacent hydroxyl group at C3 position Hbonds with 'H' atom of the –NH group of SER360(GLU361).This positioned plane of kaempferol containing phenolic hydroxyl group at C4' Hbond with intervening amino group peptide chain in GLY355-CO– **HN**-CH-PHE357-CH-**NH**- CO-ARG358. The conformer at its successive free energy of binding change its plane for phenolic hydroxyl group at C4'position in such a way Hbond with TYR666. This conformation is of supreme importance where the TYR 547 on its distant is the possible orientation but as the flexibility of the ligand changes its direction towards TYR666.

Quercetin at its lowest binding energy makes position in such a way a flexible plane, where it binds with PRO550, SER552, TYR585, and TYR666. Oxygen atom of –OH group at 4' Hbond with terminal "H' atom of –OH group of TYR 666 and H atom of –OH group at 5' position Hbond with Oxygen atom of –C=O of PRO 550. With changing of their plane, the fused ring system containing -C=O at C4 Hbond with SER552 containing –NH group. C7 branched with OH group, Hbond with O-atom of terminal OH atom of TYR 585. At their 2^{ND} conformation the flexible ligands with their fused rings in position containing hydroxyl groups at C3,C5,C7 makes Hbond with intervening , terminal –NH atom of GLU361, GLU408,ILE 407. Carbonyl group containing oxygen atom at the C4 position Hbond with the –NH group in the pyrazole ring of HIS363.The 1,3 dihydroxy phenol attached at the C2 of the fused ring change its plane and the 'O' atom of OH group at the 3' and 4' makes Hbond with GLY355.

Sitagliptin at its lowest binding energy (-8.27kcal/mol) has their angle towards binding with GLU205 and GLU206. Each hydrogen atom attached at nitrogen to form amino group next to 2,4,5 triflurophenyl moiety interacts with GLU205 and GLU206.This explains the importance of GLU205 and GLU206 interaction with the inhibitor.The specific trapping mode of the N-terminal α-ammonium group in the cationic hole formed by Glu205 and Glu206 explains the strict dipeptidyl aminopeptidase activity of DPPIV[18]. During other conformations with reasonable lowest binding energies probable interaction shows the hydrogen atom of Phenyl hydroxyl group attached to TYR666, TYR585 and HIS126,SER630 interacts with nitrogen (N2) atom of triazolo ring of sitagliptin.

Flexible docking explains the flexibility of amino acids and its importance at their active site on the binding of ligands (inhibitors)

The necessity of flexibility in the molecular representation is demonstrated well in [21], where the amount of movement in a protein clearly affects the accuracy of docking results in well-established software tools; the observation is supported by [2]. GAs Genetic algorithms lend themselves to the flexible docking problem by allowing conformations to change in as many variables as required.

Tyr 547 phenolic group contains distal O^{η} hydroxyl group that is responsible for polarization of the peptide carbonyl group in the oxyanion hole, protrudes freely into the active site without any binding with the nearby side chains. Any modeling attempts of DPP-IV inhibitors require consideration of the flexibility of TYR547 and SER630 [22]

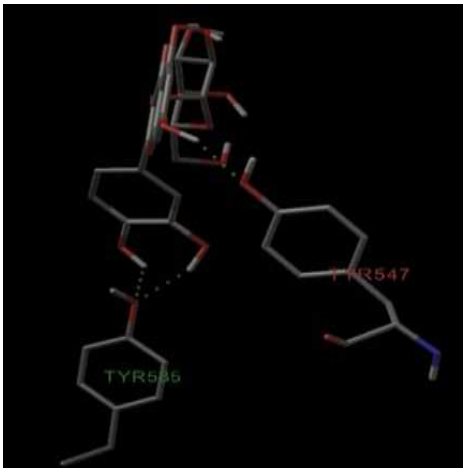

Fig 5: Showing the interaction of Tyr 547 with hyperoside during the run of Lamarckian genetic algorithm.

For e.g. the figure 6 shows the binding of TYR 547 only during the 8^{th} run and almost shows differing energy levels (-1.64kcal/mol) than the rigid mode of binding.

This type of change in energy levels supported the importance and as well as the involvement of aminoacids at the active site of S1 AND S2 site , for better affinity towards binding with the ligand.

When Tyr547, Tyr585 is made flexible, **Kaempferol** being rigid in its position interacts with other aminoacids in its vicinity but they has interaction with Tyr 585 during its aminoacid flexibility at its lowest binding (-6.43kcal/mol).Continuous amino acids GLN553 and LYS554 has its interaction with phenyl hydroxyl group of kaempferol.ASP585 Hbond with C7 of Kaempferol at this conformation. During the flexibility of various run, flexibility allowed interaction with other aminoacids like TYR666,GLN553, SER630, ARG358, HIS363, TYR547, ARG125 within the range of free energy of binding between -4.61 kcal/mol—6.43kcal/mol. When ILE407,SER552, TYR666 selected for torsions, **quercetin** possess lowest binding energy of -1.55 kcal/mol. But the Hydrogen bond formed during 6^{th} run with GLU205 and GLU206.This explains the role of ILE407, SER552, TYR666 on interaction with quercetin in bringing out increased affinity with its free energy of binding in rigid mode of interaction.

Both rigid docking and as well as flexible docking explains the importance of aminoacids at its active site from its hydrogen bond formation and values of free energy of binding .

Our approach towards *insilico* evaluation shows satisfactory docking results, when compared with standard using rigid and as well as flexible docking. Flavonoids exhibited lowest binding energy (ΔG) and differing dissociation constant (k_d) comparable with that of the standard. Further, binding site analysis of rigid and flexible mode of docking reveal that they occupy the active site with maximum positioning. All these parameters and analysis through computational studies, explains the potential DPP-IV inhibitory characteristics of *selected* flavonoids. Further invitro and invivo studies can be done in order produce a better lead in the development of DPP-IV inhibitors.

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