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**Research Article**

# MOLECULAR DOCKING STUDY ON DIPEPTIDYL PEPTIDASE-4 INHIBITORS

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**ABSTRACT**

Dipeptidyl peptidase (DPP)-IV inhibitors are a new approach to the treatment of type 2 diabetes. DPP-IV is a member of a family of serine peptidases that includes quiescent cell proline dipeptidase (QPP), DPP8, and DPP9. DPP-IV is a key regulator of incretin hormones, but the functions of other family members are unknown. To determine the importance of selective DPP-IV inhibition for the treatment of diabetes, we conducted molecular docking studies on clinical inhibitors of DPP-IV.

**Keywords:** DPP IV; Docking; Type 2 diabetes; AutoDock.

## INTRODUCTION

Glucagon-like peptide-1 (GLP-1)[1,2] is an incretin hormone secreted from the L cells of the small intestine in response to food intake. This hormone plays several biological roles including the stimulation of insulin secretion, inhibition of glucagon secretion, retardation of gastric emptying, induction of satiety and stimulating the regeneration and differentiation of islet b-cells.[3,4] However, GLP-1 (GLP-1[7-36] amide) is rapidly degraded in vivo (lifetime: about 1 min) through the action of dipeptidyl peptidase IV (DPP-IV), which cleaves a dipeptide from the N-terminus to give the inactive GLP[9-36]amide.[5,6]

DPP-IV is a serine protease cleaving the N-terminal dipeptide with a preference for L-proline or L-alanine at the penultimate position.[7] This protease is expressed in many tissues and body fluids, and exists as either a membrane-bound or a soluble enzyme. Inhibition of DPP-IV increases the level of circulating GLP-1 and thus increases insulin secretion,[8-10] which can ameliorate hyperglycaemia in type2 diabetes. A number of small molecule inhibitors of DPP-IV have been described[11-14] and several of these, including Vildagliptin (LAF237),[15] Saxagliptin (BMS477118),[16] Alogliptin (SYR-322)[17] and Sitagliptin (MK-0431),[18] are in late-stage of clinical development or approved by the U.S. Food and Drug Administration (Fig. 1).

The first X-ray structures of DPP-4 that have been published in 2003 give rather detailed information about the structural characteristics of the binding site. Many structurally diverse DPP-4 inhibitors have been discovered and it is not that surprising considering the properties of the binding site:[19] 1. A deep lipophilic pocket (S1) combined with several exposed aromatic side chains for achieving high affinity small molecule binding. 2. A significant solvent access (S2 pocket) that makes it possible to tune the physico-chemical properties of the inhibitors that leads to better

pharmacokinetic behavior. The S1 pocket relatively smaller the S2 pocket, usually smaller group such as prolin mimetic that occupies the S1 pocket.

the inhibitor binding site. The catalytic domain shows an α/β-hydrolase fold and contains the catalytic triad Ser630 - Asp708 - His740. The S1-pocket is very hydrophobic and is

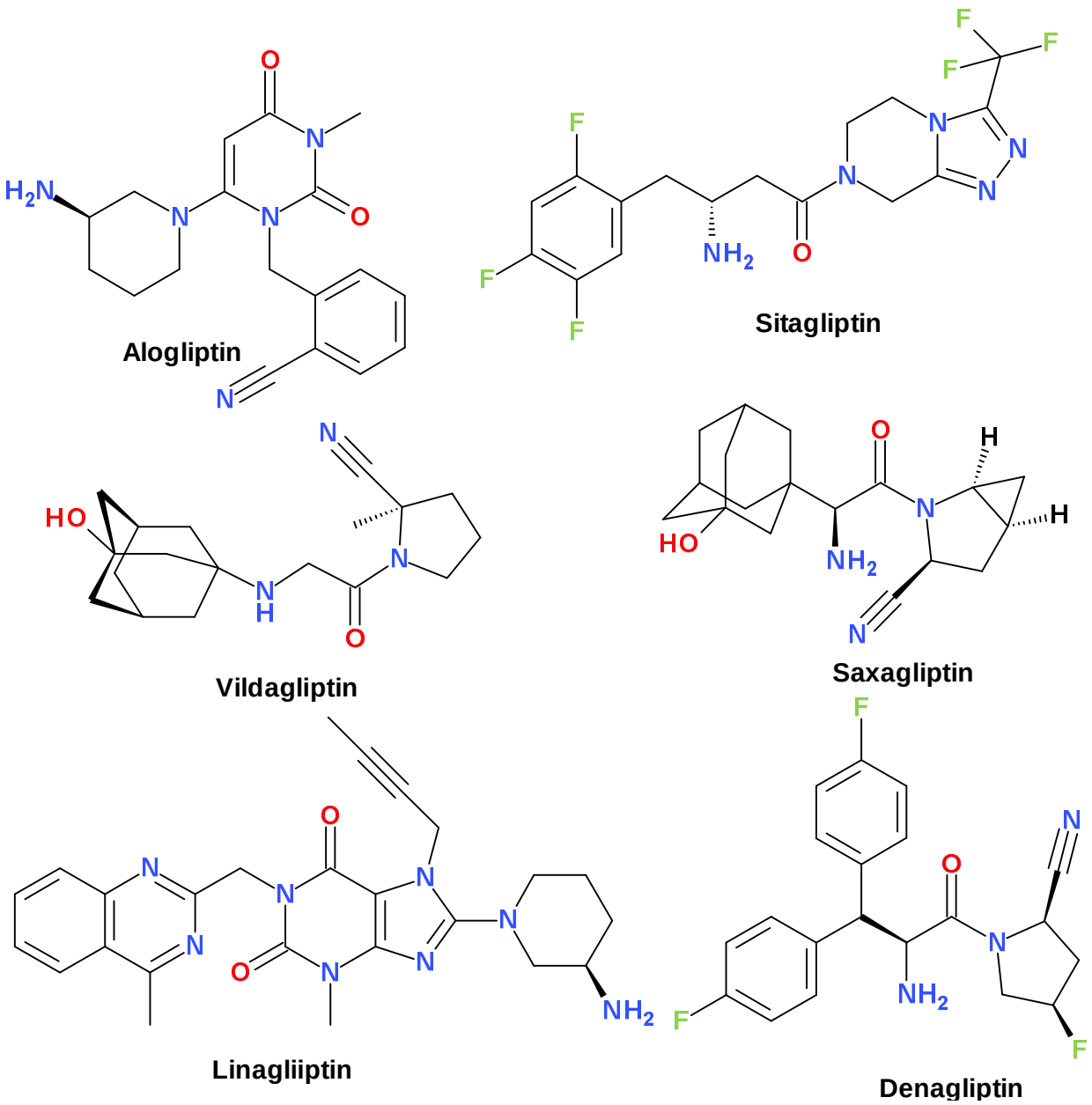



**Figure 1 Structures of Selected DPPIV Inhibitors**

DPP-4 is transmembrane glycoprotein that belongs to the prolyloligopeptidase family. It contains 766-amino acid residues, which consists of three parts; a cytoplasmic tail, a transmembrane region and an extracellular part. The extracellular part is divided into a catalytic domain and an eight-bladed β-propeller domain. The latter contributes to composed of the side chains: Tyr631, Val656, Trp662, Tyr666 and Val711. The active site of DPP-4 is shown in Figure 2 with important residues labeled. X-ray structures show that there is not much difference in size and shape of the pocket that indicates that the S1-pocket has high specificity for proline residues.[20]

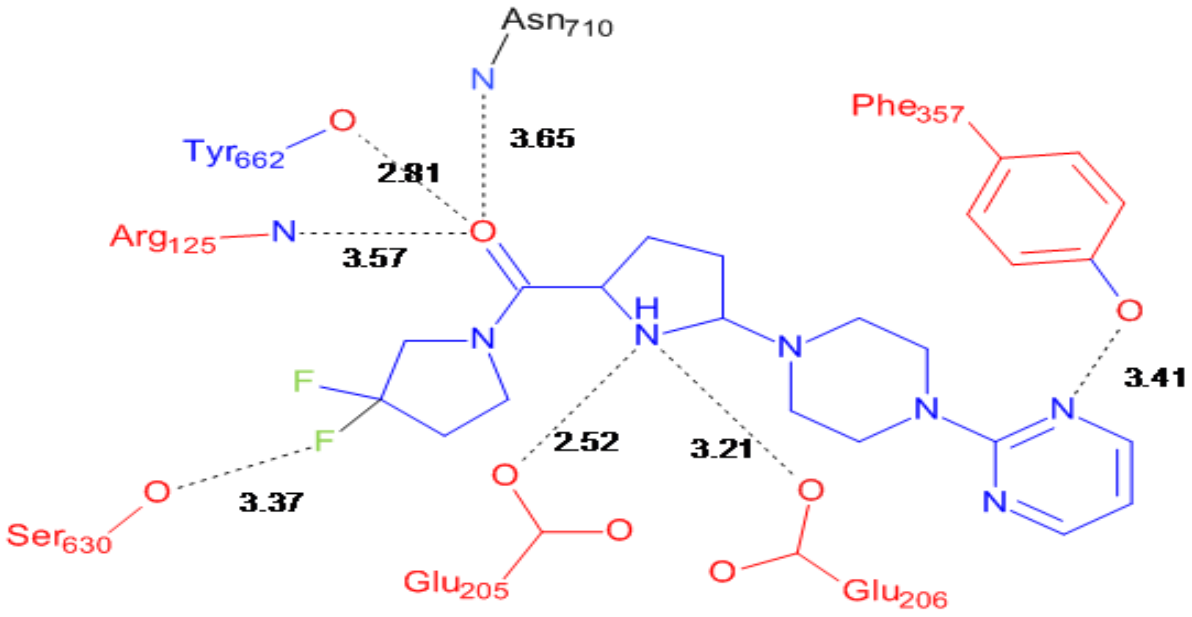



**Figure 2. Active Site interactions between the ligand and DPP-4 complex.**

DPP-4 inhibitors span diverse structural types (Fig.1). In order to know how the diverse DPP-IV ligand binds into the active site of the enzyme, we carried out molecular docking study using AutoDock program. Consequently to get new insights into the active site of DPP-IV, docking study helps to identify the relationship between the structural information of the known compounds.

**Materials and Methods**

All computational studies were carried out using AutoDock 4.0.1[21] with MGL tools 1.5.6 installed in a 8 CPU machine running on a 2.0 GHz Intel core i5 processor with 2GB RAM and 2 TB hard disk with LINUX (RED HAT 6) operating system.

**Ligand Structure Preparation**

Ligand 2D structures were drawn using ChemDraw Ultra 7.0 (ChemOffice 2002). Chem3D Ultra 7.0 was used to convert 2D structure into 3D and the energy minimized using semi empirical AM1 method. Minimize energy to minimum RMS gradient of 0.100 was set in each iteration. All structures were saved as .pdb file format for input to AutoDockTools (ADT) version 1.5.6.[22] All the ligand structures were then saved in PDBQT file format, for input into AutoDock version 1.5.6.[22]

**Protein Structure Preparation**

For the molecular docking study, protein structure was obtained from the Brookhaven protein data bank; the DPP-IV structure PDB ID was 3F8S.[23] The co crystallized ligand (PF2) in the DPP-IV structure was removed. For the protein structure, all hydrogen atoms were added, lower occupancy residue structures were deleted, and any incomplete side chains were replaced using the ADT version 1.5.6. Further ADT was used to remove crystal water, added Gagteiger charges to each atom, and merged the non-polar hydrogen atoms to the protein structure. The distance between donor and acceptor atoms that form a hydrogen bond was defined as 1.9 Å with a tolerance of 0.5 Å, and the acceptor–hydrogen–donor angle was not less than 120◦. The structures were then saved in PDBQT file format, for input into AutoDock version 1.5.6.

**Docking Protocol and their validation**

A grid box with dimension of 40 × 40 × 40 Å3 and was centred on 34.840, 6.101, 61.781 was created around the binding site of PF2 on DPP-IV protein using autodock tools. The centre of the box was set at PF2 and grid energy calculations were carried out. For the Autodock docking calculation, default parameters were used and 50 docked

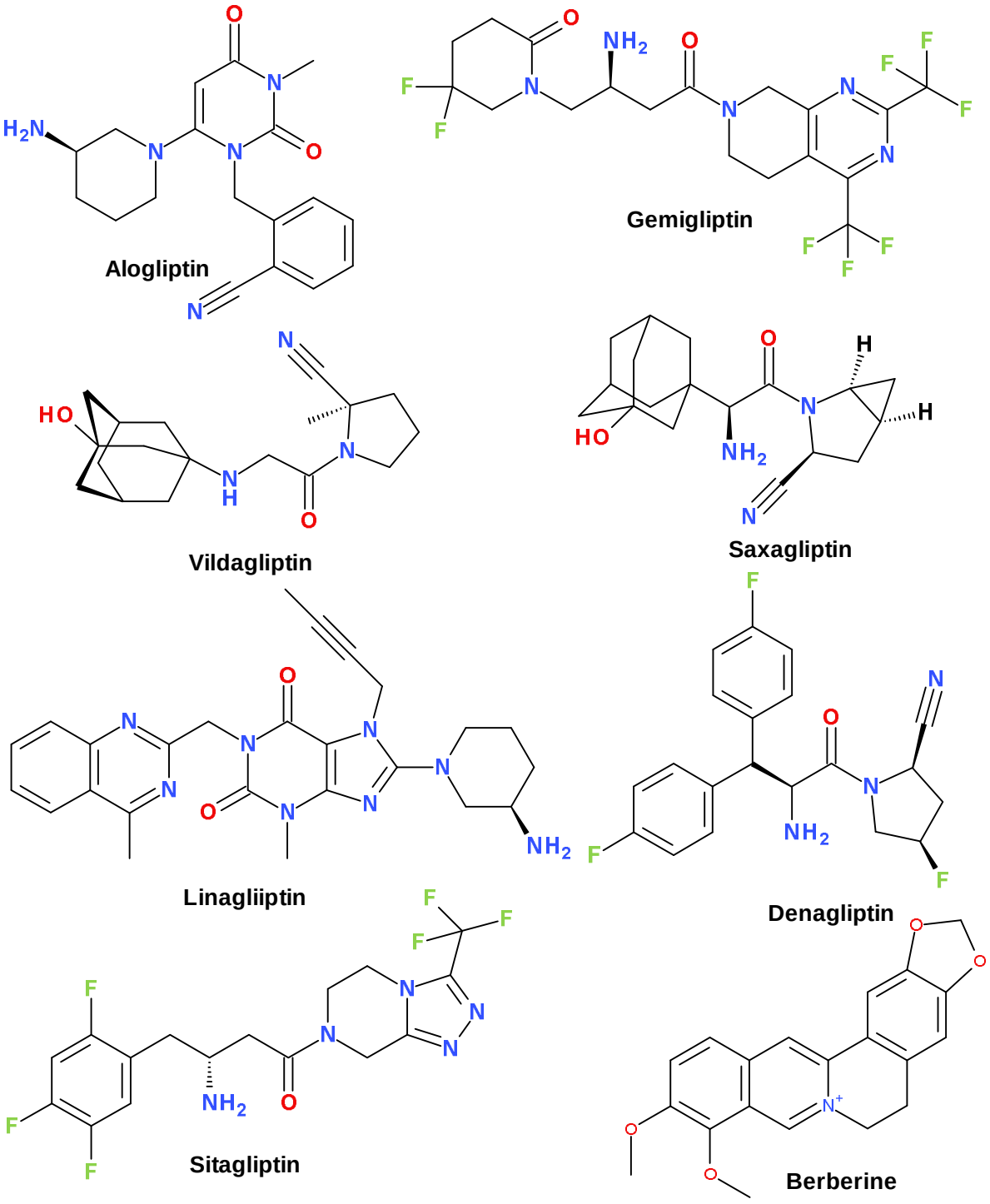



**Fig. 3 Structures of DPPIV Inhibitors used for docking study**

conformations were generated for each compound. In order to verify reproducibility of the docking calculations, the bound ligand (PF2) was extracted from the complexes and submitted for one-ligand run calculation. This reproduced top scoring conformations of 4 falling within root-mean-square deviation (rmsd) values of 0.71 to 0.74 Å from bound X-ray conformation for DPP-IV, suggesting this method is valid enough to be used for docking studies of other compounds

(Figure 4). The outputs were exported to VMD and Pymol for visual inspection of the binding modes and interactions of the compounds with amino acid residues in the active sites.

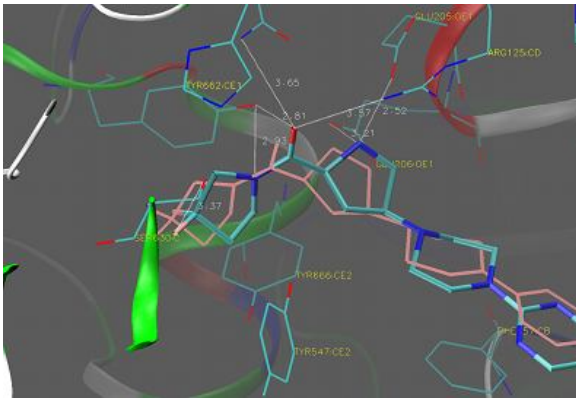


**Fig 4. The structural overlay of Docked compound (magenta) and X-ray co-crystal ligand (cyan)**

**RESULT AND DISCUSSION:**

The best ways is to fit ligand molecules (DPP IV inhibitors), into DPP IV structure, using Autodock4 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. Interaction energies between ligand-receptor are calculated with a free energy-based expression. The lowest binding energy conformation in all cluster were considered as the most favourable docking pose. 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. The docking score were generated by autodock module along with experimental $IC_{50}$ were shown in Table 1. From this study, the lowest docked-energy structure was analyzed in detail in an effort to know the common pharmacophore for DPP IV inhibitors.

The low energy docked structures of eight DPP IV inhibitors are shown in Figure 5. The top low energy structures of all eight DPP IV inhibitors had docking energies ranging from –9.06 to –6.84 kcal/mol. Only one modes of binding were observed for all eight inhibitors with the docked energy within a range of 2.2 kcal/mol of the lowest docked energy structure.

The key amino acid residues of DPP IV involved in the hydrogen bonding interaction with low energy docked structures of various ligands studied are shown in Table 2. All the eight inhibitors formed two intermolecular hydrogen bonds in the lipophilic pocket (S1) of the binding site. The Primary/secondary amino group of all DPP IV inhibitors forms hydrogen bonded with carboxyl group of Glu205 and Glu206. This S1 lipophilic pocket (Glu205 and Glu206) hydrogen bond interaction is the conserved interaction against all DPP IV inhibitors.

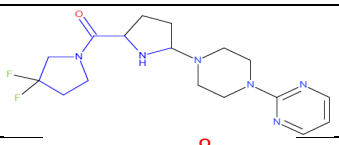
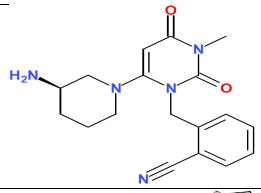
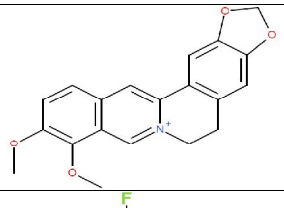
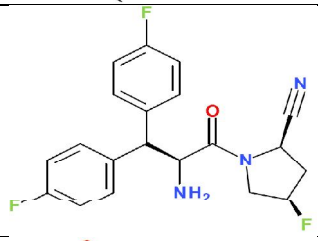
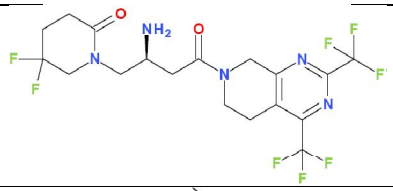
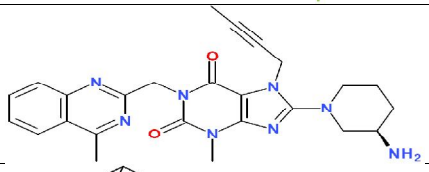
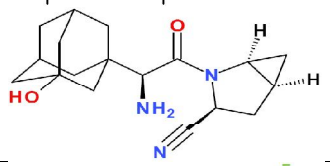
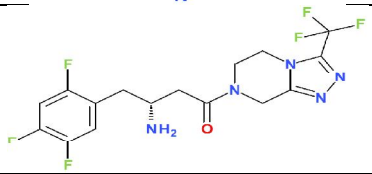
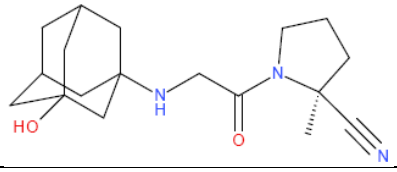
**Alogliptin:** Figure 5a shows the low energy docked structures of Alogliptin with DPP IV protein domain. The docked energies of the top 10 poses from alogliptin fall in the range of –7.92 to –6.53 kcal/mol. The lowest energy docked structure interacted with DPP IV with five hydrogen bonds, two conserved hydrogen bond interactions are from Glu205 and Glu206. The remaining three hydrogen bond interactions are from Arg125, Tyr666 and Tyr547. The dihydropyrimidin ring of alogliptin was stabilized by hydrophobic interactions with Phe357. The experimental $IC_{50}$ value of alogliptin is 10 nm,

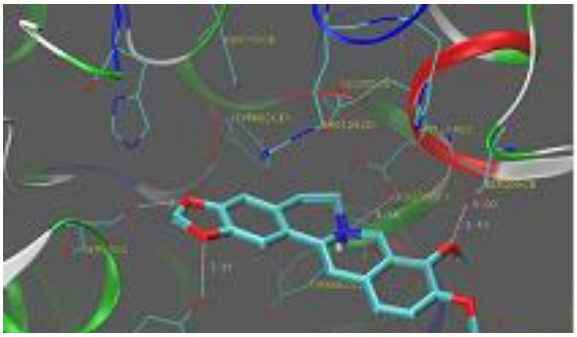
**Berberine**, the common herbal dietary supplement, too inhibits DPP IV, which at least partly explains its antihyperglycemic activity.[24] To know the binding mode berberine in the DPP IV protein we included the in the docking study. The Figure 5b shows the low energy docked structures of berberine with DPP IV protein. Berberine also binds similar to that of other DPP IV inhibitors. The top ten low energy structures had docking energies ranging from -7.21 to -6.61 kcal/mol. The lowest energy docked structure forms four hydrogen bond interactions in the active site of DPP IV are Glu206, Ser230, Tyr547 and Ser209 respectively. The dimethoxy phenyl ring of berberine forms strong pi stacking interaction with Phe357.

**Denagliptin:** Figure 5c shows the low energy docked structures of denagliptin with DPP IV protein domain. The lowest docked energy of denagliptin is -9.06 kcal/mol. The lowest energy docked structure interacted with DPP IV with five strong hydrogen bonds Glu205, Glu206, Asn710, Tyr662 and Arg125. The all hydrogen bonds hetro atom to hetro atom distance is 2.55 to 2.97 Å makes the compound into low energy compared to other DPP IV inhibitors. Further to hypothesis the low energy result is due to structure with a

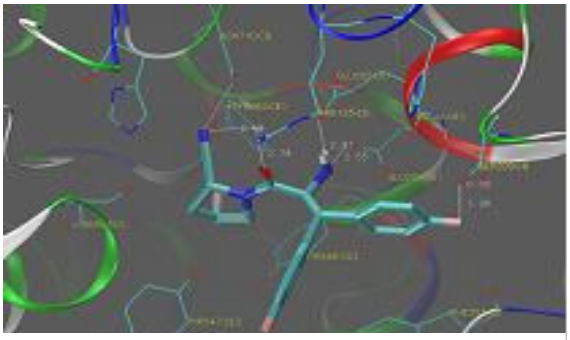
 

**Table 1. DPP IV inhibitors Name with experimental biological activities ($IC_{50}$) and final total docking energy in kcal/mol**

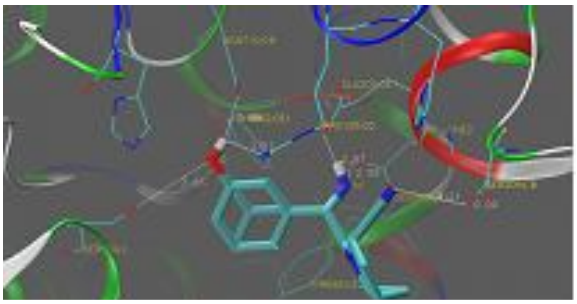
| S. No | Name | Structure | $IC_{50}$ | Docking Score (kcal/mol) |
|---|---|---|---|---|
| Std | PF2 |  | 12.9 nm | -7.71 |
| 1 | Alogliptin |  | 10 nm | -7.92 |
| 2 | Berberine |  | 13.3 nm | -7.21 |
| 3 | Denagliptin |  | 22 nm | -9.06 |
| 4 | Gemigliptin |  | 16 nm | -6.84 |
| 5 | Linagliptin |  | 1 nm | -7.91 |
| 6 | Saxagliptin |  | 4 nm | -7.61 |
| 7 | Sitagliptin |  | 18 nm | -7.10 |
| 8 | Vildagliptin |  | 3 nm | -7.43 |



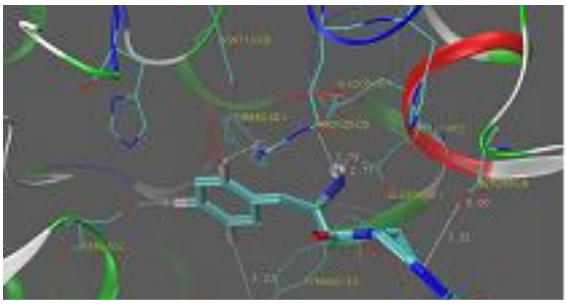
**A : Berberine**



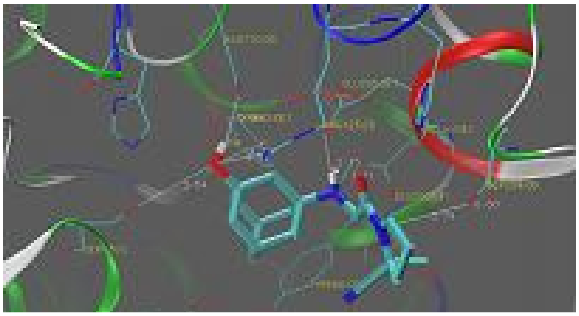
**B : Denagliptin**



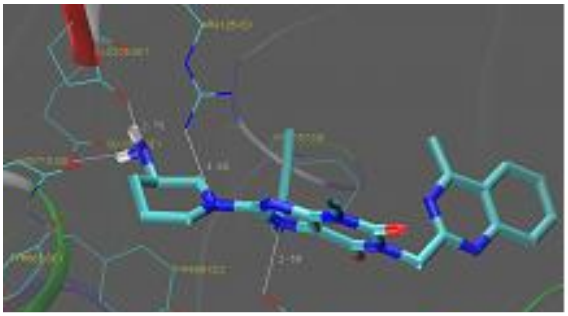
**C : Saxagliptin**



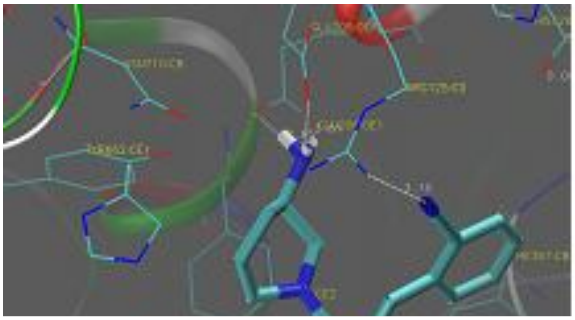
**D : Sitagliptin**



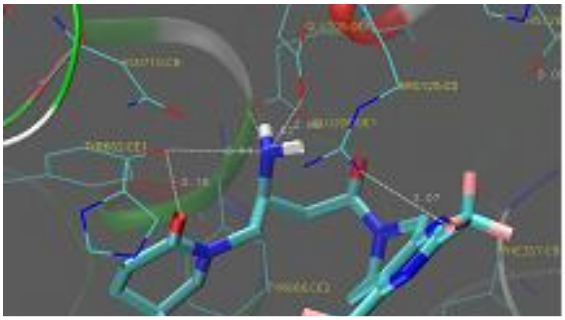
**E : Vildagliptin**



**F : Linagliptin**



**G : Alogliptin**



**H : Gemigliptin**

**Fig 5. Binding mode and hydrogen bond interaction of compounds (A-H) in the active site of DPP IV (3F8S) along with interacting amino acids residues.**

**Table 2: The key amino acid residues of DPP IV involved in the hydrogen bonding interaction with various ligands studied during molecular docking exercise.**

| S. No | DPP IV inhibitors | Glu205 | Glu206 | Asn710 | Tyr662 | Arg125 | Ser630 | Phe357 (Pi staking) | Tyr666 | Tyr547 | Ser209 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Std | PF2 | 2.52 | 3.21 | 3.65 | 2.81 | 3.57 | 3.37 | 3.41 | - | - | - |
| 1 | Alogliptin | 2.81 | 2.65 | - | - | 3.10 | - | - | 3.46 | 3.24 | - |
| 2 | Berberine | - | 3.58 | - | - | - | 2.67 | 3.94 | - | 3.61 | 3.43 |
| 3 | Denagliptin | 2.97 | 2.55 | 2.73 | 2.68 | 2.78 | - | - | - | - | 3.98 |
| 4 | Gemigliptin | 2.60 | 2.62 | - | 2.94 | 3.07 | 3.46 | - | 3.07 | 3.19 | - |
| 5 | Linagliptin | 2.75 | 2.77 | - | 3.61 | 3.60 | - | - | - | 2.68 | - |
| 6 | Saxagliptin | 2.87 | 2.59 | 1.88 | - | 3.07 | 3.65 | - | - | - | 3.21 |
| 7 | Sitagliptin | 2.70 | 2.77 | - | 3.86 | - | 3.36 | - | 3.23 | - | 3.32 |
| 8 | Vildagliptin | 2.75 | 3.11 | 2.01 | 3.79 | 3.51 | 3.54 | - | - | - | 3.74 |

branched side-chain at the P2 position, occupy the solvent exposed area make it more hydrophobic and van der Waals interaction too. In addition to this biological evaluations have shown that the S-configuration of the amino acid portion is more inhibitory activity than the R-configuration. For denagliptin, both R and S configurations were considered (branched side-chain at the P2 position) for docking. The difference in energy of docking between the R-configuration (6.17 kcal/mol) was less than ~3 kcal/mol compared to S-configuration (-9.06 kcal/mol).

**Gemigliptin:** Figure 5d shows the low energy docked structures of gemigliptin with DPP IV protein. The docked energies of the top two structures fall in the range of -6.84 to -5.93 kcal/mol. The lowest energy docked structure forms seven hydrogen bond interactions with DPP IV protein. Two hydrogen bond interactions namely Glu205 and Glu206 are conserved along with all DPP IV inhibitors. The remaining five of the hydrogen bond interactions are Tyr662, Arg307, Ser3.46, Tyr66 and Tyr547 respectively.

**Linagliptin:** Figure 5e shows the low energy docked structures of linaliptin with DPP IV protein. The primary amino group of lowest energy docked structure of linagliptin forms hydrogen bond interaction with Glu205, Glu206 and Tyr662. In addition to hydrogen bond interaction uracil group of linagliptin undergoes a pi stacking interaction with Tyr547 residue. The docking energies of the top cluster contains eight poses were in the range of -8.30 to -6.83 kcal/mol.

**Saxagliptin:** Figure 5f shows the low energy docked structures of saxagliptin with DPP IV protein domain. The docked energies of the top three structures fall in the range of -7.61 to -6.31 kcal/mol The primary amino group of top low energy docked pose of saxagliptin forms conserved hydrogen bond interaction with Glu205 and Glu206 like that of other DPP IV inhibitors. The bulky adamantly group of saxagliptin forms hydrophobic and van der Waals interaction with Tyr666, Tyr547, Phe357 and Tyr 662. The hydroxyl group on the adamantly group forms hydrogen bond interaction with Ser630, His740, Asn710 and Arg125, which gives microsomal stability as well as chemical stability of saxagliptin.

**Sitagliptin** has a novel structure which belongs to β-amino amide derivatives. The low energy docked structures of sitagliptin with DPP IV protein domain was shown in Figure 5g. The trifluorophenyl group of sitagliptin occupies the S1 lipophilic pocket of DPP IV protein. The primary amino group of sitagliptin forms hydrogen bond interaction with Glu205 and Glu206. In addition, the primary amino group of sitagliptin salt bridge with Tyr662 residue. The Ser630 and Tyr666 residues interact with triflurophenyl ring of sitagliptin. The triazolopiperazine group of sitagliptin forms hydrogen and hydrophobic interaction with Ser209 and Phe357 residues respectively. The docked energies of the top cluster structures fall in the range of -7.10 to -6.50 kcal/mol.

**Vildagliptin:** Figure 5h shows the low energy docked structure of vildagliptin with DPP IV protein binding domain. Vildagliptin is structurally very similar to that of saxagliptin forms similar binding interaction with active site of DPP IV. The in vitro $IC_{50}$ value of vildagliptin and saxagliptin are 3nm and 4nm respectively. The main difference between the two structure is saxagliptin had primary amino group and vilatagliptin had secondary amino group. As like saxagliptin

the bulky adamantly group of vildagliptin occupies the S1 lipophilic pocket of DPP IV protein. The seconary amino group forms conserved hydrogen bond interaction with Glu205 and Glu206. The lowest docking energy of the top cluster contains only one pose having the -7.53 kcal/mol.

**CONCLUSION:**

The serine protease dipeptidyl peptidase IV (DPP-IV) is a clinically validated target for the treatment of type II diabetes and has received considerable interest from the pharmaceutical industry over the last years. Molecular docking studies were performed on structurally diverse clinical DPP IV candidate using AutoDock module. Docking results suggest that optimized interactions with the two key recognition motifs, i.e. the lipophilic S1 pocket and the negatively charged Glu 205/206 pair, result in large gains in binding free energy, which can be further improved by additional favourable contacts to side chains that flank the active site.

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