***In-silico* Structure Modeling and Docking Studies Using Dipeptidyl Peptidase 4 (DPP4) Inhibitors against Diabetes Type-2**

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Abstract Recently recognized class of oral hypoglycemic, dipeptidyl peptidase (DPP4) inhibitors could block the dipeptidyl peptidase 4 (DPP4) enzymes. DPP4 is an intrinsic membrane glycoprotein and a serine exopeptidase that plays a major role in glucose metabolism and responsible for the degradation of incretins such as GLP-1, therefore providing a useful treatment to diabetes mellitus type 2. The present work focused on the study of the structural homology modeling of dipeptidyl peptidase 4 [*Homo sapiens*] (NP_001926). The Ramachandran plot of DPP4 (NP_001926.2) has 88.9% residues in the most favoured region while template 2QT9 has 96.1% residues in the most favoured region. The model was validated by using protein structure tools RAMPAGE and Procheck for reliability. Docking studies were further performed to analyze the interaction mode between selected DPP4 inhibitor anagliptin derivative SKK and receptor DPP4 by using Hex 8.0.0. The *in-silico* analysis was useful to identify the novel inhibitor that illustrates better activity than the other reported inhibitors.

Keywords Diabetes, Docking, SKK, DPP4, *In-silico* Analysis

1. Introduction

Diabetes mellitus is a prime public health problem and forthcoming epidemic all over the globe [38] disorder caused due to insufficient or ineffective insulin [2]. In India, there were approximately 40 million people suffering from diabetes and this number possibly will rise up to 300 million by 2025 [45; 46]. According to International Diabetes Federation record, worldwide the number of people with diabetes will be increase from current figure of 240 million to 380 million over the next 20 years [11; 37] and up to 642 million by 2040 [10].

The combination therapeutics or oral monotherapy with

other anti-diabetic agents used to control diabetes through clinical anti-diabetes therapy [16]. But, these anti-diabetic agents may cause adverse side effects and chronic complications [6; 25].

Dipeptidyl peptidase-4 (DPP4) encoded by the DPP4 gene and it is a membrane serine exopeptidase that cleaves X-proline dipeptides from the N-terminus of polypeptides [12; 40]. DPP-4 inhibitors recommended for use in patients with T2DM therapy, that including hypoglycaemia incidences, risks of cardiovascular complications and weight gain [14]. It is a rather indiscriminate enzyme for which a diverse range of substrates are known. Dipeptidyl peptidase 4 (DPP4) is a serine protease that [36] that degrade peptidic hormone glucagon-like peptide 1 (GLP-1). GLP-1 plays an important role in the regulation of insulin release to control the level of blood sugar in human body [18]. Several studies demonstrated that the inhibition of DPP4 could increase the amount of circulating GLP-1 to improve the secretion of insulin in the body [43]. Therefore, it has regarded as promising target to develop novel drug for treatment of type 2 diabetes.

So far, a couple of identified DPP4 inhibitors, such as sitagliptin and saxagliptin, were approved to be used clinically as antidiabetic drugs by FDA [41; 20]. However, there is still a need for more potent, selective and safer DPP4 inhibitor, which does not have the inspecificity and side effect possessed by the presently available inhibitors [13], because of worldwide problem of type 2 diabetes. The recent research on DPP4 inhibitors using *in silico* methods is focus on physicochemical properties analysis[3]. Therefore, researcher are studying new DPP4 ligands for the development of the novel anti-diabetes drug.

Homology modelling provides structural information about the protein dynamics, function, interactions with other proteins and ligands [31; 7]. In the present study, homology modelling [22] process was used to determine the structure of dipeptidyl peptidase 4 [*Homo sapiens*] (NP_001926).

The docking was performed to reveal the interaction mode and to predict the orientation [29; 30] between inhibitors

SKK and DPP4 by using Hex 8.0.0. The docking outcome demonstrated the binding conformation of anagliptin derivative SKK and DPP4. The results of the study could be used to design or predict new potent DPP4 inhibitors. *In silico* analysis is an effective way to develop the anti-diabetic drug model.

2. Material and Methods

2.1 Sequences Retrieval and Alignment

The amino acid sequence of dipeptidyl peptidase 4 [*Homo sapiens*] (NP_001926) was retrieved from NCBI (www.ncbi.nlm.nih.gov). Multiple sequence alignment was performed by using BLASTp [1; 44] against the PDB (Protein Databank), to find out the related homologues. The PDB file of DPP4 (NP_001926) was generated by using 3D-JIGSAW. The PDB file of homologous template and query were further utilized for 3D model energy validation [17].

2.2. Molecular Modelling of DPP4

The UCLA-DOE provide an analysis about the quality of a putative crystal structure for protein. RAMPAGE program was used to visualize and evaluate the Ramachandran plot of DPP4 (NP_001926) and its homologous template [23]. The models were analyzed based on various factors such as G-factor, number of residues in core, generously allowed and disallowed regions in Ramachandran plot. The validation of structure models was performed by using PROCHECK [21]. QMEAN [8] and ProSA [42] were further used for the analysis of model. The ProSA server displays the Z-score and energy plots.

The 3D structure selected as a template for constructing the model for DPP4 (NP_001926) was Human dipeptidyl peptidase (PDB access code: 2QT9). 2QT9 has 766 amino acid residues and a resolution of 2.1 Å [19]. 2QT9 was illustrated 99% identity with the target sequence (2QT9).

The quality of folding was checked by using Verify3D. The system energy and 3D alignment were monitored using Atomic Non-Local Environment Assessment (ANOLEA) [24]. Produced models of homologous template and query were ranked on QMEAN server and utilized for the ribbon structural model construction. QMEAN locate the position of active site amino acids of protein and estimates the per-residue error [9].

2.3. Docking of DPP4 (NP_001926) with SKK

The aim of protein docking is to determine 3D structure of a protein complex preliminary from its unbound components [15]. Docking was performed to analyze the interaction between selected DPP4 inhibitor anagliptin derivative SKK [Synonyms: Anagliptin, C₁₉H₂₅N₇ O₂] (Figure 1) and receptor DPP4 (NP_001926) by using Hex 8.0.0 [34]. The PDB files of DPP4 (NP_001926) and SKK was used as inputs for the protein-protein docking. Hex 8.0.0 is a docking program; that performed structural refinement, energy minimization and display the fast 3D superposition using the SPF correlation approach. Based on the energy minimization the best front of the docked complex was selected [35].

3. Results and Discussion

3.1. Building of Protein Model

The sequence alignment of dipeptidyl peptidase 4 [*Homo sapiens*] (NP_001926) revealed the sequence homology with human dipeptidyl peptidase (PDB access code: 2QT9), which has 766 amino acid residues and a resolution of 2.1 Å [19]. The 2QT9 (with 99% sequence identity) was selected as template for the model building of DPP4 (NP_001926) protein. Total 766 residues were modeled with 99% confidence by the single highest scoring template. To build the model, BLAST was run with the maximum E-value allowed for template being 0.0.

Figure 1. SKK (N-[2-((2S)-2-cyanopyrrolidin-1-yl)-2-oxoethyl]amino)-2-methylpropyl]-2-methylpyrazolo[1,5-a]pyrimidine-6-carboxamide)

Figure 2. Ramachandran Plot analysis of dipeptidyl peptidase 4 (NP_001926.2) [*Homo sapiens*] protein. (a) Total number of residues were 601 with 88.9% in most favored regions [A, B, L], 8.0 % in allowed regions [a,b,l,p], and 19% in outlier region regions. (b) Non-proline residues and non-glycine residue regions.

Figure 3. Ramachandran Plot analysis of homologous Human Dipeptidyl Peptidase IvCD26 in complex with a 4-Aryl Cyclohexylalanine Inhibitor (2QT9) protein. (a) Total number of residues were 1452 with 96.1% in most favored regions [A, B, L], 3.9 % in allowed regions [a,b,l,p], and 0.1% in outlier region (b) Non-proline residues and non-glycine residue regions

The Ramachandran plot of DPP4 (NP_001926) has only 88.9% residues in the most favoured region and its template 2QT9 [Human dipeptidyl peptidase] has higher 96.1% residues in most favoured regions, hence 2QT9 is more stable than DPP4 (NP_001926) (Table 1). A good quality Ramachandran plot has more than 90% in the most favoured regions [26] thus the 2QT9 model could be include in good quality category. For target DPP4 (NP_001926) model, energy minimization should be required to enhance stability by using application of simulated annealing, steepest descent and conjugate gradient.

3.3. Model Validation

The problems of protein structures based on energy plots could be easily seen by ProSA and displayed in a three-dimensional manner. ProSA web was used to check the three dimensional model errors of DPP4 (NP_001926) and 2QT9 (Figure 4 and Figure 5). ProSA web z-scores of protein chains were determined by X-ray crystallography (light blue) or NMR spectroscopy (dark blue) with respect to their length. The z-scores of DPP4 (NP_001926) and DPP IvCD26 (2QT9) is highlighted as large dots.

Figure 4. ProSA web service analysis of DPP4 (NP_001926) [*Homo sapiens*] protein overall model quality (a) and local model quality (b).

Figure 5. ProSA web service analysis of DPP IvCD26 (2QT9) [*Homo sapiens*] protein overall model quality (a) and local model quality (b).

The ProSA z-score was -6.21 for DPP4 (NP_001926) [Figure 4] and -11.09 for the DPP IvCD26 (2QT9) protein, indicates the overall model quality of target and template (Figure 5) measures the deviation of the total energy of the structure with respect to an energy distribution [39].

Figure 6a. The density plot for target DPP4 (NP_001926) [*Homo sapiens*] showing the value of Z-score and QMEAN score

Figure 6b. Plot showing the QMEAN value as well as Z-score (for NP_001926)

Figure 7a. The density plot for target DPP IvCD26 (2QT9) [*Homo sapiens*] showing the value of Z-score and QMEAN score

Figure 7b. Plot showing the QMEAN value as well as Z-score (for 2QT9)

QMEAN score of target DPP4 (NP_001926) model was 0.53 and Z-score was -3.99 (Figure 6a). QMEAN score of template DPP IvCD26 (2QT9) was 0.72 with Z-score was -0.55 (Figure 7a), which very closed to the value of 0. That illustrated the fine quality of the both models [42]. The reliability of the model was expected in between 0 and 1 and this could be inferred from the density plot for QMEAN scores of the reference set. A comparison between protein size and normalized QMEAN score (0.40) in non-redundant set of PDB structures plot revealed different set of z-values for different parameters such as C-beta interactions (-1.67), interactions between all atoms (-1.97), solvation (-3.02) and torsion (-1.93) [Figure 6b]. The ribbon model of DPP4 (NP_001926) and 2QT9 was generated using QMEAN server (Figure 8 and Figure 9). The per-residue error visualized using a color gradient from blue (more reliable region) to red (potentially unreliable region).

Figure 8. DPP4 (NP_001926) [*Homo sapiens*] ribbon model generated using QMEAN server. Estimated per-residue error visualized using a color gradient from blue (more reliable region) to red (potentially unreliable region).

Figure 9. DPP IvCD26 (2QT9) [*Homo sapiens*] ribbon model generated using QMEAN server. Estimated per-residue error visualized using a color gradient from blue (more reliable region) to red (potentially unreliable region).

Protein docking predicts symmetrical 3D models of protein-protein complexes with arbitrary point group symmetry [33]. Hex assigns multiple local coordinate systems to larger molecule (receptor DPP4 [NP_001926]) and docks the ligand (SKK) around each local coordinate frame on the receptor. Figure represents Hex view of DPP4 (NP_001926) protein domain and SKK in vander waals mode, with the intermolecular axis (10a) and solid surfaces form (10b).

Figure 10. A Hex scene showing the DPP4 (NP_001926) domain and SKK (below) in Vander Waals mode and with the intermolecular axis drawn in blue (a) and solid surfaces (b)

On the basis of the RMS and energy values, the best docking orientation was selected. The better RMS value of docking was -1.00. The binding sites of protein reveal chemical specificity, to determine the nature of ligand and affinity strength of chemical bond [4,5]. The 1 Clst initial Etotol, Eshape and Eforce values for the model were -304.7, -304.7 and 0.0 (Table 2).

Table 2. Clustering found 560 clusters from 2000 docking solutions in 0.05 seconds.

Clst	Soln	Models	E-total	E-shape	E-force	E-air	Bmp	RMS
1	1	000:001	-304.7	-304.7	0.00	0.0	-1	-1.00
1	2	000:001	-304.6	-304.6	0.00	0.0	-1	-1.00
1	7	000:001	-295.7	-295.7	0.00	0.0	-1	-1.00
1	8	000:001	-294.7	-295.7	0.00	0.0	-1	-1.00
1	9	000:002	-292.7	-292.7	0.00	0.0	-1	-1.00
1	11	000:001	-288.3	-288.3	0.00	0.0	-1	-1.00
1	12	000:002	-286.3	-286.3	0.00	0.0	-1	-1.00
1	21	000:002	-282.4	-282.4	0.00	0.0	-1	-1.00
1	23	000:001	-280.5	-280.5	0.00	0.0	-1	-1.00
1	28	000:002	-277.7	-277.7	0.00	0.0	-1	-1.00
1	30	000:001	-276.0	-276.0	0.00	0.0	-1	-1.00
1	37	000:001	-272.6	-272.6	0.00	0.0	-1	-1.00
1	43	000:002	-270.8	-270.8	0.00	0.0	-1	-1.00
1	44	000:002	-270.6	-270.6	0.00	0.0	-1	-1.00
1	45	000:001	-270.4	-270.4	0.00	0.0	-1	-1.00
1	49	000:001	-270.2	-270.2	0.00	0.0	-1	-1.00
1	54	000:001	-268.8	-268.8	0.00	0.0	-1	-1.00
1	65	000:001	-265.7	-265.7	0.00	0.0	-1	-1.00
1	78	000:001	-263.4	-263.4	0.00	0.0	-1	-1.00
1	87	000:001	-261.6	-261.6	0.00	0.0	-1	-1.00

Figure illustrates spherical harmonic surfaces to order $L = 12$ for the DPP4 (NP_001926) protein domain and SKK (Figure 11). Illustration of the DPP4/SKK complex shown as contoured Gaussian density surfaces and background modes (Figure 11). Figure illustrates docking control results in the form of solid models (Figure 12a) and solid surface (Figure 12b) model view of DPP4 (NP_001926) and SKK complex. These docking results suggest that SKK interacts with the DPP4 protein of and causes its inhibition [28]. The binding pocket values for DPP4 model were predicted by using Hex 8.0.0. The predicted two pockets by the software with different primary surface area and volume shown in Table 3.

Figure 11. Illustration of spherical harmonic surfaces to order $L = 12$ for the DPP4 (NP_001926) domain and SKK (a). Illustration of the DPP4 (NP_001926) (Receptor) and SKK (Ligand) complex shown as contoured Gaussian density surfaces and coloured by chain colour, drawn using perspective (keyboard P) and background (keyboard B) modes (b)

Figure 12. Docking results illustrate solid models (a) and solid surface (b) view forms of DPP4 (NP_001926) /SKK complex

Table 3. Binding site model DPP4 (NP_001926) protein. Calculated 29 surface normals (docking orientations) in 0.28 seconds. Calculating canonical orientation to L=12

Pocket	Polar Probe	Apolar probe	Primary Surface area	Primary Surface volume	Typical Edge arc	Typical edge length (Å)	Average radius (Å)	Surface area (Å)	Triangles		
									Min.	Max.	Avg.
1	0.00A	0.00A	51389.81	62777.83	4.62	2.59	32.14	20349.10	1.84	58.99	4.52
2	0.00A	0.00A	51084.10	66988.55	2.80	0.69	14.07	1.3433	0.00	37.04	1.24

Docking could be used to study the mechanism of enzymatic reaction, to identify possible binding modes for a ligand and to screen a database [27]. A few amino acids were found to be conserved in DPP4 (NP_001926), that forming the binding cavity for the SKK. Interaction of SKK with DPP4 (NP_001926), used as a guideline to design and predict new potent DPP4 inhibitors, which could be an effective way to find novel leads for the development of anti-diabetic drugs. The anaglyptic derivative improves the glycemic control; hence, SKK might be consider as novel potent DPP4 inhibitor.

4. Conclusion

The DPP4 inhibitors accepted as new drug class because it provides effective glycemic control and low risk of hypoglycemia. *In silico* modeling and docking analyses illustrated that the anaglyptin derivative SKK could be inhibit DPP4 enzymatic activity. The results of study could be used to design and predict new potent DPP4 inhibitors, which could be an efficient mode for designing of anti-diabetic drugs. The results will be further supportive to screen new DPP4 inhibitors from nature sources also. The *in silico* techniques are useful to identify the novel inhibitors for the clinical trial of diabetic patients.

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