Original Article

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Discovery of Potential Natural Dipeptidyl Peptidase-4 Inhibitors for Type-2 Diabetes Treatment via Structure-Based Virtual Screening

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Abstract

Dipeptidyl peptidase IV (DPP-4) is a serine protease that plays a crucial role in glucose metabolism; hence, it is a significant target for type II diabetes mellitus treatment. DPP-4 inhibitors decrease glucose concentrations in such patients by preventing the rapid degradation and thereby lengthening the physiological actions of hypoglycemic incretin hormones. In this study, a structure-based virtual screening strategy was applied to search for novel natural DPP4 inhibitors. From the Supernatural database, 1856 natural structures were picked up and were subjected to molecular docking analysis. Thirteen of them were identified to form more stable complexes than the co-crystallized ligand with the DPP-4 protein. The drug-likeness and pharmacokinetic properties of the top five compounds were also predicted. It was proved that the compounds were compliant with the drug-likeness rules and possess favorable pharmacokinetic properties. The proposed natural compounds can be introduced as potential DPP-4 inhibitors that might be promising leads for further drug development.

Keywords: DPP-4 inhibitor, serine protease, docking, ADME properties, drug-likeness

1. Introduction

Type 2 diabetes mellitus (T2DM) is a major metabolic disorder which considered being the "epidemic of the 21st century". It causes serious vascular complications, heart disease, renal failure, blindness, significant morbidity, and mortality. It is believed that several proteins participate in the formation and development of this chronic disease (1, 2). Dipeptidyl peptidase IV (DPP4) is a serine protease that plays a crucial role in glucose metabolism. DPP4 enzyme cleaves a dipeptide from the N-terminus of peptide substrates, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) (3, 4). Therefore, DPP4 is a promising target for T2DM treatment as it is key regulator of endogenous GLP-1, and GIP (4). It has been proved that DPP4 inhibition causes circulating GLP-1 and GIP levels to be increased, which leads to reduced levels of blood glucose, hemoglobin A1c and glucagon (5). DPP4 inhibitors including Sitagliptin, Vildagliptin, Saxagliptin, Alogliptin and Linagliptin are clinically used as antidiabetic drugs, and various candidates are developed in clinical trials (6, 7).

Human DPP-4 is a 110 kDa transmembrane glycoprotein consists of a cytoplasmic tail, a transmembrane region and an extracellular part. The extracellular region has two domains: one catalytic (residues 508-766) and other eight-bladed β-propeller chain (residues 56-497) which also contributes to the inhibitor binding site (8).

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The binding site of DPP4 consists of S1 and S2 pockets; the S1 pocket includes catalytic triad Ser630, Asp708 and His740, and the S2 pocket involves interactions with Glu205-Glu206, Arg125 and other residues. The substrate specific S1 cavity is composed of hydrophobic residues including Tyr 631, Val 656, Trp 659, Tyr 662, Tyr 666, and Val 711 and the inhibitor specific S2 subsite is surrounded by Val207, Ser209, Arg358 and Phe357 hydrophobic residues. The S2 cavity also governs the DPP-4 inhibitor selectivity over DPP-8 and DPP-9 (9, 10).

To date, lots of DPP4 crystal structures in complex with various ligands have been reported; therefore, the structure-based methods can be applied to discover novel DPP-4 inhibitors. Molecular docking is a beneficial technique to predict how small molecules interact with proteins. Based on the docking studies, virtual screening has become a powerful tool for the discovery of DPP-4 inhibitors and many DPP-4 inhibitors has been discovered via the ligand-based methods (11-15).

Natural products (NPs) have represented a source of diverse chemical scaffolds and bioactive structures for the medicinal chemists (16, 17). A number of NPs such as cyanidin, cyanidin-3-glucoside, malvidin, luteolin, apigenin, quercetin, kaempferol, flavone, hesperetin, naringenin, berberine, and galangin have been reported to exhibit promising DPP-4 inhibitory activity (18-20). Considering these ideas, we decided to apply molecular docking analysis to identify DPP-4 inhibitors from a natural product database. After docking, we determined in silico drug-likeness and pharmacokinetic properties of the proposed DPP-4 inhibitors.

2. Materials and methods

2.1. Ligand Selection and Preparation

A dataset containing 1856 NP structures were obtained from Supernatural database. NPs containing amine and amide groups were chosen as the reported DPP4 inhibitors mostly possess these moieties in their structures. All the ligands were exported in a single file format for the docking analysis.

2.2. Protein Structure for Docking

Protein target selection was done from the Protein Data bank (http://www.rcsb.org/pdb). The X-ray crystal structure of human DPP4 (PDB ID: 3HAC) was selected. After removing the co-crystallized ligand and water molecules from 3HAC, the crystal structure was then prepared for docking analysis using AutoDock Tools 1.5.4. For this purpose, hydrogens were added, nonpolar hydrogens were merged and Gasteiger charges were calculated for protein 3HAC.

2.3. Docking procedure

Docking was performed by AutoDock 4.2 and AutoDock Tools 1.5.4. The grid maps were constructed by AutoGrid and grid box dimensions were set to 60×60×60 with 0.375 Å grid spacing. The grids' center were placed on the binding site of the co-crystallized ligand (x=41.37, y=51.18, z=35.61). In order to determine the docking parameter file, rigid macromolecule and Lamarckian genetic search algorithm were chosen and the number of GA runs was set at 100. Default values were retained for the rest of the parameters. Docking validity was tested using co-crystallized inhibitor as ligand and the above-mentioned procedure.

2.4. In silico drug likeness and pharmacokinetics calculations

The drug likeness and pharmacokinetics properties of the NPs were determined using the preADMET online server (http://preadmet.bmdrc.org/).

3. Results and discussion

3.1. Virtual Screening of DPP4 Inhibitors

Virtual screening of NP libraries may facilitate the search for novel lead compounds that are suitable for further drug discovery study. The 3D structure of PDB ID 3HAC was retrieved from the Protein Data Bank and AutoDock 4.2 software was applied to screen the 1856 NPs for prediction of highly binding compounds. Docking results are listed in Table 1. Top compounds were introduced as the potential DPP4 inhibitors. Docking validation was done by removing the structure of the innate ligand and re-docking it into the receptor (self-docking). The root mean square deviation

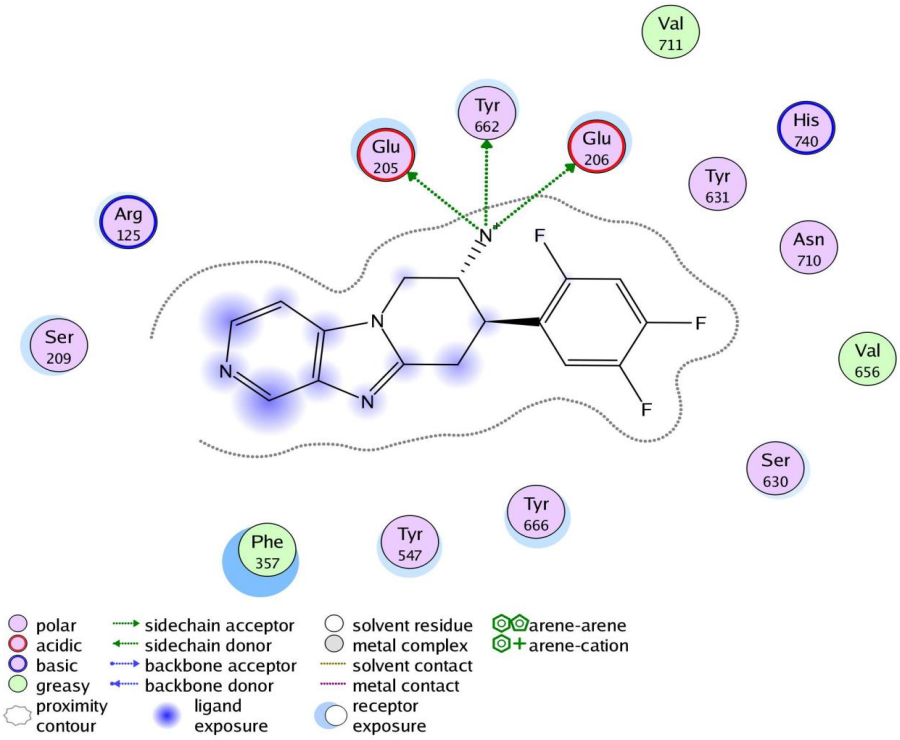

Figure 1. The interaction of co-crystallized ligand, (7R,8R)-8-(2,4,5-trifluorophenyl)-6,7,8,9-tetrahydroimidazo[1,2-a:4,5-c']dipyridin-7-amine, with the catalytic site of DPP4 (PDB code: 3HAC, resolution 2.0).

(RMSD) between the best pose of co-crystallized ligand docked into the binding site of tyrosinase and the one in the crystal structure was 1.51 Å (Figure 1). According to the docking results (Table 1), thirteen compounds showed more favorable estimated free energies of binding than the co-crystallized ligand did. Representations of the docking results for the five compounds with the most negative estimated free energies of binding including SN00027017, SN00010049, SN00016481,

Table 1. Docking results of the co-crystallized ligand and top thirteen NP compounds into the DPP4 binding site (PDB code: 3HAC).

Code	ΔG (kcal/mol)	Ki (nM)	Involved Amino acids in H-bonding
Co-crystallized Ligand	-9.2	160.15	Glu206,Glu205,Tyr662
SN00027017	-11.10	7.28	Glu205,Glu205,Arg669
SN00010049	-11.03	11.31	Tyr666,Glu205,Glu206,Tyr585,Gln553
SN00016481	-11.00	8.63	Tyr547,Glu205,Arg669
SN00027018	-10.86	11.00	Glu205,Glu205,Arg669
SN00015223	-10.58	17.71	Arg358,Ser630,Tyr631,Tyr547,Glu206
SN00091823	-10.52	19.40	Tyr547,Arg127
SN00036033	-10.35	26.04	Glu206,Ser630,Try547,Tyr666
SN00032074	-10.28	29.03	Phe357,Arg358,Glu206
SN00100126	-10.21	33.03	Glu206,Glu206,Tyr547,Tyr662,Tyr662
SN00027106	-10.13	37.30	Tyr666,Cys551,Glu205,Arg358
SN00009997	-10.09	40.30	Tyr666
SN00025160	-10.08	40.67	Glu205
SN00073066	-10.07	41.23	Glu205,Glu206

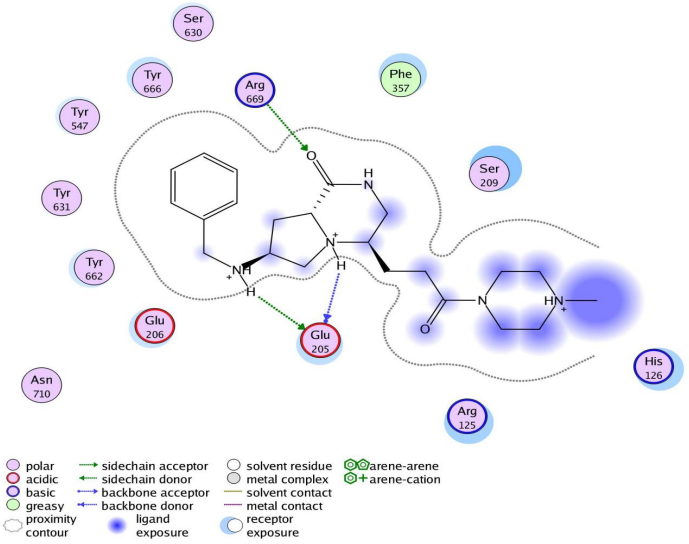

Figure 2. The interaction of compound SN00027017 with the catalytic site of DPP4 (PDB code: 3HAC, resolution 2.0).

SN00027018 and SN00015223 (ΔG=-11.10-10.07 kcal/mol) are depicted in Figures 2-6.

The binding pose of SN00027017 possessed the most negative estimated free energy of binding (ΔG=-11.10 kcal/mol) with DDD-4 enzyme is illustrated in Figure 2. Its phenyl moiety occupies the hydrophobic S1 pocket and surrounded by Tyr547, Tyr631, Tyr662 and Tyr666. The pyrrolidinamine moiety involved in three hydrogen bindings; two with Glu205 and one with Arg669. The other parts of the molecule occupied the S2 pocket including His129 and Phe357 residues. Docking results revealed that all top ten compounds listed in Table 1 (except SN00091823) established the key interaction with residues Glu205 and/or Glu206; however, due to the diverse skeleton of these compounds, the binding modes were different. The compounds well accommodated within DPP-4 active site, and different parts of the molecules oriented favorably in the S1 and S2 hydrophobic pockets. Other residues including

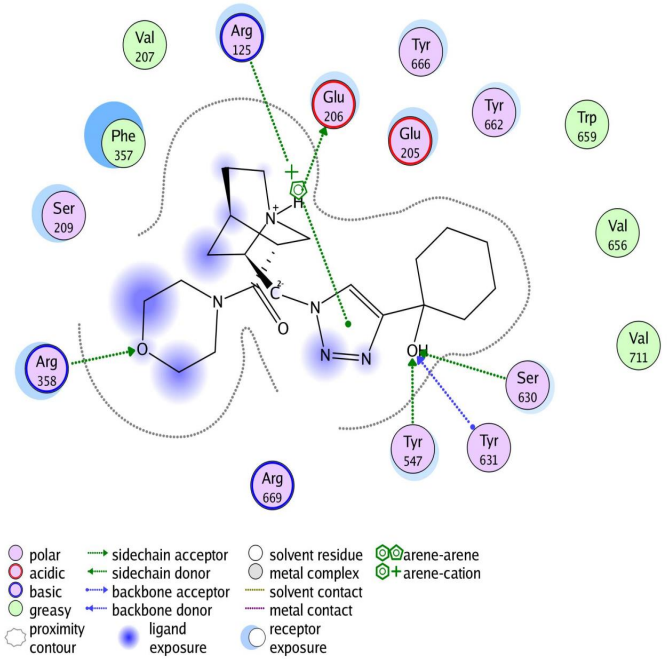

Figure 3. The interaction of compound SN00015223 with the catalytic site of DPP4 (PDB code: 3HAC, resolution 2.0).

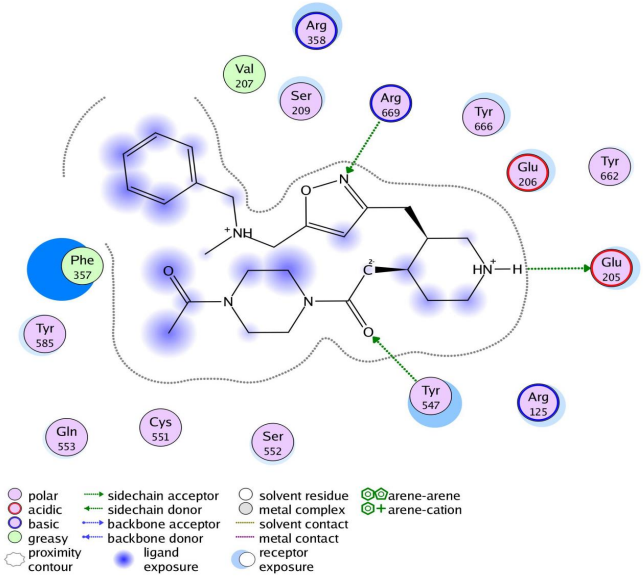

Figure 4. The interaction of compound SN00016481 with the catalytic site of DPP4 (PDB code: 3HAC, resolution 2.0).

Tyr666, Tyr585, Gln553, Tyr547, Arg358, Ser630, Tyr631, Arg127, Cys551 and Phe357 also played a significant role in the formation of stable ligand-target complexes.

3.2. Drug-likeness and ADME properties

The drug-likeness properties (including; CMC like rule, Lipinski rule of five, MDDR like rule, and WDI like rule) and pharmacokinetics scores (including; human intestinal absorption, in vitro Caco-2 (Caucasian colon adenocarcinoma) cell permeability, in vitro MDCK (Maden Darby Canine Kidney) cell permeability, in vivo BBB penetration and in vitro plasma protein binding) were predicted for the top five potential DPP4 inhibitors possessed the most negative estimated free energies of binding in the active site of DPP4. The results are summarized in Table 2 and Table 3. Tthe proposed NP compounds fulfill the drug-likeness rules; however, NP SN00016481 is an excep-

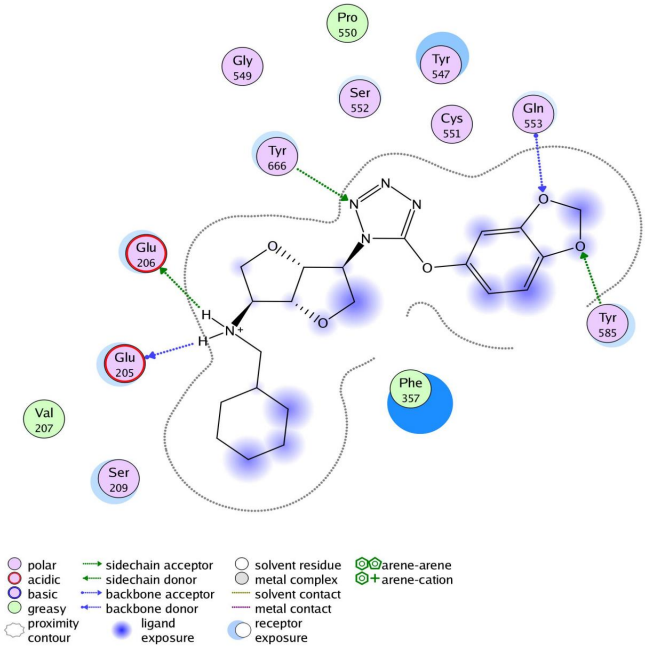

Figure 5. The interaction of compound SN00010049 with the catalytic site of DPP4 (PDB code: 3HAC, resolution 2.0).

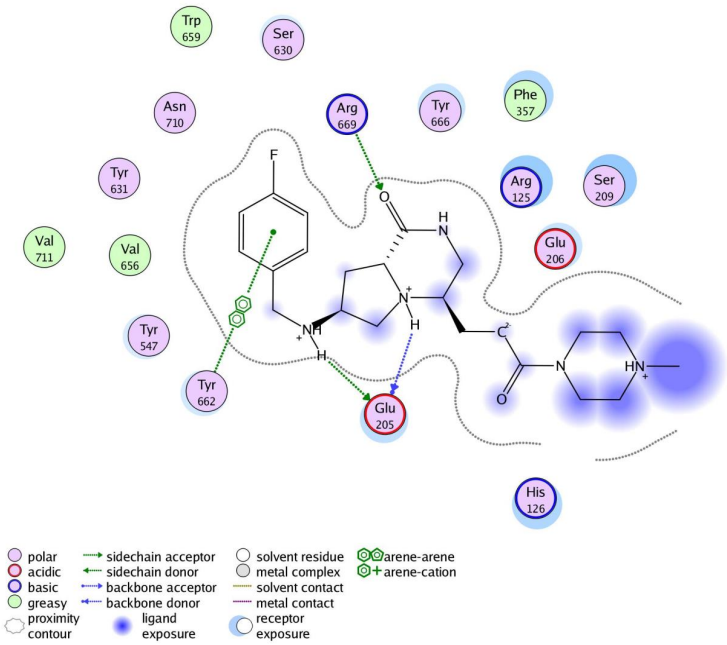

Figure 6. The interaction of compound SN00027018 with the catalytic site of DPP4 (PDB code: 3HAC, resolution 2.0).

tion as it is not compliance with the CMC like rule criteria (Table 2). The data in Table 3 reveals that the compounds exhibit favorable ADME properties. It is predicted that the compounds showed well intestinal absorption and bind weakly to plasma proteins demonstrating that they may be developed in oral dosage forms. Moreover, the proposed NPs are predicted to have low blood–brain barrier permeation; therefore, they may not cause neurotoxicity.

Table 2. *In silico* Lipinski rule of five, CMC like rule, MDDR like rule, and WDI like rule prediction for the top five potential DPP4 inhibitors.

Code	CMC like rule	MDDR like rule	Lipinski's rule of five	WDI like rule
SN00010049	Qualified	Drug-like	Suitable	In 90% cutoff
SN00015223	Qualified	Mid-structure	Suitable	In 90% cutoff
SN00016481	Not qualified	Drug-like	Suitable	In 90% cutoff
SN00027017	Qualified	Drug-like	Suitable	In 90% cutoff
SN00027018	Qualified	Drug-like	Suitable	In 90% cutoff

4. Conclusion

The molecular docking analysis of 1856 structure from Supernatural database identified thirteen natural compounds as potential DPP-4 inhibitors. The in silico drug-likeness and ADME

Table 3. *In silico* ADME profiling of the top five potential DPP4 inhibitors.

	Absorption			Distribution	
Code	Human intestinal absorption HIA (percentage)	In vitro caco-2 cell permeability (nm s-1)	In vitro MDCK cell permeability (nm s-1)	In vitro plasma protein binding (percentage)	In vivo blood–brain barrier penetration (C.brain/C.blood)
SN00010049	94.89	10.95	99.85	67.27	0.08
SN00015223	96.65	23.78	6.66	36.56	0.11
SN00016481	97.26	46.71	0.66	11.01	0.09
SN00027017	93.99	40.07	1.08	10.54	0.02
SN00027018	94.00	40.36	2.07	10.76	0.03

calculations revealed that the compounds show compliance with the drug-likeness rules and have desired pharmacokinetic properties. Therefore, it could be suggested that the proposed NPs introduced as potential DPP-4 inhibitors; however, complementary biological evaluations will be the subject of future studies.

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Conflict of Interest

None declared.

5. References

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