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Research Article

Dipeptidyl Peptidase IV Inhibitory Activity of Berberine and Mangiferin: An *In Silico* Approach

Abstract

Background: Dipeptidyl peptidase-IV (DPP-IV) Inhibitors may represent single anti-diabetic drugs, the multiple actions of which may translate into demonstrable therapeutic benefits in diabetes. The marketed synthetic DPP-IV Inhibitors are expensive drugs and have been reported to cause unacceptable adverse effects such as pancreatitis, angioedema, thyroid and pancreatic cancers. In this scenario, research to identify DPP-IV Inhibitors from alternative sources is desirable.

Aims and Objective: The study was designed to elucidate the DPP-IV Inhibitory activity of Berberine and Mangiferin, determine the binding sites and affinity of Berberine and Mangiferin for DPP- IV enzyme using *in silico* studies and compare it to synthetic DPP-IV Inhibitor: Vildagliptin.

Material and Methods: The crystal structure of human DPP IV (PDB Id: 2QT9) was downloaded from Protein Databank. Berberine and Mangiferin were computationally designed and screened through *in silico* docking studies against crystal structure of DPP-IV. Computational *in silico* studies were used to identify the sites as well as amino acid residues on DPP-IV enzyme to which these natural DPP-IV Inhibitors binds.

Results: The DPP-IV Inhibitory activity of Mangiferin was found to be comparable to synthetic marketed DPP-IV Inhibitors; Vildagliptin and Sitagliptin. Like Vildagliptin, Berberine and Mangiferin bind within the active site pocket (the 1st largest pocket) of DPP-IV enzyme whereas, Sitagliptin prefers to bind in the second largest pocket of DPP-IV enzyme. Berberine prefers to bind to the active site pocket of DPP-IV enzyme. Berberine binds very close to Glu205 and Glu206. As delineated using *in silico* binding energy results, Mangiferin, possess superior DPP-IV inhibitory activity as compared to Sitagliptin and Vildagliptin.

Conclusion: *In silico* studies demonstrates that Berberine and Mangiferin possess significant DPP-IV Inhibitory activity comparable to marketed synthetic DPP-IV Inhibitors.

Introduction

Recently, one of the additions to this anti-diabetic armament of drugs is dipeptidyl peptidase-IV Inhibitors (DPP-IV) [1], DPP-IV Inhibitors increase incretin levels: Glucagon-like peptide-1 (GLP-1) and Glucagon inhibitory peptide (GIP), which inhibit glucagon release, which in turn increases insulin secretion, decreases gastric emptying, and decreases blood glucose levels [2,3]. A recent study has shown beneficial effects of DPP-IV Inhibitor on metabolic parameters in type 2 diabetic patients [4], DPP-IV Inhibitors have a large number of beneficial effects in the subgroup of metabolic syndrome with diabetes. Apart from glycemic control, they are known to lower blood pressure, are weight neutral, improve dyslipidemia, reduce inflammatory markers, diminish oxidative stress,

improve endothelial function and reduce platelet aggregation in patients with type II diabetes [5], Therefore, it is plausible to explore the therapeutic potential of DPP-IV Inhibitors in experimental diabetes with metabolic syndrome.

As a drug class, DPP-IV Inhibitors are widely used clinically because of their low risk of hypoglycemia, once daily dosing, pharmacological effects such as weight neutral and preservation of beta cell mass [6], However, in spite of their beneficial effects, they have limitations: high cost of therapy and unacceptable adverse effects such as pancreatitis, pancreatic cancer, angioedema and thyroid cancer. In this scenario, it would be desirable to identify novel DPP-IV Inhibitors from alternative sources, that share the beneficial effects of the available synthetic DPP-IV Inhibitors and at the same time are cost effective. In this light, the DPP-IV Inhibitors obtained



from natural source may be an alternative that needs to be explored, considering the rich biodiversity India is bestowed with. With these point in view, the present study was designed.

Asian countries such as India and China are already known for their contributions toward the usage of plant medicine in preventing and treating various diseases [7], Mangiferin, a major phytochemical in *Mangifera indica* has been reported to possess DPP-IV inhibitory activity. It belongs to family Anacardiaceae Mangiferin. Being a glucosylxanthone, it possesses strong antioxidant, anti-lipid peroxidation, immunomodulation, anti-diabetic cardiotonic, hypotensive, wound healing, anti-hyperlipidemic, anti-atherogenic and anti-degenerative properties [8], Mangiferin showed significant antihyperlipidemic and antiatherogenic activities as evidenced by significant alteration of lipid profile level and diminution of atherogenic index in diabetic rats [9], Although several benefits of Mangiferin have been reported by multiple pathways, there is no experimental evidence presently available in literature with regard to its DPP-IV inhibitory activity.

Berberine, an isoquinoline alkaloids originally isolated from the root of *Berberis aristata* belongs to family Berberidaceae. It has shown a wide array of pharmacological activities including antimicrobial, antitumor, anti-inflammation and antidiabetic activity [10], in 1988, the hypoglycemic effect of Berberine was found when Berberine was used to treat diarrhoea in diabetic patients in China. Since then, Berberine has been used as an anti-hyperglycemic agent also in a recent single-blind clinical observation, the study showed that Berberine supplementation was beneficial in correcting lipid metabolism disorders and reducing cardiovascular risk factors. Berberine has been reported in the several literatures to have beneficial effects in human type II diabetes. Experimentally, Berberine was found to inhibit human recombinant DPP-IV *in vitro*. The findings suggest that DPP-IV inhibition is, at least, one of the mechanisms that explain the anti-hyperglycemic activity of Berberine [11].

The DPP-IV Inhibitory activities of Berberine and Mangiferin were reported from the laboratory [12]. The results of *in vitro* and *in vivo* studies in the experimental model of diabetes co-existing with metabolic syndrome confirmed the DPP-IV Inhibitory activity of Berberine and Mangiferin. Serum DPP-IV levels were measured and was found to directly correlate to the antidiabetic efficacy of Berberine and Mangiferin in experimental rats [13], to further elucidate the binding sites and affinity of Mangiferin and Berberine for DPP-IV enzyme *in silico* docking studies were designed. Computational *in silico* studies were used to confirm the DPP-IV Inhibitory activity of Berberine and Mangiferin and identify the sites as well as amino acid residues on DPP-IV enzyme to which these natural DPP-IV Inhibitors binds.

Materials and Methods

The crystal structure of human DPP IV (PDB Id: 2QT9) [14], was downloaded from Protein Databank [15], which has a resolution of 2.1 Å. This was considered as a receptor for docking

studies. The ligand selected includes sitagliptin, vildagliptin, mangiferin and berberine. All of them were downloaded from Pubchem Database [16]. The later was obtained from Chemfaces (www.chemfaces.com). All these structures were retrieved in SDF file format which was further converted into 3D format using Frog v2.14 (FRee On line druG conformation generation) [17]. In the Frog online server, the input was maintained as '1D to 2D' and the input drug description was 'SDF' file. In the calculation parameters the output format was maintained as 'PDB'. The minimize option was opted as 'yes'. In the produce option we selected 'single'. The total number of conformers generated was kept as 100. While rest of the options were left as default. The active site of DPPIV was retrieved through literature search and also predicted based on CASTp online server to identify pockets [18]. Now the receptor and the 3D generated ligands were considered for docking using Hex software 8.0.0 [19], it is an interactive molecular graphics program for calculating and displaying feasible docking modes which uses spherical polar Fourier (SPF) correlations to accelerate the calculations. During docking, in the docking parameter settings, we opted for 'shape and electro' for correlation type. Sampling method was 'range angles'. Post processing was OPLS minimization. The rest of the options were left default. Here the 2QT9 was loaded along with their inhibitor 4-aryl cyclohexylalanine in complex state. Now this was considered as the reference pose for the docking of DPPIV of *Homo sapiens* against all the available chemical compounds. Top ten docked poses were downloaded and considered for further analysis using Swiss pdb viewer and CHIMERA software [20].

Results

In this study, the crystal structure of DPP IV was considered as receptor which was docked against 14 chemical compounds. The available chemicals in 2D format were converted into 3D format and further minimized using Frog2 software. All these optimized chemical compounds were considered for docking against DPPIV based on the reference structure of DPPIV against 4-aryl cyclohexylalanine in complex state (2QT9). Basically, DPPIV is active in homo dimer form. The active site is a deep cleft in DPPIV which can be accessed via the opening of the propeller domain or through side opening formed at the interface of the β-propeller and hydrolase domains. Furthermore, the β propeller is a funnel shaped tunnel which extends to the active site (Figure 1a). All these chemical compounds were docked in the active site pocket (Figure 1b). In addition to this, the active sites were also predicted using CASTp software. This online server listed 198 pockets with area and volume. The first predicted pocket was the largest pocket with an area of 6086.4 Å² and a volume of 16471 Å³. The second largest pocket has an area of 574.3 Å² with a volume of 1171.8 Å³. Third largest pocket is observed proximal to the first largest pocket with an area 274.4 Å² and a volume of 191.5 Å³ (Figure 2).

All these listed compounds were docked against the reference docked pose of 4-aryl cyclohexylalanine inhibitor binding site from 2QT9 crystal structure. Berberine prefers the



active site pocket of DPP-IV enzyme. Of these, in particular, Berberine binds very close to Glu205 and Glu206 (Figure 3). Compounds like Vildagliptin and Mangiferin prefers to bind near the interface region of the DPPIV as their biological active forms are homodimer (Figure 4a,b). Sitagliptin binds near the α/β hydrolase domain (Figure 5). It was observed that few of compounds preferred to bind within the active pocket, whereas others prefer to interact with interface region, β propeller and α/β hydrolase domains. However, as per CASTp prediction, in addition to the active site, the region of interface and the α/β hydrolase and β propeller domain combo is also considered as

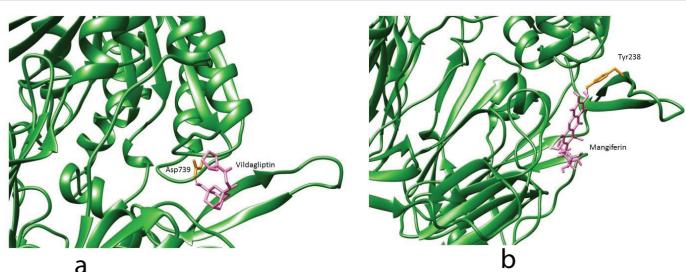


Figure 4: The docking of chemical compounds in the interface region of DPPIV receptor. (a) Vildagliptin (b) Mangiferin. Images generated using CHIMERA.

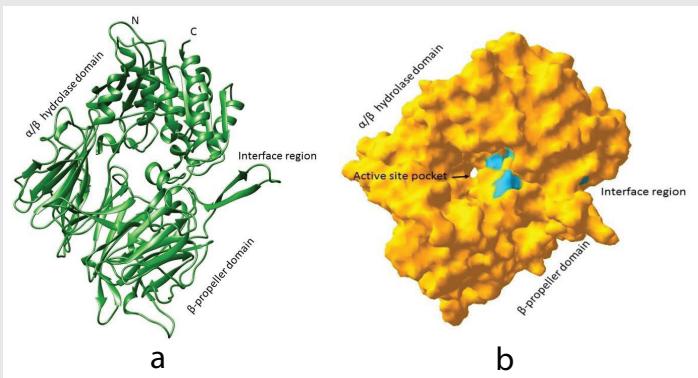


Figure 1: The crystal structure of DPPIV (a) the Ribbon format showing the α/β hydrolase domain, β propeller region, active site pocket and the interface region. (b) The surface generated structure showing the active site pocket in blue colour. Images were generated using Swiss pdb viewer.

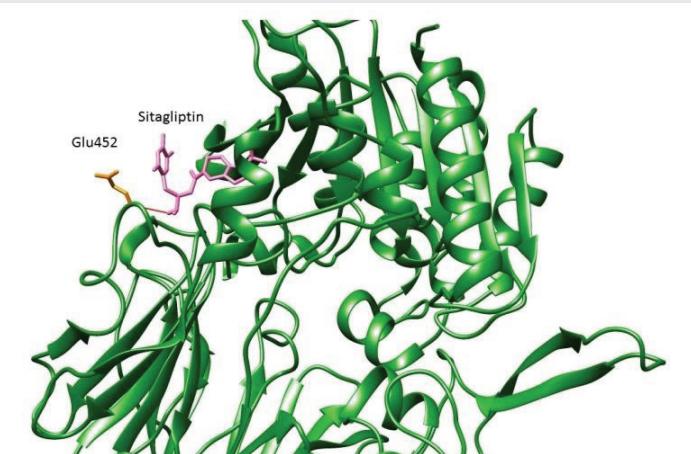


Figure 5: The docking of Sitagliptin near α/β hydrolase domain and beta propeller domain of DPPIV receptor. Images generated using CHIMERA.

39-	TRKTYTLLTDY	LNTYTRKLKY	SLRWISDHEI	LYKEENNILV	FNAEYGNSSV
89-	FLENSTPDEF	GHSINDWYS	PDDOFILLEY	NNVKRWRHSY	TASYDIYDNLN
139-	KRQLSTTEIRI	PNTQWVWTWS	PVGHKLAYVW	NNNDIVVKIEP	NLPSYRITWT
189-	GKEDIIYNG	TDWVYEEEVF	SAYSALWWSP	NGTFFLAYAQF	NDTEVPLIE
239-	SFYSDESLQY	PATVVRVPYPK	AGAVNPTVKF	FVVNTDSLSS	VTNATSQIT
289-	APASMLIGDH	YLQODVTWATO	ERISLQWLRR	IQNTSYMDIC	DYDESSGRWN
339-	CLVARQHIE	MSTTGWVGRFR	PSEPHFTLQDG	NSFVXISNE	EGYRHICGYZQ
389-	IDK KDC	TFIT	ALTSDLYYYI	SNEYHGMPGG	RNLHYKIQLSD
439-	YTKVTC	LSL	VSFSKEAKYY	QLRCGPGPLP	LYTLHSSVND
489-	KGLRLVEDNS	ALDKMLQNVO	MPSKHLDFII	LNEETHFWYQM	ILPYPFDKSK
539-	KYPUPLL	DVVA	FRLNWATYLA	STENIIVASF	DGRGSGYQQGD
589-	KIM	HAINRRL	AARQFSKMGF	VDNKRIAI	WG WSY GGYVTSM
639-	VLGGSGGV	CGIAVAPVSR	WEYVDSVYSTE	RYMLGLPTED	NLDHYRNSTV
689-	MSRAENFKQV	EYLLIHGTAD	DNVHFGQSAQ	ISKALVDVGV	DFQAMWYTYDE
739-	DHGIASS	TAH QHIYTHM	SHF IKQCFSLP		

Figure 2: The active site pocket prediction using CASTp software. The dark green colour denotes the largest pocket residues. The aqua denotes the second largest pocket residues.

part of the largest active site pocket. Berberine and Vildagliptin prefers to bind within the active site pocket (the 1st largest pocket). Mangiferin prefers to bind in the second largest pocket and Sitagliptin prefers to bind with the third largest pocket.

Discussion

Several anti-diabetic drugs are available for the treatment of type 2 diabetes mellitus. A relatively new class of oral hypoglycemic agent, DPP-IV Inhibitors has recently been introduced. DPP-IV Inhibitors increase incretin levels {GLP-1 (glucose dependent insulinotropic polypeptide) and GIP (glucagon-like peptide-1)}, which in turn increases insulin secretion and decreases blood glucose levels. DPP-IV Inhibitors work by enhancing the sensitivity of β cells to glucose, and have also been shown to improve markers of β cell function. Since the introduction of DPP-IV Inhibitors in 2006, they are flourishing as monotherapy and also used in combination with commonly prescribed anti-diabetic agents [22]. However, in spite of potential multiple beneficial effects of DPP-IV Inhibitors, they do have certain limitations: high cost of therapy and unacceptable adverse effects. The marketed synthetic DPP-IV Inhibitors are expensive drugs when indicated for long term therapy for a chronic disease like diabetes and are also reported to cause unacceptable adverse effects like pancreatitis, upper respiratory infections, anaphylactic reactions and pancreatic cancer (411). In this scenario, research to explore DPP-IV Inhibitors from alternate sources is of paramount importance. The present study was developed to identify DPP-IV Inhibitors

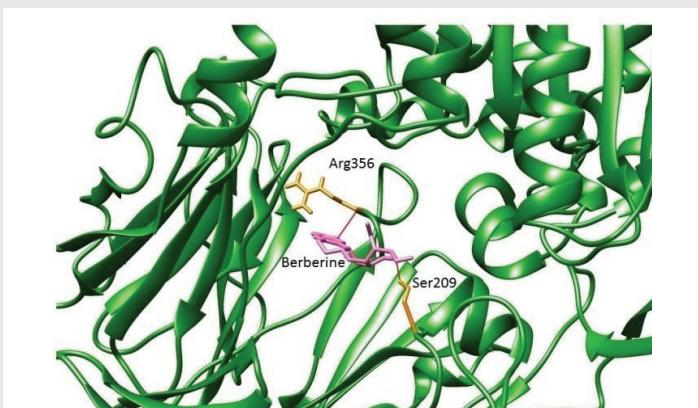


Figure 3: The docking of Berberine in the active site pocket of DPPIV receptor. Images generated using CHIMERA.



from indigenous sources which may work in concert with body's own defense mechanism, have fewer adverse effects and would be more affordable.

Computational *in silico* studies were used to confirm the DPP-IV Inhibitory activity of Berberine and Mangiferin and identify the sites as well as amino acid residues on DPP-IV enzyme to which these natural DPP-IV Inhibitors binds. The active principles Berberine and Mangiferin were computationally designed and screened through *in silico* docking studies against crystal structure of DPP-IV. The *in silico* methods have been demonstrated to be useful in predicting the potential of proteins as precursors of peptides in various bioactivities, such as DPP-IV. Such *in silico* computational methodologies have been widely used for virtual ligand and target based screening and profiling to predict biological activity. They can also be used to explore the target structures for possible active sites, generate candidate molecules, check for their drug generated likeness, dock these molecules with the target, rank them based on variations on the structures according to their binding affinities and further optimize the molecules to improve binding characteristics [23].

The crystal structure of DPP-IV shows that the enzyme 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 Leu455-Val55). Catalytic domain and propeller domain together embrace an egg-shaped cavity of approximate dimensions 40 Å X 20 Å X 20 Å, which harbours the active centre [23].

The crystal structure of DPP-IV was considered as receptor which was docked against the Berberine, Mangiferin, Sitagliptin and Vildagliptin. All these optimized chemical compounds were considered for docking against DPP-IV based on the reference structure of DPP-IV against 4-aryl cyclohexylalanine in complex state (2QT9). Basically, DPPIV is active in homo dimer form. The active site is a deep cleft in DPPIV which can be accessed via the opening of the propeller domain or through side opening formed at the interface of the β -propeller and hydrolase domains. Furthermore, the β propeller is a funnel shaped tunnel which extends to the active site. The propeller domain gets well packed against the hydrolase domain, and the catalytic triad (S630, H740, and D708) which is at the interface of the two domains [23]. The active site cavity is well guarded by residues like W659, Y631, Y547, P550, W629, Y752, F357, Y666, E205, E206, Y662, N710, R125, V656, S630 and V711 [24]. All these chemical compounds were docked in the active site pocket. In addition to this, the active sites were also predicted using CASTp software. This online server listed 198 pockets with area and volume. The first predicted pocket was the largest pocket with an area of 6086.4 Å² and a volume of 16471 Å³. The second largest pocket has an area of 574.3 Å² with a volume of 1171.8 Å³. Third largest pocket is observed proximal to the first largest pocket with an area 274.4 Å² and a volume of 191.5 Å³.

Berberine and Mangiferin showed significant inhibition of DPP-IV enzyme. Berberine binds to the active site pocket, very close to Arg 356 and ser 209 of DPP-IV receptor. Mangiferin binds near the interface region near Tyr 238 of the DPP-IV receptor. Vildagliptin preferred to bind to Asp739 amino acid near the interface region of the DPP-IV as their biological active forms are homodimers and Sitagliptin binds near the α/β hydrolase domain. It was observed that few of the ligands preferred to bind within the active pocket, whereas others prefer to interact with interface region, β propeller and α/β hydrolase domains. However, as per CAST prediction, in addition to the active site, the region of interface and the α/β hydrolase and β propeller domain combo are also considered as part of the largest active site pocket. The synthetic DPP IV inhibitors: Sitagliptin binds to amino acids: Glu452 and Vildagliptin to Asp739. Berberine and Mangiferin bind to the amino acid residues: Ser 209, Arg356 and Tyr 238. The binding energy is inversely proportional to the DPP-IV inhibitory activity. Based on the binding energy results, it was found all the ligands (Berberine, Mangiferin and Vildagliptin) studied had superior DPP-IV binding affinity as compared to Sitagliptin.

Previous *in vitro* and *in vivo* experimental studies from the laboratory demonstrated the DPP-IV Inhibitory activities of Berberine and Mangiferin. Results demonstrated that DPP-IV inhibition is one of the mechanisms attributing to the therapeutic efficacy of Berberine and Mangiferin in experimental diabetes with metabolic syndrome [12]. In the present study the DPP-IV inhibitory activity of Berberine and Mangiferin has been delineated using *in silico* docking studies against crystal structure of DPP-IV and compared to the synthetic DPP-IV Inhibitors. *In silico* results emphasize the potential of developing Berberine and Mangiferin as natural alternative to synthetic DPP-IV Inhibitors (Table 1).

Conclusion

Vildagliptin, Mangiferin prefers to bind near the interface region of the DPP-IV as their biological active forms are homodimer. Berberine prefers to bind to the active site pocket of DPP-IV enzyme. Berberine binds very close to Glu205 and Glu206. Sitagliptin binds near the α/β hydrolase domain of DPP-IV enzyme. Berberine, Mangiferin and Vildagliptin prefer to bind within the active site pocket (the 1st largest pocket) of DPP-IV enzyme whereas Sitagliptin prefers to bind in the second largest pocket of DPP-IV enzyme.

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Table 1: Details of chemical compounds with their IC50 value, binding energy and their interacting residues against DPPIV.

S.No	Chemical compound	IC50	Binding energy	Amino acids
1	Sitagliptin	18nm	-139.91	Glu452
2	Vildagliptin	3nm	-237.57	Asp739
3	Mangiferin		-296.76	Tyr238
4	Berberine	13.3nm	-229.45	Ser209,Arg 356



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