

Research Article

# *In silico* discovery of potential inhibitors against Dipeptidyl Peptidase-4: A major biological target of Type-2 diabetes mellitus

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## Abstract

**Objectives:** Type-2 diabetes mellitus, caused by impaired secretion of insulin, is becoming one of the health hazardous threats to human lives across the world. Its prevalence is rising with time. In this study, 2750 phytochemicals, that are considered to have great ability to eliminate diseases caused by different viruses and bacteria, are obtained from different medicinal plants and discovery of inhibitors through *in silico* method was performed against Dipeptidyl peptidase-4 (DPP4).

**Method:** The pharmacological assessment and pharmacokinetics of phytochemicals, molecular docking and density functional theory (DFT) analysis helped to explore the inhibitory action of phytochemicals against DPP4. Total forty-nine phytochemicals were screened initially to reduce the number of compounds to be analyzed further based on a threshold of binding affinity  $\geq -5.5$  kcal/mol and were considered for further computational studies to analyze their inhibitory effects for DPP4. For comparison and validation of the results of present study, various previously reported and experimentally validated compounds were docked with the DPP4. For these dockings, binding affinity was predicted and compared with those of phytochemicals to check if these phytochemicals are competent enough to be used as an inhibitor in the treatment of diabetes mellitus in the future.

**Results:** Only four phytochemicals showed binding affinity greater than those of experimentally validated compounds. These included two phytochemicals from *Silybum marianum*, i.e. Diprenylerydiol and Taxifolin and while other two phytochemicals from *Santolina insularis* and *Erythrina Varigatae* i.e. Papraline and Osajin respectively. The reactivity levels for these four phytochemicals with the binding site residues of DPP4 were obtained by DFT based analysis, in which ELUMO, EHOMO and band energy gap were computed.

**Conclusion:** Based on these results, it is concluded that these four phytochemicals, after passing through *in vitro* and *in vivo* validation, can be utilized as potential DPP4 inhibitors as they have strong properties as compared to those of various experimentally validated inhibitors.

## Introduction

Type-2 diabetes is part of a complicated metabolic syndrome, a long-term disorder that is characterized by abnormal insulin secretion, high blood sugar, insulin resistance or lack of insulin [1,2]. It has emerged as one of the commonly found diseases in the world and with epidemic prevalence

with about 300 million cases is to be expected worldwide by 2025 [3]. Both genetic and environmental factors are involved in pathogenicity of type-2 diabetes. It may result in many other disorders like obesity, increased thirst, frequent urination and physical inactivity [4]. There are many symptoms of type-2 diabetes but one of the main symptoms is of raising the blood glucose levels, which is usually caused by impaired

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production of insulin by the pancreas or improperly uptake of glucose. It may occur when insulin utilization in the muscles does not work properly or by the improper production of glucose in the liver. Thus, it may lead to hyperglycemia also. All of the mentioned conditions can lead to type-2 diabetes mellitus and reduce the progression of this rapidly spreading disease, there are many treatments, currently available for the management of hyperglycemia (e.g. metformin and other exogenous insulin) while some are in developmental process. Symptoms like weight gain, severe hypoglycemia, obesity, other complications and the lack of long-term efficacy could also be found. So to overcome this situation there comes a need for the identification of some new therapeutic targets that can not only treat type-2 diabetes but also accommodate these issues [5,6].

Dipeptidyl peptidase 4 (DPP4) is considered as a multifunctional glycoprotein, having the role of serine dipeptidase activity, and it is present both in circulation and on the cell surface. In the early 1990s, DPP4 was found to be involved in the inactivation of glucagon-like peptide 1 (GLP-1) [7,8] and glucose-dependent insulinotropic polypeptide (GIP) [9]. While both of these peptides GLP-1 and GIP (produced by intestine) is responsible for several glucose-mediated actions like glucose-induced insulin biosynthesis and secretion. Activities like inhibition of glucagon secretion, regulation of gene expression, slowing the process of gastric emptying and also trophic effects on  $\beta$ -cells are linked with GIP. So it is important for GIP and GLP-1 both to be active for the production of insulin by  $\beta$ -cells. These are significant to maintain the blood glucose level and regulation; also control appetite and body weight but unfortunately got attacked by DPP4 glycoprotein [10].

On the other hand, DPP4 inhibition play important role to increase GLP-1 and GIP circulations in humans, which leads to decrease in glucose level in blood and many other advantages related to antidiabetic therapies e.g. lowered risk of hypoglycemia, the potential for weight loss, and the potential for regeneration and differentiation of pancreatic  $\beta$ -cells etc [11,12]. Dipeptidyl peptidase-4 (DPP4), also known as adenosine deaminase i.e. complex of CD26 protein and considered as a marker for activated T-cell, is encoded by the DPP4 gene [13]. It belongs to the proline family. However, the exact functioning of these enzymes is still missing but the only point that cannot be ignored is their presence in some of the biological processes that are regulated by proline-specific amino-terminal processes [14].

In the present time, screening through *in vitro* and *in vivo* analysis is becoming comparatively much complicated, costly, time taking and large problems of the risk of wastage of investments are also present, when number of compounds to be analyzed is high. The *in silico* methods, comprised of computational techniques, facilitate the process of drug discovery by making the analysis worthwhile, efficient and also saves resource consumption [15,16]. Drug discovery, through computational analysis, helps to identify the potent

medicinal compounds having inhibitory potential and high efficiency [17]. The main purpose of this study was to discover some novel and potent inhibitors having good pharmacological profiles and reactivity. A total of 2750 phytochemicals were searched and obtained from medicinal plants of local region of Pakistan and India, and were analyzed against DPP4.

## Material and methods

The present study targets the Dipeptidyl peptidase-4 (DPP4) protein for type-2 diabetes mellitus. The method initiates by retrieving the structure of the protein, collecting phytochemicals and terminates at screening those compounds which are effective and potent against DPP4. For targeting DPP4 protein and to discover its inhibitors first of all retrieval of the structure of DPP4 was required, which was retrieved from RCSB Protein Databank (PDB ID: 4A5S). Binding pocket was confirmed through the already present inhibitor i.e. N7F (6-[(3S)-3-AMINOPIPERIDIN-1-YL]-5-BENZYL-4-OXO-3-(QUINOLIN-4-YLMETHYL)-4,5-DIHYDRO-3H-PYRROLO[3,2-D]PYRIMIDINE-7-CARBONITRILE), and later on, it was removed for preparing the structure for docking.

### Collection of phytochemicals and their pharmacological assessment

Total 2750 phytochemicals were selected through a literature survey. In which according to classification 1292 were flavonoids, 488 were sesquiterpene, 475 were terpenoids and 495 were alkaloids [18,19]. These medicinal plants and their important chemical compounds were searched by using different terms like plant names, their origin etc. It took the duration of 4-6 months for the complete searching period. Those plants were chosen for selecting phytochemicals, localized to India and Pakistan only [20,21]. After selecting plants, the phytochemicals of these selected plants were found and with the help of PubChem and DrugBank, their structures were retrieved. The phytochemicals were filtered through ADMET to predict drug-like properties of the protein by using PreADMET server [22] the pharmacological properties [23] of these phytochemicals were analyzed according to a criteria i.e. Lipinski's violations must be 0, solubility should be high or moderate, GI (gastrointestinal) absorption must be high, there should be no BBB-permeability while toxicity and carcinogenicity must be zero or nil.

### Molecular docking and estimation of binding energies

Molecular docking was performed to estimate binding energies of compounds with DPP4. The ligand-receptor complexes were attained with optimized conformation and to observe the behaviour of small molecule usually inhibitors in the binding pocket of the target protein. Retrieved protein was prepared for docking by further evaluations, performed on AutoDock Tools [24]. Molecular docking of DPP4 (Dipeptidyl peptidase-4) was performed with selected phytochemicals, one by one, using AutoDock Vina [25]. Model of DPP4 was prepared by doing some important editing steps just like doing the addition of polar hydrogen bonds in Auto dock tools that helped in modifying the interactions between



phytochemicals and DPP4. Then a three-dimensional (3D) grid was designed for DPP4 with a size of  $20 \times 20 \times 22 \text{ \AA}$ . This grid helped in defining the binding space for phytochemicals to be docked against DPP4. Similarly, phytochemicals (chemical compounds) were also prepared for docking by removing non-standard residues and using the same methodology [26]. For comparison and validation of the results of present study, various previously reported and experimentally validated compounds were docked with the DPP4. For these dockings, binding affinity was predicted and compared with those of phytochemicals to check if these phytochemicals are competent enough to be used as an inhibitor in the treatment of diabetes mellitus in the future [27,28].

The  $K_i$  (inhibitor constant) values of these compounds was determined by using a formula:

$$K_i = e^{\Delta G/RT}$$

Where  $\Delta G$  is binding energy,  $R$  is the gas constant while  $T$  is temperature constant.  $K_i$  values helped in the indication of how potent the inhibitors are.

### Analysis of reactivity using Density functional theory (DFT)

Density functional theory (DFT) analysis was used for studying and predicting the reactivity of ligands with interacting residues of DPP4 [29-32]. By applying the Becke, 3-parameter, Lee Yang-Parr (B3LYP) correlation function of DFT, highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energy were used [33]. The energy calculations were performed using the ORCA program [34].

## Results

### Pharmacological properties of phytochemicals

Before docking, all the phytochemicals were passed through ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) analysis to assess the pharmacology and pharmacokinetics. There were 1061 compounds out of 2750 phytochemical found to fulfil the Lipinski's rule of five. While the other compounds were filtered on the basis of different parameters i.e. 1689 phytochemicals were filtered and separated on the basis of high GI absorption, the permeability of BBB (blood-brain barrier), solubility rate, Toxicity and carcinogenicity. Out of total phytochemicals, only 108 passed the criteria that were set before, of being drug likeliness, having suitable ADMET profiles and further moved for docking. **Table S1** shows the results of some important ADMET parameters of phytochemicals i.e. ESOL class (solubility); all were soluble, GI (gastrointestinal) absorption; all phytochemicals showed high absorption, BBB (Blood-brain barrier) penetration; all phytochemicals were of no BBB penetration, Lipinski violations were zero of all compounds, there were carcinogenicity and toxicity observed of all phytochemicals.

ADMET of experimentally validated inhibitors was also done to perform a comparison between these compounds and the selected phytochemicals. ADMET analysis of these experimentally validated inhibitors revealed that gemigliptin, kombiglyze, teneligliptin and trelagliptin have the ability to penetrate the blood-brain barrier and saxagliptin showed low gastrointestinal absorption. Besides, there were some experimental compounds having very good ESOL class property (Table 1).

### Molecular docking of phytochemicals and their comparative validation

Binding energies and inhibitory constant ( $K_i$ ) values were obtained by docking all of the 108 phytochemicals with DPP4. The results of molecular docking revealed that when different phytochemicals made interactions with interacting residues or binding sites of the protein, they exhibit different behaviours. A threshold of  $-5.5 \text{ kcal/mol}$  (binding affinity) was set for the ligand-protein complexes for screening the effectively docked phytochemical. A total of forty-nine phytochemicals exhibited binding affinity  $\geq -5.5 \text{ kcal/mol}$ , showing the great inhibition against DPP4. In which only two compounds i.e. Papraline and Osajin obtained from the plant source *Santolina insularis* and *Varigatae* respectively showed the highest binding affinity of  $-6.5 \text{ (kcal/mol)}$  against DPP4, having  $K_i$  value  $16.989 \text{ \mu M}$  and made interactions with Val78, Ile63, Ser87, Asn85, Glu67 and Val78, Glu67, Pro109, Ile63, Leu69, Phe89, Ile10, respectively for Papraline and Osajin.

To compare the result of binding energies of the phytochemicals, certain experimentally validated inhibitors were searched out that were reported in different studies, and were docked against DPP4 (Table 2). Upon docking with DPP4, these inhibitors showed various binding affinities; and

**Table 1:** Pharmacological assessment of experimentally validated inhibitors.

Phytochemicals	ESOL Class	GI absorption	BBB Penetration	Lipinski violations	Carcinogenicity	Toxicity
Alogliptin	Soluble	High	No	0	No	No
Anagliptin	Soluble	High	No	0	No	No
Berberine	Soluble	High	No	0	No	No
Denagliptin	Soluble	High	No	0	No	No
Evogliptin	Moderately soluble	High	No	0	No	No
Gemigliptin	Soluble	High	Yes	0	No	No
Gosogliptin	Soluble	High	No	0	No	No
Kombiglyze	Very soluble	High	Yes	0	No	No
Linagliptin	Soluble	High	No	0	No	No
Melogliptin	Very soluble	High	No	0	No	No
N7F	Soluble	Low	No	1	No	No
Omarigliptin	Soluble	High	No	0	No	No
Phenylethylamine	Soluble	High	No	0	No	No
Saxagliptin	Very soluble	Low	No	0	No	No
Sitagliptin	Soluble	High	No	0	No	No
SSR162369	Soluble	High	No	0	No	No
Teneligliptin	Soluble	High	Yes	0	No	No
Trelagliptin	Soluble	High	Yes	0	No	No
Vildagliptin	Very soluble	High	No	0	No	No
Zomarist	Soluble	High	No	0	No	No

**Table 2:** Results for experimentally validated inhibitors docked against DPP4.

Inhibitors	Interaction Plot	Affinities kcal/mol	Ki Values( $\mu$ M)
Melogliptin		-6.3	23.820
Linagliptin		-6.0	39.545
N7F		-6.0	39.545
Gemigliptin		-5.9	46.824
Sitagliptin		-5.8	55.444
Vildagliptin		-5.8	55.444
Omarigliptin		-5.8	55.444

**Table 2:** Results for experimentally validated inhibitors docked against DPP4.

Inhibitors	Interaction Plot	Affinities kcal/mol	Ki Values( $\mu$ M)
Evogliptin		-5.7	65.650
Denagliptin		-5.7	65.650
Teneligliptin		-5.6	77.735
SSR162369		-5.6	77.735
Gosogliptin		-5.6	77.735
Berberine		-5.3	129.051
Saxagliptin		-5.1	180.936

**Table 2:** Results for experimentally validated inhibitors docked against DPP4.

Inhibitors	Interaction Plot	Affinities kcal/mol	Ki Values( $\mu$ M)
Kombigzyle		-5.1	180.936
Zomarist		-5.0	214.243
Trelagliptin		-4.8	300.379
Alogliptin		-4.7	355.674
Anagliptin		-4.7	355.674
Phenylethylamine		-3.9	1374.380



the highest affinity in experimentally validated inhibitors was -6.3 kcal/mol. This was set as threshold to screen potent and novel inhibitors from the set of phytochemicals, selected in this study. By comparing affinities of both sets, there were four compounds found with binding affinity  $\geq -6.3$  kcal/mol, which were Papraline and Osajin with binding affinity -6.5 kcal/mol; and Diprenyleriodictyol and SchizolaenoneB with binding affinity -6.3 kcal/mol (Table 3).

### Analysis of reactivity using DFT

The DFT result exhibited that the 4 selected phytochemicals have high reactivity against the DPP4. The energy gap values ranged from 0.109 kcal/mol to 0.128 kcal/mol and proved their high reactivity abilities. The values of ELUMO and EHOMO were also low, exhibiting the fact that lower band energy gaps result in high affinity of the inhibitors for the target proteins. Among these phytochemicals, Papraline showed the highest reactivity against the DPP4, because the lowest band energy was exhibited among all of the phytochemicals i.e. 0.109 kcal/mol (Table 4).

**Table 4:** Band energy gaps of selected phytochemicals.

Phytochemicals	LUMO (kcal/mol)	HOMO (kcal/mol)	Band Energy Gap (kcal/mol)
Papraline	-0.302	-0.411	0.109
Osajin	-0.290	-0.40	0.110
Diprenyleriodictyol	-0.139	-0.257	0.118
SchizolaenoneB	-0.310	-0.438	0.128

## Discussion

Diabetes is considered as epidemic worldwide and the majority of them have type-2 diabetes, which typically diagnosed in middle or later life. It is usually found associated with being overweight or obese. The trend to treat Type 2 diabetes is through lifestyle (diet and exercise) and oral antidiabetic agents (OADs) [35]. Since ancient times the plants and their phytochemical have been used as drugs for the treatment of type-2 diabetes mellitus in humans [36]. Earlier studies have shown that phytochemicals, that are safe and have no hazardous impact on human health, are considered as good therapeutic agents to cure many diseases [37,38]. There are some inhibitors of DPP4, which are already approved and

**Table 3:** Results for molecular docking of phytochemicals showing binding affinities  $\geq -6.3$ kcal/mol against DPP4.

Source Plants	Phytochemicals	Interaction plot	Binding Affinities (kcal/mol)	Ki ( $\mu$ M)
Santolina insularis	Papraline	<p><b>Interactions</b></p> <ul style="list-style-type: none"> <li>Conventional Hydrogen Bond</li> <li>Carbon Hydrogen Bond</li> <li>Pi-Anion</li> <li>Pi-Alkyl</li> </ul>	-6.5	16.989
Erythrina Varigatae	Osajin	<p><b>Interactions</b></p> <ul style="list-style-type: none"> <li>Conventional Hydrogen bond</li> <li>Pi-Anion</li> <li>alkyl</li> <li>Pi-Alkyl</li> </ul>	-6.5	16.989
Silybum Marianum	Diprenyleriodictyol	<p><b>Interactions</b></p> <ul style="list-style-type: none"> <li>Conventional Hydrogen Bond</li> <li>Upholeable Acceptor-Acceptor</li> <li>alkyl</li> <li>Pi-alkyl</li> </ul>	-6.3	23.820
Silybum Marianum	SchizolaenoneB	<p><b>Interactions</b></p> <ul style="list-style-type: none"> <li>Conventional Hydrogen Bond</li> <li>Pi-Anion</li> <li>Pi-Donor Hydrogen Bond</li> <li>Pi-Pi Stacked</li> <li>alkyl</li> <li>Pi-alkyl</li> </ul>	-6.3	23.820



have been used with diet and diabetic patient suggested to do exercise to control high blood sugar in adults with type-2 diabetes mellitus as prescribed medicines [39]. Medicinal class of DPP4 inhibitor includes sitagliptin, saxagliptin, linagliptin, and alogliptin most probably. They are available as both single-ingredient drugs and also in combination with some other diabetes medicines such as metformin (24). Lipinski's rule considered as a very critical rule, used for gaining drug-like properties of phytochemicals. These can be consumed by a patient for the treatment of disease [23]. In this approach, the phytochemicals once passing through the Lipinski's parameter, then leads to further evaluations on the basis of blood-brain barrier penetration behaviour. *In silico* screening method consists of such important parameters that help to explore the drug-like properties of all chemical compounds. BBB is called a semipermeable membrane barrier; its main function is to keep blood circulation separates from the fluid in the central nervous system (CNS). The drugs are more preferred that not reaching the CNS [40]. On the other hand, it was observed that the compounds that showed non-penetrating behaviour also represent another important parameter i.e. gastrointestinal absorption. A good phytochemical should have either high GI absorption that is found to be linked with epithelial cells [41].

Sitagliptin, also known as JANUVIA, is a derivative of triazolopiperazine, developed by Merck and Co., was approved in 2006 and considered to be a potent and selective inhibitor of DPP4. It could be used in combination with metformin or pioglitazone, giving different results. Sitagliptin is considered to be weight and lipid, neutral agent. It has been reported that Melogliptin also known as GRC-8200, found to be one of the potent and strong inhibitors of DPP4, developed by Glenmark Pharmaceuticals Ltd. [42]. It showed the highest binding potential to DPP4 among other experimentally validated inhibitors. According to another study, melogliptin is reported with 50% - 95% bioavailability i.e. with good pharmacokinetic profile and approved as safe and efficient for weight neutral agent. Another study reported that Linagliptin (BI-1356) also called as Ondero, is another experimental inhibitor that based on dihydropurinedione, developed by Boehringer Ingelheim [43]. It helps to weight loss and also enhances beta cell functioning [44]. Further studies reported that Vildagliptin a derivative of cyanopyrrolidine. Its medicinal name is GALVUS developed by Novartis. It is considered as second DPP4 inhibitor approved for human use and is indicated in type 2 diabetes [45]. It is one of the orally bioavailable DPP4 potent inhibitors. Some adverse impacts of Vildagliptin are a headache and dizziness [46,47]. Another type of gliptin Saxagliptin was reported as a derivative of methanoproline nitrile. Its pharmaceutical name is ONGLYZA, developed by Bristol/Myers Squibb (BMS). This one is considered as one of the most important because it is not only potent and selective but also versatile and long-acting DPP4 inhibitor with better stability and several different benefits as compared with other gliptins [48-51]. But a high dose of

saxagliptin can cause an adverse effect on human health. Although these were previously reported inhibitors, in which some are approved as commercial dose against type-2 diabetes mellitus just like Sitagliptin by name JANUVA, Vildagliptin by name GALVUS, Saxagliptin by name ONGLYZA and Linagliptin by the name TRADJENTA while some are approved as free of health hazardous impacts and some are under developmental studies but considered as good to treat against diabetes.

A comparison was made between affinities of screened phytochemicals and some experimentally validated inhibitors reported before. There were some types of gliptins found very important as oral drugs against the disease. In fact, many of them have been used as the commercial dose for the treatment of type-2 diabetes mellitus. According to this study, some inhibitors (like gemigliptin) were found that can penetrate the blood-brain barrier while one of them was found with less GI absorption i.e. saxagliptin. This was revealed by ADMET analysis of experimentally validated inhibitors; it might be the reason behind the serious side effects, which comes with the utilization or of these inhibitors as a drug. It led to the need for the development of some newer OADs, the DPP4 inhibitors with less or no side effects. It was observed that the highest affinity of experimentally validated inhibitors i.e. Melogliptin was -6.3 (kcal/mol) while that of the list of phytochemicals identified in this study, the highest binding affinity observed was of -6.5(kcal/mol) by the phytochemical Papraline originated from plant *Santolina insularis*. Also, the two more compounds Diprenyleriodictyol and SchizolaenoneB emerged showing the binding affinity of -6.3 (kcal/mol). It was observed that later on identified phytochemicals have shown stronger binding potential than experimental one thus after making it sure that these phytochemicals are free of hazardous impacts, these can be utilized for the treatment of type-2 diabetes mellitus.

## Conclusion

The main purpose of this study was computational drug discovery against DPP4 (Dipeptidyl peptidase-4). In this study, out of 2750 phytochemicals were selected from different medicinal plants of different countries, 108 were screened on by good ADMET profiles and docked while forty-nine were selected on the basis of having a strong binding affinity. The DFT results helped to access the reactivity of the selected compounds. Another important factor of this study was a comparison with previously reported experimentally validated inhibitors (phytochemical). Four compounds with strong binding affinities were determined through this study. It was found that Papraline from *Santolina insularis* plant and Osajin from *Varigatae* plant were the most reactive phytochemicals among all of the forty-nine phytochemicals against DPP4. The other two phytochemicals were taken from *Silybum marianum*, which gave second highest affinities. These four phytochemicals i.e. Papraline, Osajin, Diprenyleriodictyol and SchizolaenoneB having equal and





greater binding affinities than that of the experimentally validated inhibitors. After passing through some in-vivo and in-vitro developmental phases and further evaluations to determine their efficacy and safety as proper and suitable drug against DPP4, having no health-hazardous issues.

### Informed consent

All authors are aware of and approve the manuscript.

### References

- Taylor SI. Deconstructing type 2 diabetes. *Cell*. 1999; 97: 9-12.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/10199397>
- Miranda PJ, De Fronzo, Ralph C, Robert M, Guyton JR. Metabolic syndrome: Definition, pathophysiology, and mechanisms. *Am Heart J*. 2004; 149: 33-45.
- Diabetes Atlas. 2003. <http://www.eatlas.idf.org/prevalence>
- Hussain A, Claussen B, Ramachandran A, Williams R. Prevention of type 2 diabetes: a review. *Diabetes Res Clinic Pract*. 2007; 76: 317-326.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/17069920>
- Panunti B, Ali J, Fonseca VA. Mechanisms and therapeutic targets in type 2 diabetes mellitus. *Drug Discovery Today: Disease Mechanisms*. 2004; 1: 151-157.
- Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. *Lancet*. 2005; 365: 1333-1346.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/15823385>
- Mentlein R, Gallwitz B, Schmidt WE. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36) amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur J Biochem*. 1993; 214: 829-835.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/8100523>
- Deacon CF, Johnsen AH, Holst JJ. Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. *J Clin Endocrinol Metab*. 1995; 80: 952-957.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/7883856>
- Deacon CF. Therapeutic Strategies Based on Glucagon-Like Peptide 1. *Diabetes*. 2004; 53: 2181-2189.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/15331525>
- Zerilli T, Pyon EY. Sitagliptin phosphate: A DPP4 inhibitor for the treatment of type 2 diabetes mellitus. *Clin Ther*. 2007; 29: 2614-2634.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/18201579>
- Deacon CF, Carr RD, Holst JJ. DPP4 inhibitor therapy: new directions in the treatment of type 2 diabetes. *Front Biosci*. 2008; 13: 1780-1794.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/17981667>
- Ahren B. DPP-4 inhibitors. *Best Pract Res Clin Endocrinol Metab*. 2007; 21: 517-533.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/18054733>
- Kameoka J, Tanaka T, Nojima Y, Schlossman SF, Morimoto C. Direct association of adenosine deaminase with a T cell activation antigen CD26. *Science*. 1993; 261: 466-469.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/8101391>
- Chen WT, Kelly T, Ghersi G. DPP-IV, seprase, and related serine peptidases in multiple cellular functions. *Curr Top Dev Biol*. 2003; 54: 207-232.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/12696751>
- Rasool N, Hussain W. Three Major Phosphoacceptor Sites in HIV-1 Capsid Protein Enhances its Structural Stability and Resistance against Inhibitor: Explication through Molecular Dynamics Simulation, Molecular Docking and DFT Analysis. *Comb Chem High Throughput Screen*. 2019.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/31838993>
- Rasool N, Hussain W, Mahmood S. Prediction of Protein Solubility using Primary Structure Compositional Features: A Machine Learning Perspective. *J Proteo Bioinform*. 2017; 10: 324-328.
- Wadood A, Ahmed N, Shah L, Ahmad A, Hassan H, et al. In-silico drug design: An approach which revolutionarised the drug discovery process. *Drug Des Devel Ther*. 2013; 1: 3.
- Rasool N, Hussain W, Khan YD. Revelation of enzyme activity of mutant pyrazinamidases from *Mycobacterium tuberculosis* upon binding with various metals using quantum mechanical approach. *Computational Biology and Chemistry*. 2019; 83:107108
- Rasool N, Jalal A, Amjad A, Hussain W. Probing the Pharmacological Parameters, Molecular Docking and Quantum Computations of Plant Derived Compounds Exhibiting Strong Inhibitory Potential Against NS5 from Zika Virus. *Brazilian Arc Biol Technol*. 2018; 61.
- Amjad H, Hussain W, Rasool N. Molecular Simulation Investigation of Prolyl Oligopeptidase from *Pyrobaculum Calidifontis* and In Silico Docking With Substrates and Inhibitors. *Open Access J Bio Engineering Biosciences*. 2018; 2: 185-194.
- Arif N, Subhani A, Hussain W, Rasool N. In Silico Inhibition of BACE-1 by Selective Phytochemicals as Novel Potential Inhibitors: Molecular Docking and DFT Studies. *Curr Drug Discov Technol*. 2019.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/30767744>
- Lee SK, Park SH, Lee IH, No KT. PreAD-MET Ver. v2.0. BMDRC: Seoul. 2007.
- Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*. 1997; 23: 3-25.
- Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J Comput Chem*. 2010; 31: 455-461
- Gill PM, Johnson BG, Pople JA, Frisch MJ. The performance of the Becke-Lee-Yang-Parr (B-LYP) density functional theory with various basis sets. *Chem Phys Lett*. 1992; 197: 499-505.
- Hussain W, Amir A, Rasool N. Computer-aided study of selective flavonoids against chikungunya virus replication using molecular docking and DFT-based approach. *Structural Chemistry*. 2020; 1-12
- Akhtar A, Hussain W, Rasool N. Probing the Pharmacological Binding Properties, and Reactivity of Selective Phytochemicals as Potential HIV-1 protease Inhibitors. *Univ Sci*. 2019; 24: 441-464.
- Hussain W, Ali M, Sohail Afzal M, Rasool N. Penta-1,4-Diene-3-One Oxime Derivatives Strongly Inhibit the Replicase Domain of Tobacco Mosaic Virus: Elucidation Through Molecular Docking and Density Functional Theory Mechanistic Computations. *J Antivir Antiretrovir*. 2018; 10.
- Akhtar A, Amir A, Hussain W, Ghaffar A, Rasool N. In Silico Computations of Selective Phytochemicals as Potential Inhibitors Against Major Biological Targets Of Diabetes Mellitus. *Curr Comput Aided Drug Des*. 2019; 15: 401-408.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/30706825>
- Hussain W, Qaddir I, Mahmood S, Rasool N. In silico targeting of non-structural 4B protein from dengue virus 4 with spiroprazolopyridone: study of molecular dynamics simulation, ADMET and virtual screening. *Virusdisease*. 2018; 29: 1-10



31. Qaddir I, Rasool N, Hussain W, Mahmood S. Computer-aided analysis of phytochemicals as potential dengue virus inhibitors based on molecular docking, ADMET and DFT studies. *J Vector Borne Dis.* 2017; 54: 255-262.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/29097641>
32. Rasool N, Ashraf A, Waseem M, Hussain W, Mahmood S. Computational exploration of antiviral activity of phytochemicals against NS2B/NS3 proteases from dengue virus. *Turkish Journal of Biochemistry.* 2019; 44: 261-277.
33. Morris GM, Huey R, Olson AJ. Using AutoDock for ligand-receptor docking. *Curr Protoc Bioinformatics.* 2008; 24.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/19085980>
34. Neese F. The ORCA program system. *Wiley Interdisciplinary Reviews: Rev Comput Mol Sci.* 2012; 2: 73-78.
35. Nathan DM, Buse JB, Davidson MB, Heine RJ, Holman RR, et al. Management of hyperglycaemia in type 2 diabetes, a consensus algorithm for the initiation and adjustment of therapy. A consensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetologia.* 2006; 49: 1711-1721.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/16802130>
36. Greenwell M, Rahman PK. Medicinal plants, their use in anticancer treatment. *Int J Pharm Sci Res.* 2015; 6: 4103-4112.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/26594645>
37. Seal A, Aykkal R, Babu RO, Ghosh M. Docking study of HIV1 reverse transcriptase with phytochemicals. *Bioinformation.* 2011; 5: 430-439.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/21423889>
38. Senthilvel P, Lavanya P, Kumar KM, Swetha R, Anitha P, et al. Flavonoid from *Carica papaya* inhibits NS2B-NS3 protease and prevents Dengue 2 viral assembly. *Bioinformation.* 2013; 9: 889-895.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/24307765>
39. Yokota K, Igaki N. Sitagliptin (DPP4 inhibitor)-induced rheumatoid arthritis in type 2 diabetes mellitus, a case report. *Intern Med.* 2012; 51: 2041-2044.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/22864134>
40. Tran N. Blood-brain barrier. In: Kreutzer Jeffrey, DeLuca John, Caplan Bruce, (eds.). *Encyclopedia of Clinical Neuropsychology*, 1 edn, Springer. 2011; 426.
41. Kawabata Y, Wada K, Nakatani M, Yamada S, Onoue S. Formulation design for poorly water-soluble drugs based on biopharmaceutics classification system: Basic approaches and practical applications. *Int J Pharm.* 2011; 420: 1-10.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/21884771>
42. Badyal DK. Sitagliptin: a New Class of Oral Drug for Type 2 Diabetes. *JK Science.* 2008; 10: 97-98.
43. Huttner S, Graefe-Mody EU, Withopf B, Ring A, Dugi K. Safety, tolerability, pharmacokinetics and pharmacodynamics of single oral doses of BI 1356, an inhibitor of dipeptidyl peptidase 4, in healthy male volunteers. *J Clin Pharmacol.* 2008; 48: 1171-1178.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/18812608>
44. Del Prato S, Barnett AH, Huisman H, Neubacher D, Woerle HJ, et al. Effect of linagliptin monotherapy on glycaemic control and markers of beta-cell function in patients with inadequately controlled type 2 diabetes: a randomized controlled trial. *Diabetes Obes Metab.* 2011; 13: 258-267.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/21205122>
45. Seshadri KG, Kirubha MHB. Gliptins: A new class of Oral Antidiabetic Agents. *Ind J Pharm Sci.* 2009; 71: 608-614.
46. Villhauer EB, Brinkman JA, Naderi GB, Burkey BF, Dunning BE, et al. 1-[[[3-Hydroxy-1adamantyl]amino]acetyl]-2-cyano-(S)pyrrolidine, A Potent, Selective, and Orally Bioavailable Dipeptidyl Peptidase IV Inhibitor with Antihyperglycemic Properties. *J Med Chem.* 2003; 46: 2774-2789.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/12801240>
47. Gupta R, Walunj SS, Tokala RK, Parsa KV, Singh SK, et al. Emerging Drug Candidates of Dipeptidyl Peptidase IV (DPP IV) Inhibitor Class for the Treatment of Type 2 Diabetes. *Current Drug Targets.* 2009; 10: 71-87.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/19149538>
48. Augeri DJ, Robl JA, Betebenner DA, Magnin DR, Khanna A, et al. Discovery and Preclinical Profile of Saxagliptin (BMS477118) A Highly Potent, Long-Acting, Orally Active Dipeptidyl Peptidase IV Inhibitor for the Treatment of Type 2 Diabetes. *J Med Chem.* 2005; 48: 5025-5037.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/16033281>
49. Ahre'n B. Vildagliptin, an inhibitor of dipeptidyl peptidase-4 with antidiabetic properties. *Expert Opin Investig Drugs.* 2006; 15: 431-442.  
**PubMed:** <https://www.ncbi.nlm.nih.gov/pubmed/16548792>
50. International Diabetes Federation. *Diabetes Atlas*, 3rd edn, Brussel: IDF. 2006; 322.
51. Webb B, Sali A. Comparative protein structure modeling using modeller. *Curr Protoc Bioinformatics.* 2014.