Study on Exploration and Evaluation of Novel phyto components Targeting DPP-4 Enzyme in the treatment of type-1 diabetes by Molecular Docking Analysis

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Abstract: It is currently estimated that number of people with diabetes in India will reach 80 million by the year 2025. The incidence of T1DM especially in the young and active population is having negative impact in the economic up-growth of India and is hence a serious problem requiring immediate solution.T1DM is chronic pathological condition in which the pancrease is unable to secrete sufficient insulin. It is characterized by the failure of tissues to respond to a normal concentration of glucose available in the blood, resulting in reduced glucose intake into the peripheral tissue. It was evident through recent research that selective inhibition of the enzyme Dipeptidyl peptidase 4 (DPP-4) in insulin-resistant skeletal muscle causes improvements in insulin-stimulated glucose transport activity. Based on the literature survey about 23 biologically active phytocomponents were selected for DPP-4 enzyme inhibition analysis by molecular docking study. The activities of each component were compared with known DPP-4 inhibitor known as sitagliptin. Results of the present investigation clearly indicates that the compound Diosgenin ranked 1st with 8 potential interactions. Compounds Asarinine, Genistein and Sitagliptine ranked 2nd with 7 potential interactions. Compounds Cinamaldehyde and Stigmasterol ranked 3rd with 6 potential interactions. Compounds Gingerol and Aloeresin ranked 4th with 5 potential interactions. Compounds beat sitosterol , curcumin and Campesterol ranked 5th with 4 potential interactions. From the result analysis it was concluded that DPP-4 inhibitors are the new class of therapeutic agent which effective in treating T1DM. The greater advantage of using these herbal lead with DPP-4 inhibition potential lies in its multiple mode of action on increasing insulin release and also has wide margin of safety.

Key Words: Type 1 Diabetes Mellitus, Dipeptidyl peptidase 4, DPP-4 Inhibitor, Insulin-resistant, Diosgenin.

1. INTRODUCTION

The prevalence of Type 1 Diabetes Mellitus (T1DM) is steadily increasing around the world, especially in South Asia.Type 1 diabetes mellitus (T1DM) is also on increase like type 2 diabetes, even though not in the same proportion, but still with a trend of 3–5% increase/year. India has three new cases of T1DM/100,000 children of 0–14 years.

According to the statistics of 2014 nearly 29.1 million people in the US had diabetes. More than 1 in 4 of them is really not aware that they had the disease. Diabetes affects 1 in 4 people over the age of 65

Developing countries like India accounts more of the children with T1DM in South-East Asia. India has 3 new cases of T1DM/100,000 children of 0–14 years [1]. The prevalence of diabetes in India is variable, and three sets of data show 17.93 cases/100,000 children in

Karnataka, 3.2 cases/100,000 children in Chennai, and 10.2 cases/100,000 children in Karnal (Haryana) [2,[3],[4] .The bottom line remains that T1DM is quite prevalent and common.

Molecular docking and computational analysis attains considerably greater importance mainly because of the reliability and promising simulation in the results and also paves a new way for the research focus towards the alternative animal models. In-silico docking analysis continues to hold great promise in the field of computer based drug design that screens 3D structure of the phyto components by orienting and scoring them in the binding site of a specific protein. As a result novel ligands from herbs for receptors of known structure were designed and their interaction energies were calculated using the scoring functions. Docking score were utlised to estimate the ligand-binding energies. Apart from these, other input parameters for docking are also considered for evaluating the compounds inhibition efficacy. It is estimated that docking programs currently dock 70 – 80% of ligands correctly [5].

There are some metabolic hormones known as incretins which includes intestinal peptides glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (also known as: glucose-dependent insulinotropic polypeptide or GIP) both GLP-1 and GIP generally regulates and decreases the blood glucose level. The most important fascinating role played by these GLP-1 and GIP is to stimulate the release of insulin from pancreatic beta cells of the islets of Langerhans in pancreas by a blood glucose-dependent mechanism. According to the literature incretins further inhibit glucagon release from the alpha cells of the islets of Langerhans.

Dipeptidyl peptidase-4 (DPP-4) is an enzyme mainly involved in rapid degradation of both GLP-1 and GIP. Hence it was a proven fact the inhibition of this enzyme DPP-4 prolongs the action of both GLP-1 and GIP hence it directly increases the level of insulin releases and thereby decreases the blood glucose level by insulin mediated cell glucose transport mechanism.

Drugs currently used for the treatment of T1DM include metformin, sulphonylureas, thiazolidinediones, meglinitides etc., whose long term usage is known to cause side effects like heart failure, myocardial infarction, anxiety,nervousness,seizures, palpitation, and depression. Hence there is a need for alternative therapies that can overcome the limitations of conventional anti-hyperglycemic medications.

The major advantage of using DPP-4 inhibitors in treating T1DM lies in its multiple mechanism of action that greatly increases the insulin release from pancreas and effectively decrease the glucagon level which potentially benefit the patients with T1DM.Present research

work mainly focuses on the evaluation of new DPP-4 inhibitors from herbal origin to treat insulin resistance in T2DM with adequate computational analytical studies. Outcome of this research would substantially decrease the total health care expenditure of the patients suffering with T1DM.

Drug that involved DPP-4 enzyme inhibition acts by binding with biologically important amino acid either by forming H-bond interactions or by hydrophobic interactions. According to the literature it was found that Try226, Glu205, and Glu206 were involved in H-bond formation, while 10 other amino acids (Try547, Try667, Asn710, Val711, His740, Ser630, Ser209, Arg358, Phe357, and Val207) were involved in hydrophobic interactions.

As per the literature survey about 14 potential lead molecules such Gingerol, beta sitosterol,Asarinine,Capsaicin,Curcumin,Piperine,Aloeresin,Campesterol,Chlorogenic acid,Cinamaldehyde,Diosgenin,Genistein,Morindone,Stigmasterol and standard drug Sitagliptine were selected from the docking analysis

Gingerol- It is known to exhibit a variety of biological activities including anticancer, anti-inflammation, and anti-oxidation [6]. Beta- Sitosterol a known anti-cancer agent. Capsaicin with analgesic and anti-inflammatory property. Curcumin cardiovascular disease, arthritis, uveitis, ulcerative proctitis, Crohn's disease, ulcerative colitis, irritable bowel disease, tropical pancreatitis, peptic ulcer, gastric ulcer [7],[8]. Piperine, a major alkaloid constituent of black pepper, has diverse physiological actions including killing of cancer cells. Piperine, a dietary phytochemical, inhibits angiogenesis [9]. Aloeresin an anti-inflammatory [10]. Chlorogenic acid is a potential anti diabetic and anti lipidemic agent [11]. Cinamaldehyde possess promising anti diabetic agent [12]. Biological activity of diosgenin that contributes to Anti-inflammatory, antioxidant and antiangiogenic activity [13]. Genistein is an isoflavone that is described as an angiogenesis inhibitor and a phytoestrogen. Morindone a known anthraquinone derived from morinda species especially investigated for its anti-microbial and anti-cancer activity [14]. Stigmasterol is a well explored anti-osteoarthritic agent [15]. Isolation and evaluation of anticancer efficacy of stigmasterol in a mouse model of DMBA-induced skin carcinoma [16].

2.MATERIALS AND METHODS

2.1.Software's required

Several docking tools were been used in recent times which works behind structure-based drug design strategies one among which is auto dock 4 a componential software tools used to analyze

the protein DPP-4 and to study the binding energy properties with the following lead component such as Gingerol, beta sitosterol,Asarinine,Capsaicin, Curcumin, Piperine,Aloeresin,Campesterol,Chlorogenic acid,Cinamaldehyde,Diosgenin,Genistein,Morindone andStigmasterol.Dipeptidyl peptidase-4 (DPP-4) enzyme with PDB code 2P8S sequence was obtained from protein data bank (www.pdb.org/pdb/). To get insight the intermolecular interactions, the molecular docking studies were done for the above mentioned phytoconstituents along with standard Sitagliptine (DPP-4 Inhibitor) at the active site 3D space of enzyme of interest DPP-4 using online DOCKING SERVER web tool module.

2.2.Ligand preparation

The ligands such as Gingerol, beta sitosterol,Asarinine,Capsaicin,Curcumin,Piperine,Aloeresin,Campesterol,Chlorogenic acid,Cinamaldehyde,Diosgenin,Genistein,Morindone,Stigmasterol and standard drug Sitagliptine were built using Chemsketch and optimized using Docking server online web tool as shown in Figure 1 and 2 for docking studies by using Geometry optimization method MMFF94 and charge calculation was carried out based on Gasteiger method at PH 7 as shown in Table 1.

2.3.Protein preparation

The target protein Dipeptidyl peptidase-4 (DPP-4) enzyme with PDB code 2P8S which is a Human dipeptidyl peptidase IV/CD26 in complex with a cyclohexalamine was retrieved from protein Data Bank (www.rcsb.org) and the drug molecule cyclohexalamine, crystallographic water molecules were removed from the protein. The chemistry of the protein was corrected for missing hydrogen followed by correcting the disorders of crystallographic structure by filling the valence atoms using alternate conformations and valence monitor options. As shown in Figure 3.

2.4.Active Site Prediction

Active site of enzyme was obtained by LIGSITE web server by using the automatic identification of pockets on protein surface given 3D coordinates of protein.The potential ligand binding sites in DPP-4 target protein is identified using grid space of 1 and probe of radius 5.0 angstrom [17]. Ligand site prediction was performed by using online tool GHECOM and the respective pockets calculations [18],[19]. As shown in Figure 4.

2.5.Docking Methodology

Docking calculations were carried out using Docking Server [20],[21]. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out based on the binding free energy on the following compounds like Gingerol, beta sitosterol,Asarinine,Capsaicin,Curcumin,Piperine,Aloeresin,Campesterol,Chlorogenic acid,Cinamaldehyde,Diosgenin,Genistein,Morindone,Stigmasterol, standard drug Sitagliptineand their binding affinity towards the target protein DPP-4 PDB code . Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of Auto Dock tools. Affinity (grid) maps of Å grid points and 0.375 Å spacing were generated using the Autogrid program. Auto Dock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method [22]. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied [23].

3.RESULTS

3.1.Dock scores

In recent time herbs become an integral part of the human health care system. It seems like clinical management of several dreadful disease is now focused towards rejuvenating potential of herbal drug belongs to Indian system of traditional medicines. Several researches finding further substantiates significant contribution of herbal leads and its application towards communicable and non-communicable disease. Potential interaction of 3D lead with the selected target was basically measured with factor called binding energy. As per the logarithmic principle higher the value on binding energy falls on negative is more the affinity of the lead towards the target. In the present investigation interaction of phyto components with DPP-4 enzyme was evaluated using docking server analytical tool. The different docking score includes binding free energy, inhibition constant, intermolecular energy and electrostatic energy values represented in Table 2.

The results showed that all the selected compounds showed binding energy ranging between -2.45 kcal/mol to -6.28 kcal/mol when compared with that of the standard sitagliptine (-5.35 kcal/mol). Electrostatic energy (-1.68 kcal/mol to -0.01 kcal/mol) of the ligands also coincide with the binding energy. All the selected phytocomponents contributed significant DPP-4 enzyme inhibitory activity because of its structural and functional parameters.

The results of docking analysis reveals that all fourteen phytocomponents at the active sites of DPP-4 revealed that the compounds bound to the active site of enzyme with lower docking (D energy) when compared with standard DPP-4 inhibitor.

Compound Diosgenin exhibited quite tight binding against DPP-4 enzyme with binding energy --6.28 Kcal/mol and ranks first in the compound series. The second best score was ranked by compound Beta sitosterol with binding energy -5.95 Kcal/mol followed by this compound Campesterol with binding energy -5.75 Kcal/mol when compared with standard Sitagliptine DPP-4 Inhibitor with binding energy -5.35 Kcal/mol.

Inhibition potential of the lead molecules was evaluated by using core factor called

Inhibitory constant contributes more to Inhibition constant and further it's directly proportional to binding energy. The results of the present investigation reveals that inhibition constant of the selected compounds ranges from (5.61 mM to 25.07 μM). Thus from the report it was clear that all the phytoconstituents having promising DPP-4 inhibition activity when compared to that of the standard with inhibition constant 118 μM. Intermolecular energy of all fourteen compounds ranging between -4.02 to -7.59 kcal/mol which was lesser when compared to the standard -7.25 Kcal/mol. Intermolecular energy is also directly proportional to binding energy. We found a decrease in intermolecular energy of all the selected compounds coincide with the binding energy.

3.2.Hydrogen bond interaction

Research predicts that hydrogen bond interaction of the lead projects biologically significant information on structural activity relationship and behavior / orientation of the lead with respect to target on its surface. The Hydrogen bonding interaction of the compounds (Fig 5 - 19) was analyzed for possible involvement of hydrogen bond formation with amino acid residues on receptor protein surface.

The result obtained from the hydrogen bond interaction study shows that the all fourteen phytoconstituents such as signifies promising DPP-4 inhibition activity by binding with the active site pocket on target protein. Further these compounds may have a direct action on target enzyme by binding to the potentially active amino acid residue in the same way as that of the standard DPP-4 Inhibitor as listed in the Table 3. According to the present molecular docking analysis it was found that the following phyto components are ranked top among others as it possess binding on the significantly active amino acids. In which the compound Diosgenin ranked 1st with 8 potential interactions. Compounds Asarinine, Genistein and Sitagliptine (Known reference compound) ranked 2nd with 7 potential interactions. Compounds Cinamaldehyde and Stigmasterol ranked 3rd with 6 potential interactions. Compounds Gingerol and Aloeresin ranked 4th with 5 potential interactions. Compounds beat sitosterol , curcumin and Campesterol ranked 5th with 4 potential interactions.

4.DISCUSSION

Docking is a modern scientific approach which involves the prediction of valuable lead towards specific drug target. Enzyme being a primary target in the pathology of most dreadful disease either under expression or over expression of this precursor involves change in normal physiology. Docking fundamentally works behind the logic of target (enzyme/protein) lead (drug) interaction. Most of the drug acts either by antagonistic or agonistic action. Both of these mechanisms of drugs rely on binding of functional group present in the drug with the biologically active amino acid present in the target protein. Hence drug likeness is the most important property to predict the mode of bind of drug with that of the receptor.

Identification of active site on to the surface of the target seems to be significant step as this predicts the actual docking score of the molecule. In recent time various online tools available to predict the drug likeness, ADMET pathway, BBB crossing including structural activity relationship of the potential of the lead with high accuracy The reason for which the docking is considerable important as it aids in identification of promising lead by involving logical application, active site prediction, mode of drug action and above all it narrow down the research by decreasing the time spent on need less molecules [24].

5.CONCLUSION

The data's obtained from the present study clearly reflects that novel leads with herbal origin may be considered as a promising drug candidate for the development of new category of anti-diabetic agents. In conclusion, the results obtained from the current investigation substantiates that molecular docking analysis of Sitagliptine and selected leads has promising DPP-4 inhibition potential

These results clearly indicates that the leads especially Diosgenin, Asarinine, Genistein, Cinamaldehyde, Stigmasterol, Gingerol ,Aloeresin, beat sitosterol , curcumin and Campesterol shows similar binding sites and interactions with DPP-4 enzyme compared to the standard drug Sitagliptine. Further investigations on the above compounds on preclinical and clinical studies are necessary to develop potential drug candidate for the treatment of metabolic disorders like diabetes mellitus.

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6.References

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Table 1: Ligand Properties of the selected Lead

Compounds	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds	Log P
Gingerol	294.39	C17H26O4	2	4	10	2.5
Beta sitosterol	414.71	C29H50O	1	1	6	9.3
Asarinine	354.35	C20H18O6	0	6	2	2.7
Capsaicin	305.41	C18H27NO3	2	3	9	3.6
Curcumin	368.38	C21H20O6	2	6	8	3.2
Piperine	285.33	C17H19NO3	0	1	3	2.97
Aloeresin	540.52	C28H28O11	5	11	8	1
Campesterol	400.69	C28H48O	1	1	5	8.8
Chlorogenic acid	354.31	C16H18O9	6	9	5	-0.4
Cinamaldehyde	132.16	C9H8O	0	1	2	1.9
Diosgenin	414.63	C27H42O3	1	3	0	5.7
Genistein	270.24	C15H10O5	3	5	1	2.7
Morindone	270.24	C15H10O5	3	5	1	2.7
Sitagliptine	407.32	C16H15F6N5O	1	10	4	0.7
Stigmasterol	412.7	C29H48O	1	1	5	8.6

Table 2: Summary of the molecular docking studies of compounds against DPP-4 Enzyme

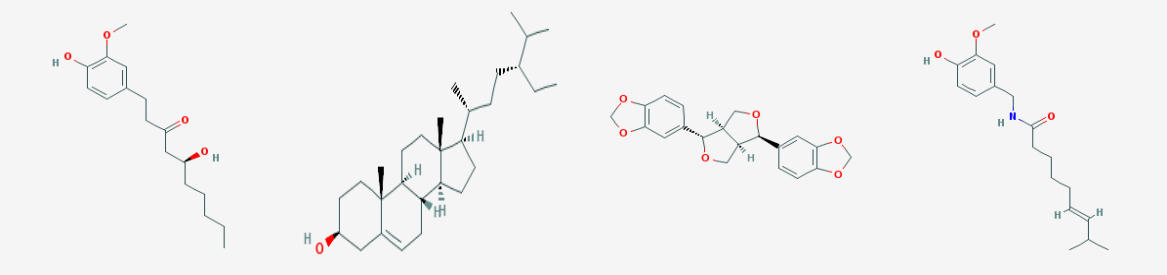
Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki μM (*mM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Gingerol	-2.45	15.97 *	-0.1	-5.38	621.67
Beta sitosterol	-5.95	43.61	-0.07	-7.59	878.14
Asarinine	-5.45	100.99	-0.21	-5.84	711.3
Capsaicin	-3.37	3.38*	-0.06	-5.28	675.29
Curcumin	-3.07	5.61*	0.07	-4.77	908.47

Piperine	-5.34	121.8	0.04	-5.89	681.54
Aloeresin	-4.89	277.82	-0.01	-5.79	780.9
Campesterol	-5.75	60.57	-0.03	-7.33	896.89
Chlorogenic acid	-3.38	1.56*	-0.12	-4.76	671.64
Cinamaldehyde	-4.09	999.81	-0.18	-4.68	436.43
Diosgenin	-6.28	25.07	-0.13	-6.58	836.89
Genistein	-4	1.17*	-0.1	-4.59	629.54
Morindone	-4.72	348.96	-0.12	-4.02	499.06
Sitagliptine	-5.35	118.9	-1.68	-7.25	585.73
Stigmasterol	-5.34	121.84	0.01	-6.92	892.85

Table 3

Interaction of lead compounds with active site amino acid residue of DPP-4 Enzyme

Compounds	**Target binding Amino acid residue**							
Gingerol	205 Glu	206 Glu	209 Ser	357 Phe	358 Arg	-	-	-
Beat sitosterol	205 Glu	206 Glu	209 Ser	357 Phe	-	-	-	-
Asarinine	205 Glu	206 Glu	209 Ser	357 Phe	358 Arg	547 Try	630 Ser	-
Capsaicin	358 Arg	630 Ser	740 His	-	-	-	-	-
Curcumin	209 Ser	357 Phe	358 Arg	547 Try	-	-	-	-
Piperine	209 Ser	357 Phe	358 Arg	-	-	-	-	-
Aloeresin	226 Tyr	206 Glu	209 Ser	357 Phe	358 Arg	-	-	-
Campesterol	206 Glu	209 Ser	357 Phe	358 Arg	-	-	-	-
Chlorogenic Acid	209 Ser	357 Phe	358 Arg	-	-	-	-	-
Cinamaldehyde	205 Glu	206 Glu	630 Ser	710 Asn	711 Val	740 His	-	-
Diosgenin	205 Glu	206 Glu	209 Ser	357 Phe	358 Arg	547 Try	630 Ser	710 Asn
Genistein	205 Glu	206 Glu	209 Ser	357 Phe	547 Try	630 Ser	711 Val	
Morindone	209 Ser	357 Phe	358 Arg	-	-	-	-	-
Stigmasterol	205 Glu	209 Ser	357 Phe	358 Arg	547 Try	630 Ser	-	-
Sitagliptine	205 Glu	206 Glu	207 Val	209 Ser	357 Phe	358 Arg	667 Try	-



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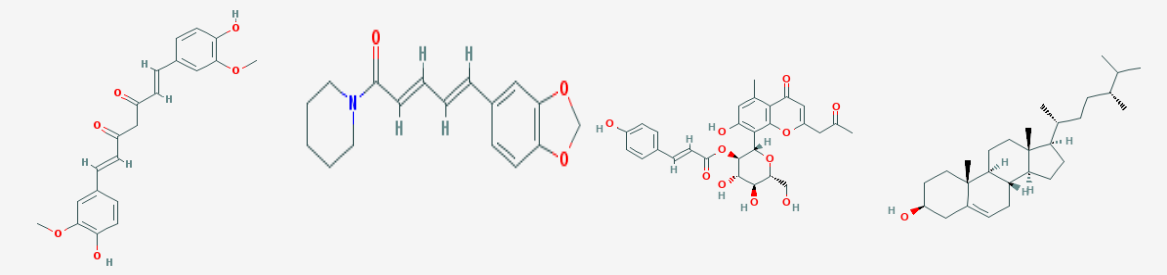

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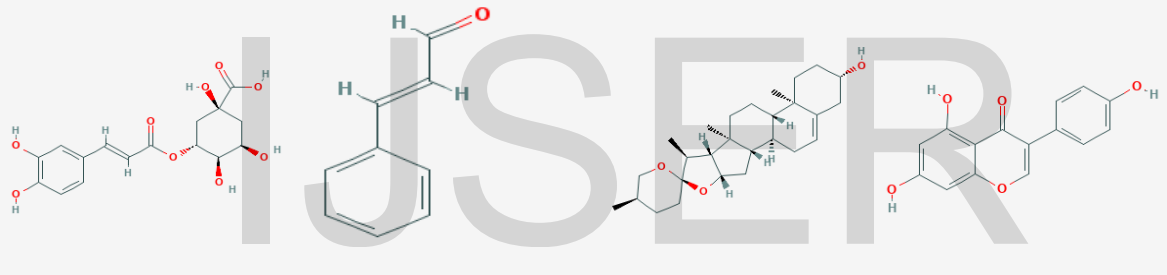
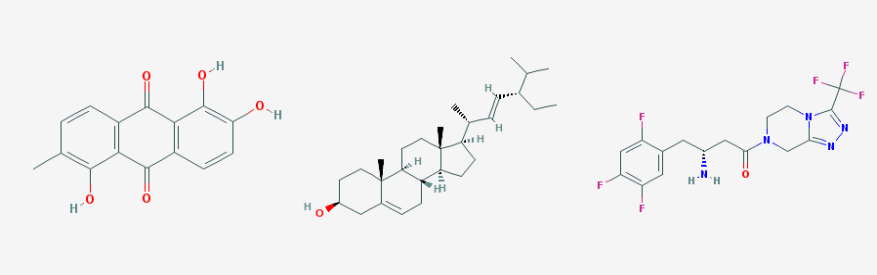


9 **10** **11** **12**



13 **14** **15**

Fig 1: 2D Structure of lead 1.Gingerol 2.Beta sitosterol 3.Asarinine 4.Capsaicin 5.Curcumin 6.Piperine 7.Aloeresin, 8.Campesterol 9.Chlorogenic acid 10.Cinamaldehyde 11.Diosgenin 12.Genistein 13. Morindone 14.Stigmasterol 15. Sitagliptine (Standard DPP-4 Inhibitor)

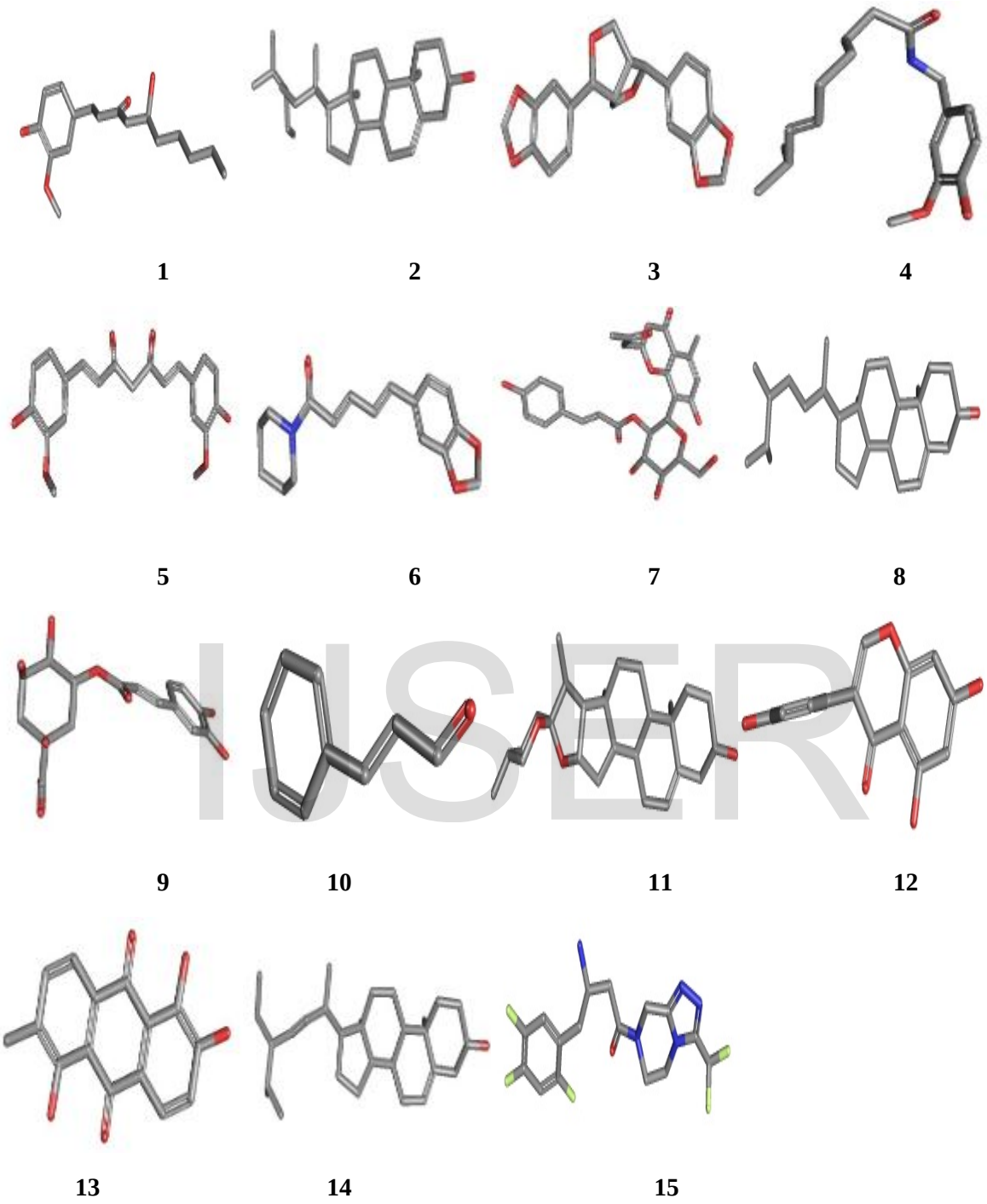


Fig 2: 3D Structure of lead 1.Gingerol 2.Beta sitosterol 3.Asarinine 4.Capsaicin 5.Curcumin 6.Piperine 7.Aloeresin, 8.Campesterol 9.Chlorogenic acid 10.Cinamaldehyde 11.Diosgenin 12.Genistein 13. Morindone 14.Stigmasterol 15. Sitagliptine (Standard DPP-4 Inhibitor)

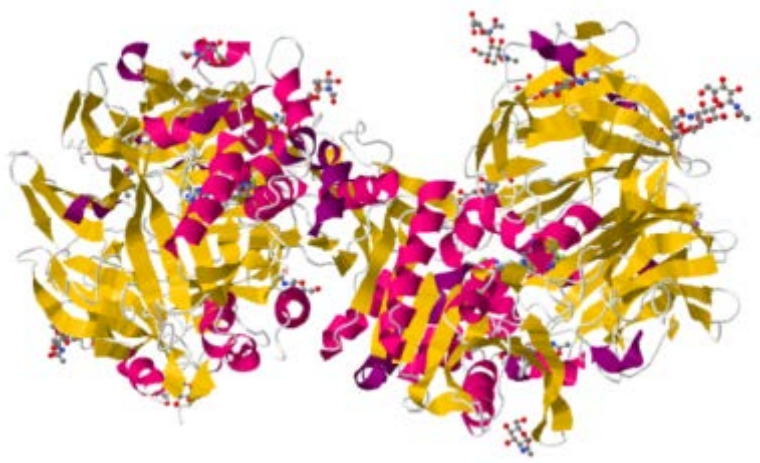


Fig 3:Target protein Dipeptidyl peptidase-4 (DPP-4) enzyme PDB code 2P8S.

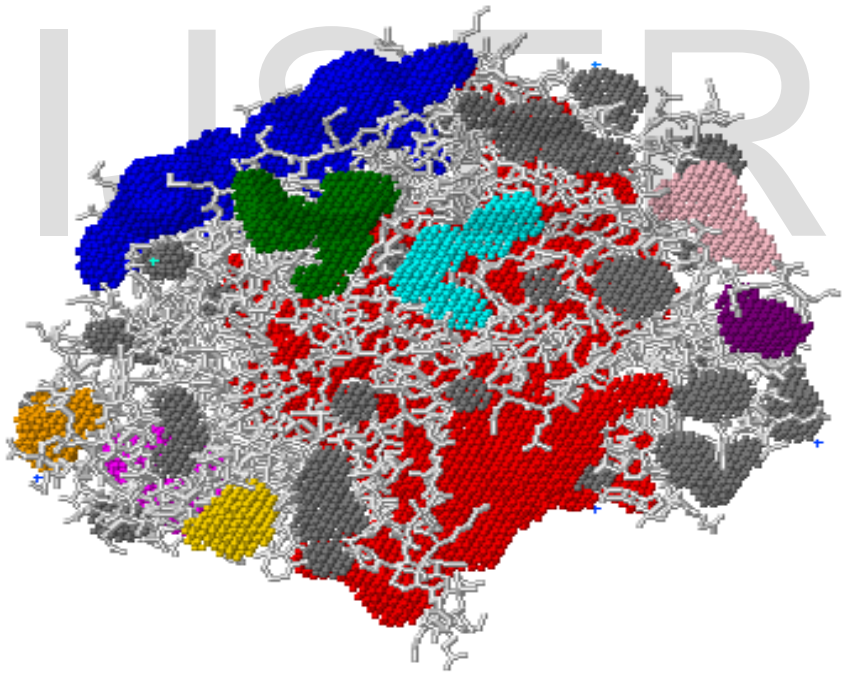


Fig 4 : Possible ligand binding pockets on the surface of target enzyme Dipeptidyl peptidase-4 (DPP-4) enzyme with PDB code 2P8S. Pockets calculated by GHECOM.

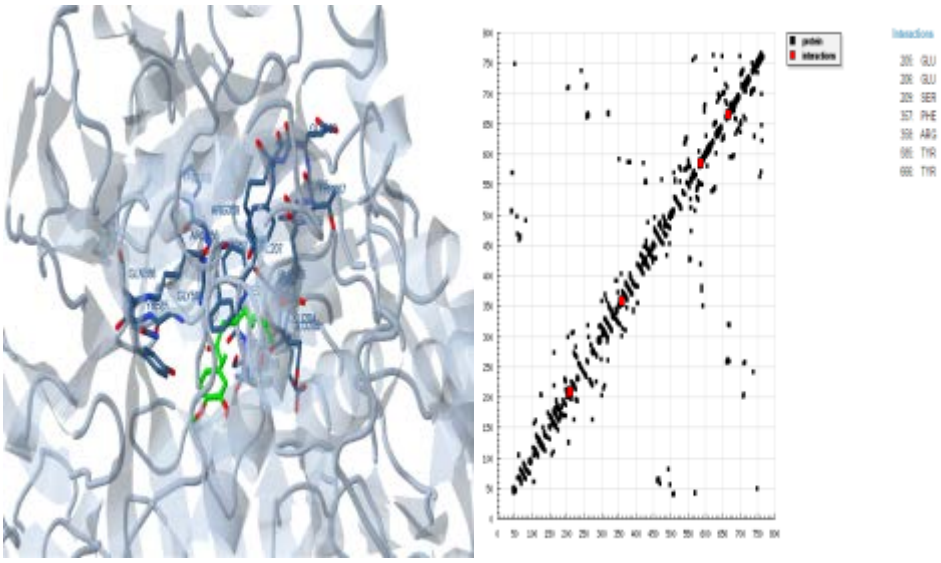


Fig 5: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme DPP-4 (2P8S) and the ligand Gingerol involved in hydrogen bond formation with amino acid residues on the protein like 205 Glu ,206 Glu,209 Ser,357 Phe and 358 Arg. Total interaction surface of about 621.67.

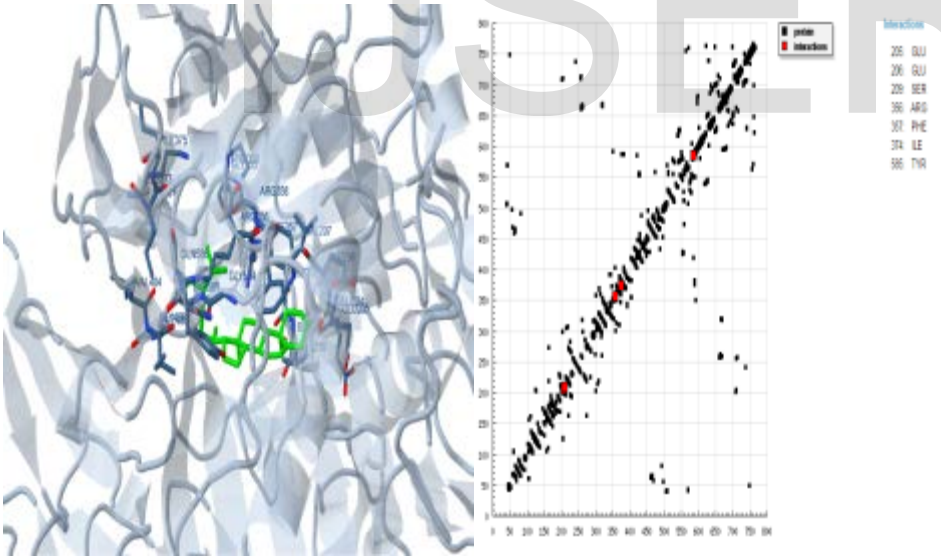


Fig 6: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme DPP-4 (2P8S) and the ligand Beta sitosterol involved in hydrogen bond formation with amino acid residues on the protein like 205 Glu, 206 Glu , 209 Ser and 357 Phe. Total interaction surface of about 878.14.

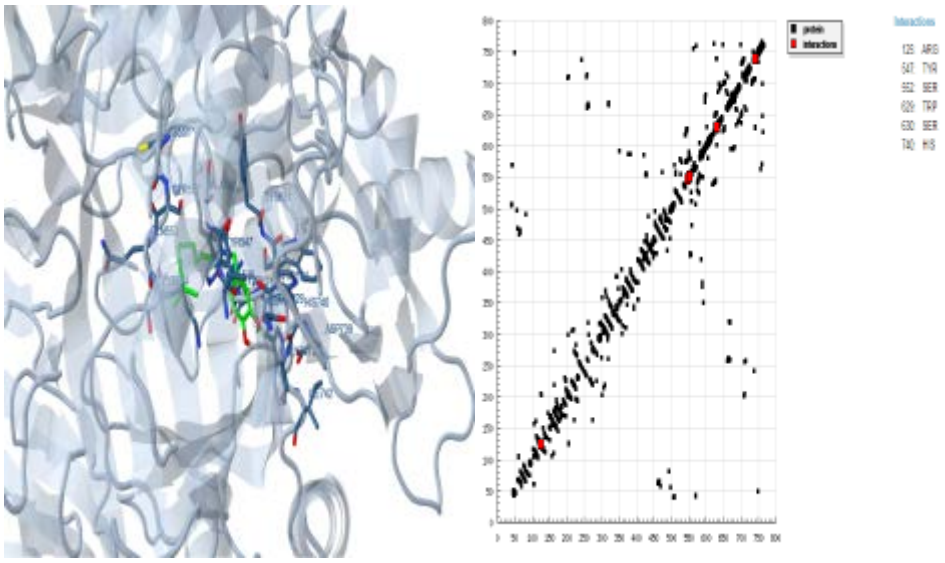


Fig 7: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme DPP-4 (2P8S) and the ligand Asarinine involved in hydrogen bond formation with amino acid residues on the protein like 205 Glu ,206 Glu, 209 Ser,357 Phe,358 Arg,547 Try and 630 Ser . Total interaction surface of about 711.3.

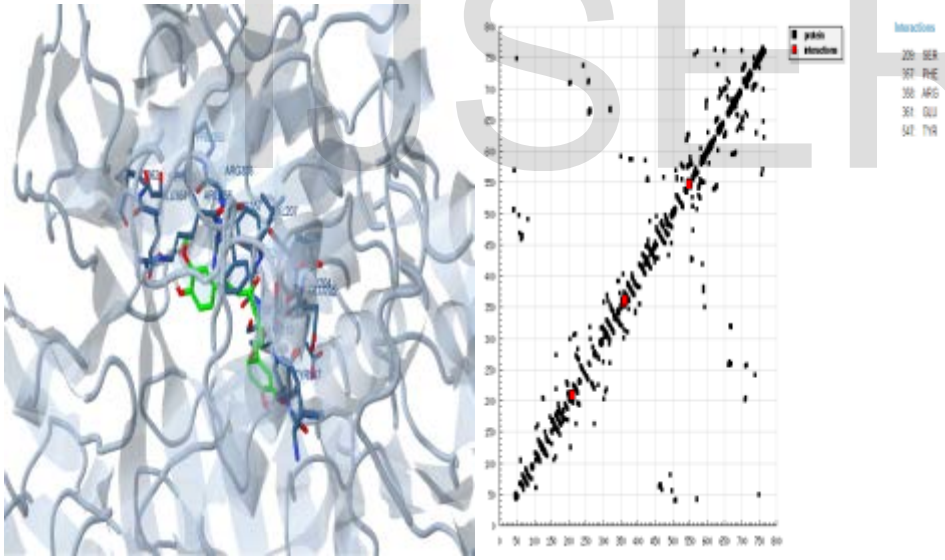


Fig 8: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme DPP-4 (2P8S) and the ligand Capsaicin involved in hydrogen bond formation with amino acid residues on the protein like 358 Arg , 630 Ser and740 His. Total interaction surface of about 675.29.

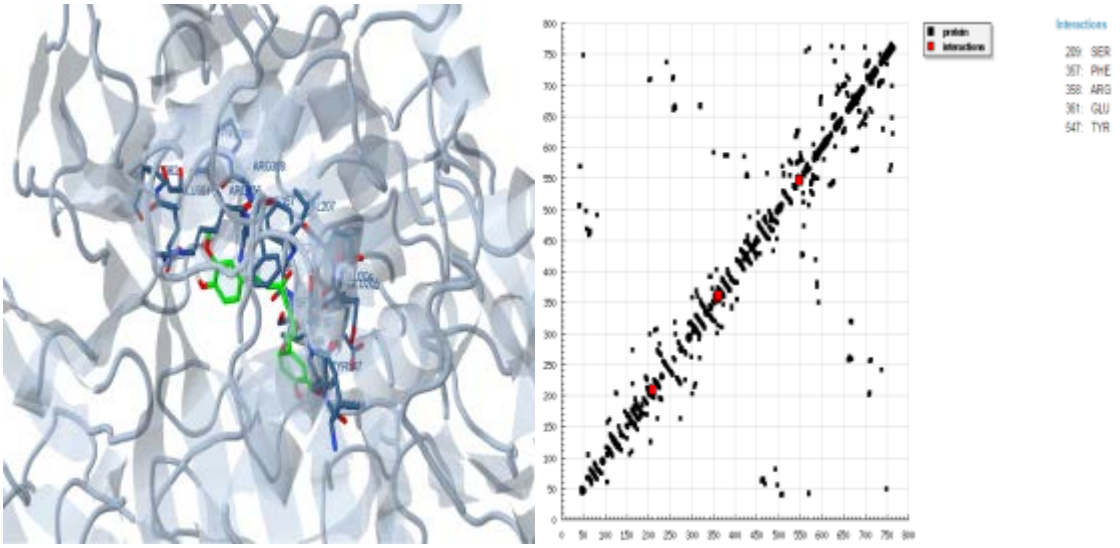


Fig 9: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme DPP-4 (2P8S) and the ligand Curcumin involved in hydrogen bond formation with amino acid residues on the protein like 209 Ser ,357 Phe, 358 Arg and 547Try. Total interaction surface of about 908.47.

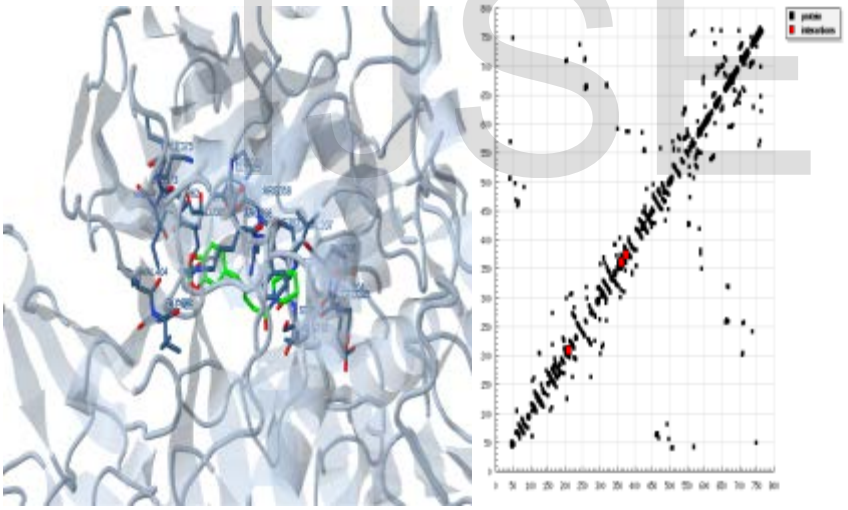


Fig 10: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme DPP-4 (2P8S) and the ligand Piperine involved in hydrogen bond formation with amino acid residues on the protein like 209 Ser , 357 Phe and 358 Arg. Total interaction surfaces of about 681.54.

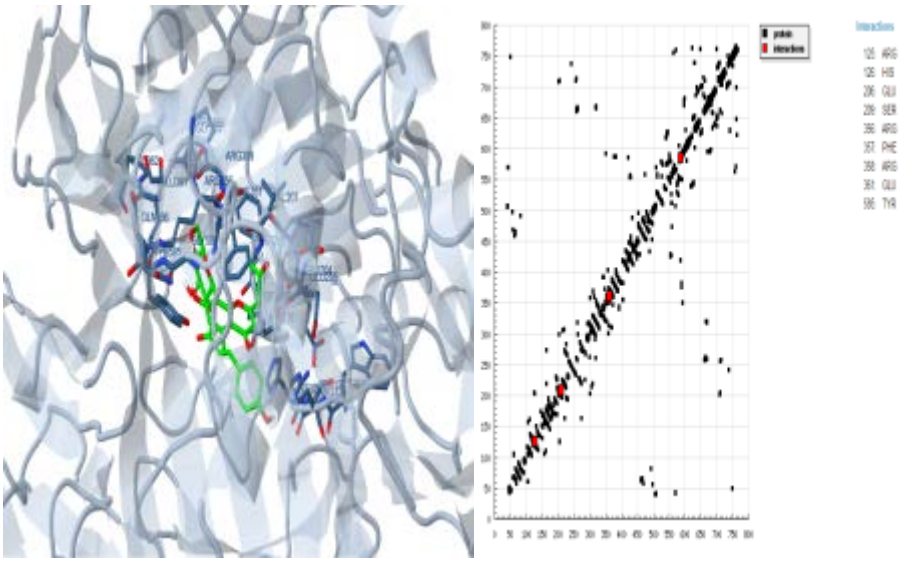


Fig 11: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme DPP-4 (2P8S) and the ligand Aloeresin involved in hydrogen bond formation with amino acid residues on the protein like 226 Tyr 2,06 Glu ,209 Ser,357 Phe and 358 Arg. Total interaction surface of about 780.9.

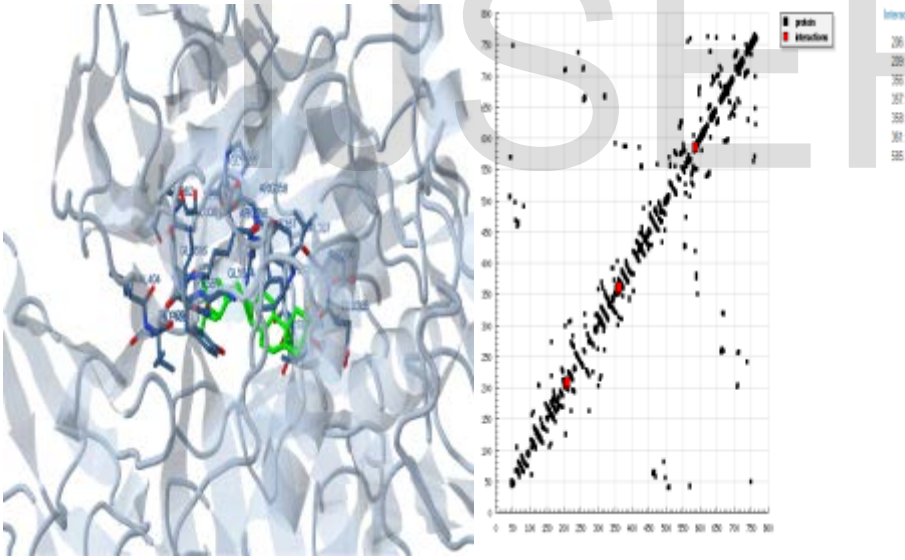


Fig 12: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme DPP-4 (2P8S) and the ligand Campesterol involved in hydrogen bond formation with amino acid residues on the protein like 206 Glu ,209 Ser, 357 Phe and 358Arg. Total interaction surface of about 896.89.

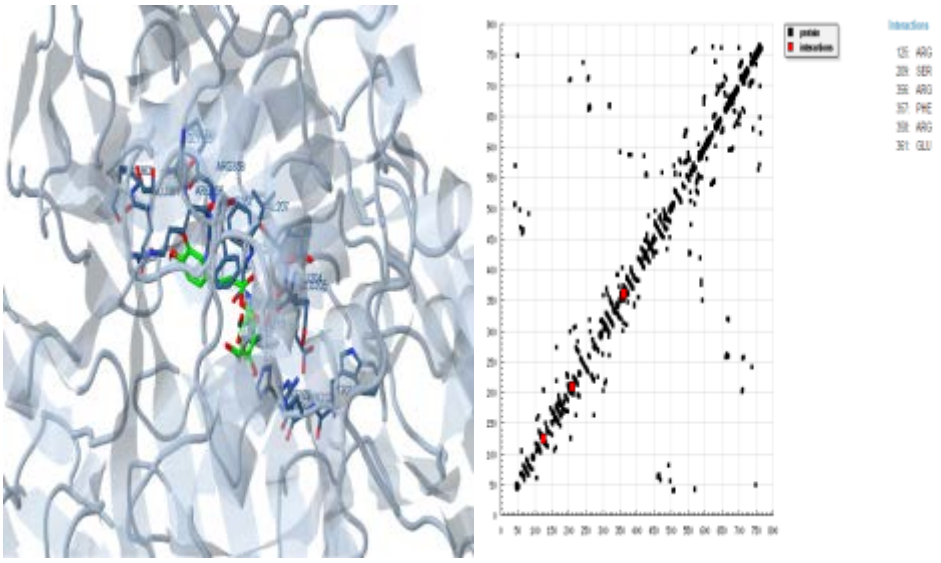


Fig 13: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme DPP-4 (2P8S) and the ligand Chlorogenic acid involved in hydrogen bond formation with amino acid residues on the protein like 209 Ser, 357 Phe and 358 Arg. Total interaction surface of about 671.64.

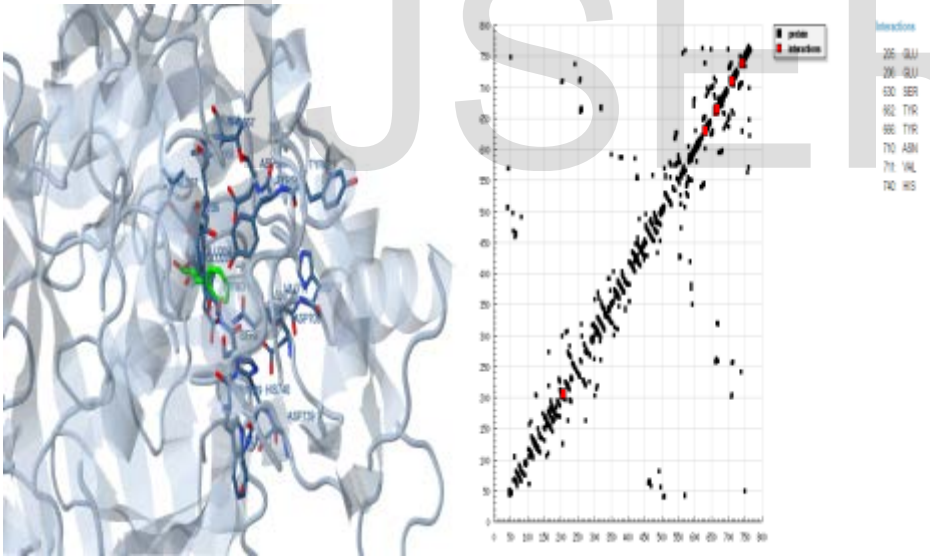


Fig 14: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme DPP-4 (2P8S) and the ligand Cinamaldehyde involved in hydrogen bond formation with amino acid residues on the protein like 205 Glu,206 Glu 630,Ser 710 Asn,711Val and 740 His

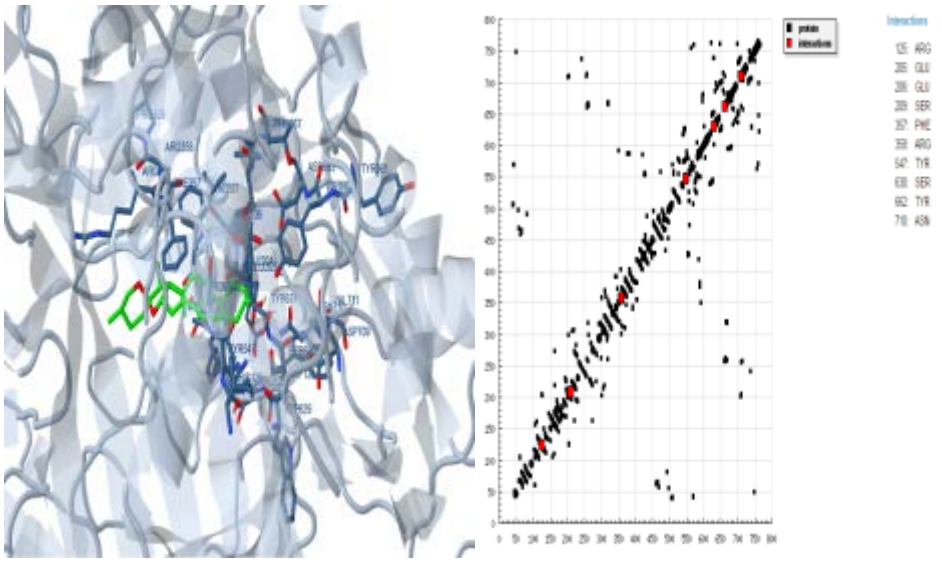


Fig 15: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme DPP-4 (2P8S) and the ligand Diosgenin involved in hydrogen bond formation with amino acid residues on the protein like 205 Glu, 206 Glu,209 Ser,357 Phe,358 Arg,547 Try,630 Ser and 710 Asn .Total interaction surface of about 836.89.

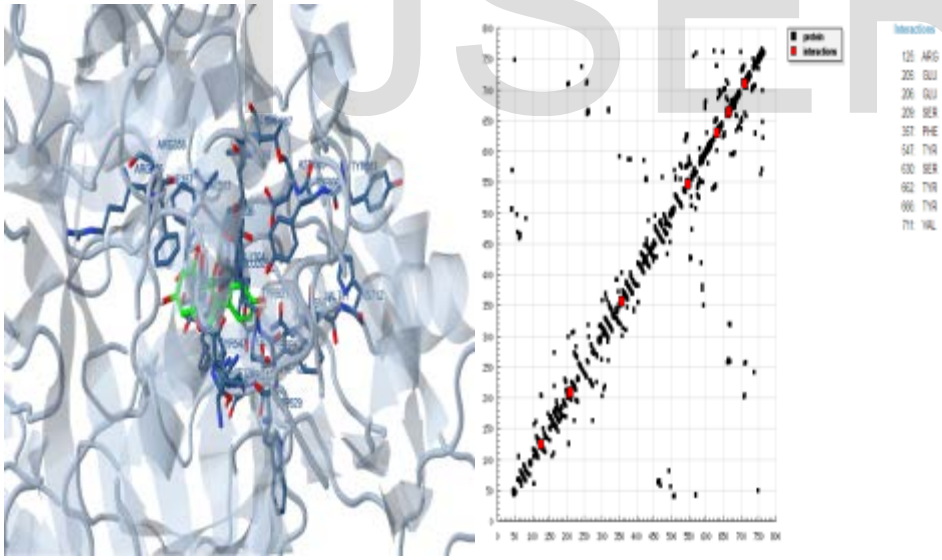


Fig 16: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme DPP-4 (2P8S) and the ligand Genistein involved in hydrogen bond formation with amino acid residues on the protein like 205 Glu , 206 Glu,209 Ser,357 Phe , 547 Try,630 Ser and 711 Val. Total interaction surface of about 629.54.

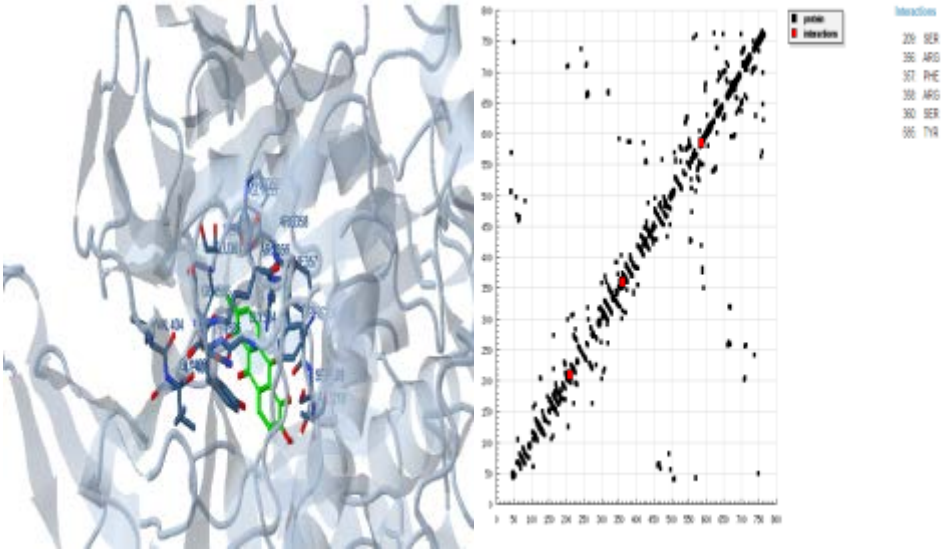


Fig 17: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme DPP-4 (2P8S) and the ligand Morindone involved in hydrogen bond formation with amino acid residues on the protein like 209 Ser ,357 Phe and 358Arg. Total interaction surface of about 499.06.

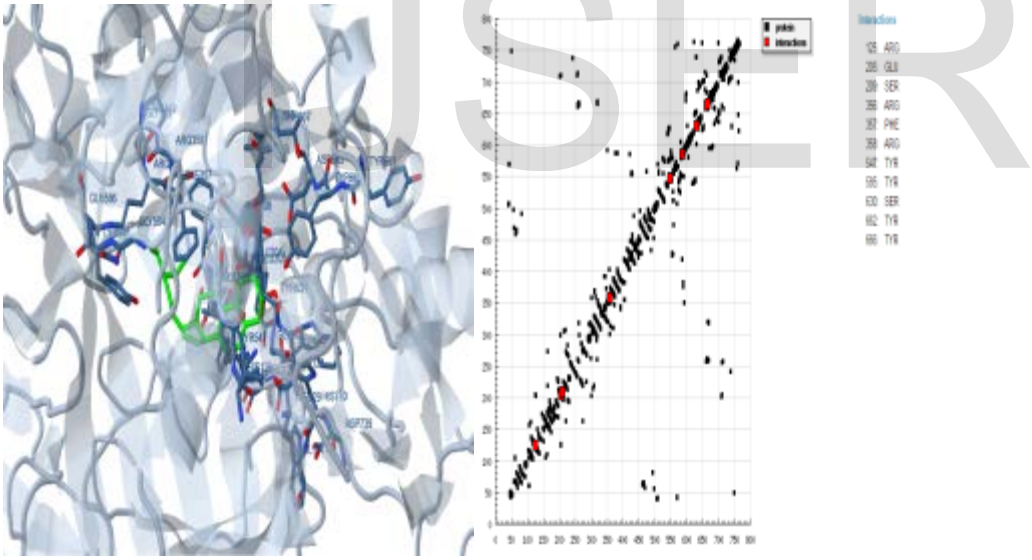


Fig 18: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme DPP-4 (2P8S) and the ligand Stigmasterol involved in hydrogen bond formation with amino acid residues on the protein like 205 Glu, 209 Ser,357 Phe,358 Arg,547 Try and 630 Ser. Total interaction surface of about 585.73.

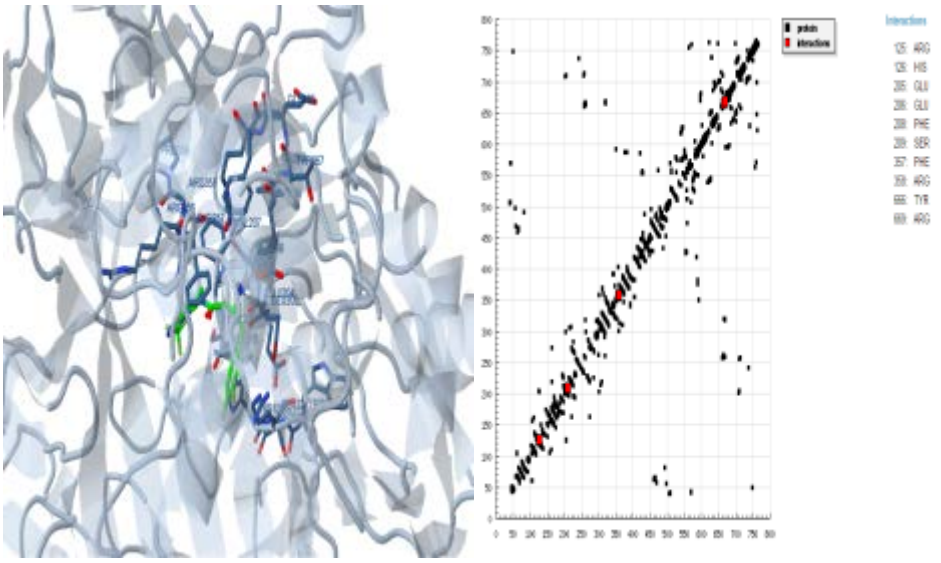


Fig 19: Shows the amino acid residues involved in hydrogen Bond interactions with enzyme DPP-4 (2P8S) and the ligand Sitagliptine involved in hydrogen bond formation with amino acid residues on the protein like 205 Glu , 206 Glu,207Val,209Ser,357 Phe,358 Arg and 667 Try. Total interaction surface of about 892.85.