ejpmr, 2024, 11(6), 11-25

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

COMPUTATIONAL EXPLORATION OF PSIDII GUAJAVAE FOLIUM BIOACTIVE COMPOUNDS AS NOVEL DPP-IV INHIBITORS FOR TYPE 2 DIABETES MELLITUS INTERVENTION

Folasade O. Ajao[a], Adeola F. Ehigie[b], Tope T. Odunitan[b], Opeyemi E. Atanda[b], Paul B. Ayoola[c] and Lamidi W. B. Olaniyan*[b]

[a]Department of Physiology, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria.
[b]Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria.
[c]Department of Science Laboratory Technology, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria.

***Corresponding Author: Lamidi W. B. Olaniyan**

Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria.

Article Received on 03/04/2024 **Article Revised on 24/04/2024** **Article Accepted on 15/05/2024**

ABSTRACT

Type 2 diabetes mellitus (T2DM) presents a significant global health challenge, necessitating the exploration of novel therapeutic interventions. In this computational study, we investigated Psidii Guajavae Folium (PGF) bioactive compounds as potential inhibitors of Dipeptidyl Peptidase IV (DPP-IV), a key enzyme implicated in T2DM pathophysiology. A total of 60 bioactive compounds, including the reference drug Teneligliptin, were subjected to molecular docking against the DPP-IV protein target. Thirteen compounds exhibiting pronounced binding affinities (-7.73 Kcal/mol to -6.6 Kcal/mol) compared to Teneligliptin (-6.5Kcal/mol) were further evaluated for drug-likeness and ADMET properties. Among these, four phytochemicals (Emetine, Linoelaidic Acid, Doconexent, and Clionasterol) demonstrated commendable drug-like characteristics and specificity, adhering to Lipinski's rule of five. Molecular dynamics simulations provided insights into the stability and dynamic behavior of the ligand-protein complexes, with Emetine emerging as the most promising inhibitor, followed closely by Linoelaidic Acid. MMGBSA calculations highlighted Emetine's robust total binding energy (-41.6889Kcal/mol) and favorable interaction profile, suggesting its potential as an effective DPP-IV inhibitor for T2DM intervention. Our findings underscore the promise of PGF-derived compounds as novel therapeutics for managing T2DM and warrant further experimental validation.

1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a major global health concern, with rising prevalence, morbidity, and mortality rates globally. This metabolic illness is characterized by insulin resistance, decreased insulin secretion, and chronic hyperglycemia, which can result in a variety of consequences such as cardiovascular disease, neuropathy, nephropathy, and retinopathy.[1,2] Despite advances in pharmacological therapies and lifestyle adjustments, the prevalence of T2DM is increasing[3], emphasizing the crucial need for novel therapeutic options to successfully control the disease and its consequences.

In recent years, there has been an increased interest in investigating natural products as potential sources of bioactive molecules with therapeutic capabilities for a variety of disorders, including diabetes. Psidii guajavae folium, often known as guava leaves, has been used in traditional medicine for its putative anti-diabetic properties.[4] Numerous studies have found bioactive chemicals in guava leaves, such as flavonoids, polyphenols, and triterpenes, that have shown promising antidiabetic efficacy through a variety of mechanisms, including the inhibition of key enzymes involved in glucose metabolism.[5,6] Among the beneficial chemicals found in guava leaves, researchers have focused on those that inhibit dipeptidyl peptidase-IV (DPP-IV), an enzyme involved in glucose regulation.[7] DPP-IV inhibitors are a type of antidiabetic medicine that works by extending the activity of incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide.[8] DPP-IV inhibitors boost insulin secretion, reduce glucagon release, improve pancreatic β-cell activity, and insulin sensitivity, resulting in better glycemic control in T2DM patients.[9] Given the remarkable antidiabetic potential of Psidii guajavae folium bioactive compounds, there is a rising interest in applying computational techniques to understand their molecular interactions with DPP-IV and assess their pharmacological effects.

Molecular docking and molecular dynamics simulations are useful for predicting ligand-receptor interactions, calculating binding affinities, and studying the structural dynamics of protein-ligand complexes.[10] Researchers can use computational analysis to find lead compounds with superior binding properties and potential therapeutic efficacy, offering useful insights for future experimental validation and drug development efforts.[11]

In this study, we report a complete computational analysis of Psidii guajavae folium bioactive components as possible DPP-IV inhibitors for Type 2 diabetes treatment. Using molecular modeling tools, we hope to clarify the molecular mechanisms behind guava leaf compounds' antidiabetic efficacy, forecast their pharmacokinetic features, and select good candidates for future preclinical and clinical studies. Through this interdisciplinary approach, we hope to contribute to the discovery of innovative therapies for T2DM management, addressing an unmet need in diabetic care and enhancing the quality of life for people suffering from this debilitating disease.

Overall, this study aims to bridge the gap between traditional medicine and modern drug development by leveraging the potential of natural products to battle the rising burden of T2DM and pave the road for more effective and accessible diabetes treatments. We intend to speed the translation of Psidii guajavae folium bioactive chemicals into clinically viable therapies by combining computational approaches and experimental validation, providing new hope to people living with Type 2 diabetes.

2. METHODOLOGY

The design and selection of the receptor structure during the first step of molecular docking screening are key stages that determine the study's success.[12] The receptor structure is designed with the study's specific goals in mind, such as understanding the ligand's mode of action and selectivity profile. Several factors are evaluated, including the resolution of the receptor structure, the state of its conformation (active or inactive), and the pharmacological properties of bound ligands.

2.1. Extraction and preparation of ligands

Diverse studies have found bioactive substances in guava leaves, including flavonoids, polyphenols, and triterpenes that have shown promising antidiabetic effect.[4-7] A thorough literature search revealed 60 bioactive components from Psidii guajavae folium that have been shown to have anti-diabetic properties. The structures of these compounds and the reference medication (Tenegliptin) were downloaded in sdf fromat from pubchem (https://pubchem.ncbi.nlm.nih.gov/). The sdf format of each compounds was converted to pdb format using OpenBabel software. The reference compound (teneligliptin) was also modified in the same approach as previously stated. The building of the chemical library is dependent on a variety of factors. Our primary goal in the virtual screening campaign is to identify a high-affinity molecule with drug-like qualities that adhere to Lipinski's rule of 5 (Figure 1).

2.2. Retrieval and Preparation of the Receptor Structure

Our research focuses on human dipeptidyl peptidase IV (DPP-4), a key protein in diabetes mellitus.[14] The three-dimensional X-ray crystallographic structures of the DPP-4 target were obtained from the Protein Data Bank (ID: 3VJM) (http://www.rcsb.org/pdb/home/home.do). Chimera was utilized to examine the protein, which was then generated using molecular operating environment (MOE). After importing the protein structure, MOE's structure preparation wizard was used to resolve all of the difficulties. This included two crucial steps: (a) Missing Residue Addition: The MOE tool was used to insert any missing residues into the protein structures, ensuring that the protein chains were complete. (b) Energy Minimization: An energy minimization process was carried out utilizing MOE, with the goal of improving the protein's geometric arrangement while minimizing unfavorable interactions and strain. This thorough construction of the protein structures paved the way for future computational investigations, assuring their precision and appropriateness for molecular docking simulations. During energy minimization, a 0.01 gradient was employed to calculate atomic coordinates representing local minima in the molecular energy function.[15,16] This approach is designed to find low-energy conformations and prepare the protein for molecular dynamics simulations.

2.3. Molecular docking

The current research employed in-silico molecular docking via Molecular Operating Environment (MOE). Prior to docking, the protein were subjected to necessary preparation steps using the structure preparation module. The AMBER 99 force field was utilized throughout the docking process, with additional optimization of bioactive compounds using AMBER 10. This optimization includes the addition of hydrogen bond, partial charges, and energy minimization of peptides. Protonation of the target protein structure was conducted using the Protonate3D module in MOE before setting it as the receptor. Active binding sites within the target protein were identified using the Site Finder module within MOE. The docking methodology involved docking a database of 60 phytochemicals into the binding site using alpha triangle docking placement technology. Poses were generated by superimposing ligand atoms and receptor site points in triplets. Five docking conformations were produced for each ligand, and these were ranked using the London dG scoring function to assess the free energy of binding for each position.[17,18] The pose with the lowest score was selected as the final option, and the binding orientations were thoroughly scrutinized for each docking operation.

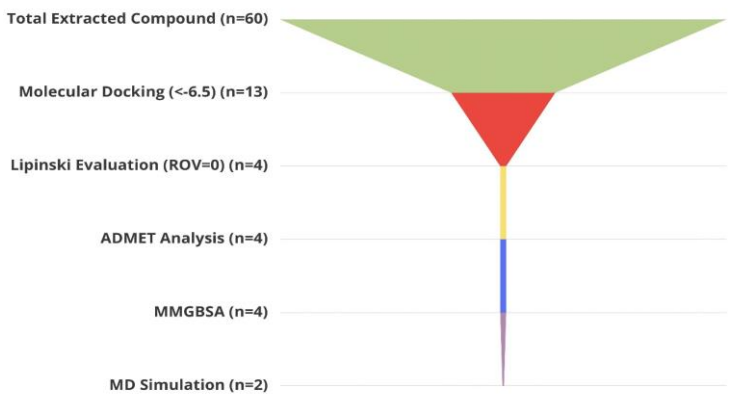


Figure 1: General workflow of phytochemicals screening and validation procedure.

2.4. Pharmacokinetics Evaluation

2.4.1. Drug-likeness properties

The lipinski's Rule of 5 (RO5) was used to evaluate the drug-likeness characteristics of the bioactive compounds. This was done using admetSAR and swissADME. This rules explores crucial molecular features that are significant for oral bioavailability, this includes molecular weight, octanol-water partition coefficient (logP), hydrogen bond acceptors and donors. This criterion, which is a refinement of drug-likeness, helps predict whether a chemical compound possesses pharmacological or biological activity suitable for oral administration in humans.[19]

2.4.2. ADME Prediction

The evaluation of small molecules in medicinal chemistry and pharmacokinetics necessitates critical Absorption, Distribution, Metabolism, and Excretion (ADME) studies. pkCSM, admetSAR, and swissADME were used to investigate the ADME properties of possible therapeutic candidates. Key parameters such as Central nervous system (CNS) permeability, Human Intestinal Absorption (HIA), mutagenicity, carcinogenicity, cytochrome P450 enzyme inhibition, and skin sensitization were assessed.[19]

2.4.3. Toxicity analysis

Toxicity analysis is an important part of drug development, this predict the impact of compounds on important organs such as the liver, heart. ProTox-II website was used to examine the LD50 values and toxicity classifications of the compounds. The term LD50 refers to the fatal dose at which 50% of the tested population succumbs to the drug. The pkCSM webserver was also used to evaluate the compounds' toxicology-related information, such as Ames positive, mutagenicity, skin sensitivity, and potential effects on liver functions, which was done by entering the Simplified Molecular Input Line Entry System (SMILES) from PubChem into pkCSM.[20]

2.5. Molecular Mechanics (MM-GBSA)

Largely accepted in computational studies, Generalized Born and Surface Area (MM-GBSA) methods in synergy with Molecular Mechanics energies are frequently used to estimate the free energy involved in the binding of small ligands to various biological macromolecules.[21-23] To evaluate the relative binding free energies of the four lead compounds following molecular docking analysis, Prime MM-GBSA, which is integrated into Maestro v12.5, was used in this investigation. Electrostatic interactions, Van der Waals forces, hydrogen bonds, and solvation energies were included in the breakdown of each frame potential energy into discrete energy contributions. The binding free energy calculation involved summing these energy terms, as expressed in the equation:

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{GB} + \Delta G_{SA} \quad (1)$$

$$\Delta E_{MM} = E_{ele} + E_{vdw} + E_{Hbond} \quad (2)$$

The binding free energy is represented by $\mathbf{\Delta G_{bind}}$, the molecular mechanical energy is denoted by $\mathbf{\Delta E_{MM}}$, the polar contribution to the solvation energy, as calculated by the Generalized Born (GB) technique, is represented by $\mathbf{\Delta G_{GB}}$, and the contribution from nonpolar components to the solvation energy is shown by $\mathbf{\Delta G_{SA}}$. The molecular mechanical energy $\mathbf{\Delta E_{MM}}$ is calculated by summing contributions from hydrogen bond energy (E_{Hbond}), electrostatic energy (E_{ele}), van der Waals energy (E_{vdw}), and torsional angle energy (Eint). These are all evaluated using the same force field that is used in the Molecular Dynamics simulations.[21-23]

2.6. Molecular Dynamics Simulations

The methodology employed in this study involved Molecular Dynamics (MD) simulations to investigate the binding stability, conformational dynamics, and interaction modes between ligands and target proteins. These simulations were conducted using GROMACS software version 2021, a well-established tool capable of accurately simulating proteins, lipids, and nucleic acids. To initialize the simulations, the topologies of the protein and ligands were generated utilizing CHARMM36 force fields, followed by refinement using protocols provided by GROMACS and CgenFF.[24-27] The resulting protein-ligand complex was then placed within a dodecahedron box filled with counterions and simple point charge (SPC) water molecules to create a neutralized solvated

environment suitable for simulation. To ensure the stability of the system, an iterative energy minimization process was performed until the maximum force reached a threshold of less than 100 kJ/mol/nm, employing optimization algorithms such as steepest descent and conjugate gradient. Subsequently, the equilibration of the system was achieved through positional-restrained dynamics simulations under both NVT (constant number of particles, volume, and temperature) and NPT (constant number of particles, pressure, and temperature) ensembles. This involved running the simulations for 5000 steps under NVT conditions followed by 50000 steps under NPT conditions, all at a temperature of 300 K. Once equilibrium was established, a production MD run of 50 nanoseconds duration was conducted at 300 K and 1 bar pressure to capture the long-term dynamics of the system. Throughout the simulation, various structural and dynamic properties were analyzed using GROMACS, including Root Mean Square Deviation (RMSD), Solvent Accessible Surface Area (SASA), and Root Mean Square Fluctuation (RMSF), providing insights into the behavior of the protein-ligand complex over time.

3. RESULTS AND DISCUSSION

3.1. Molecular Docking, Drug-likeness and binding Site analysis

In our study, we conducted a comprehensive evaluation involving a total of 60 bioactive compounds, alongside the reference compound teneligliptin, all of which were subjected to docking against the protein target Human Dipeptidyl Peptidase (DPPIV). The molecular docking analysis revealed a range of interactions, including hydrogen bonding, pi-pi, and hydrophobic interactions, which were meticulously identified and assessed. Compounds were then ranked based on their binding poses, with lower binding energy indicating higher binding affinity and vice versa. Among the 60 compounds, 13 exhibited pronounced negative binding energies, ranging from -7.73 kcal/mol to -6.6 kcal/mol against the selected targets compared to the standard with a binding score of -6.5 kcal/mol (Figure 2). Consequently, only compounds demonstrating significantly lower binding energy than the standard (Teneligliptin) were retained for further scrutiny, encompassing drug-likeness and ADMET evaluation. The binding affinities and interacting amino acids of the ultimately selected compounds are reported in Table 1. The meticulous drug-likeness analysis of the 13 compounds delineated the identification of 4 phytochemicals: emetine, linoelaidic acid, doconexent, clionasterol, alongside the reference drug (teneligliptin), all exhibiting commendable drug-like characteristics (molecular weight < 500 Da, no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, and an octanol-water partition coefficient not surpassing 5) (Table 2). These compounds strictly adhere to Lipinski's guidelines for drug-likeness. In the course of virtual screening, it is imperative to discern compounds susceptible to yielding false-positive results, typified by "pan-assay interference compounds" (PAINS), which exhibit a propensity to target multiple biological targets instead of a singular one.[28-29] The compounds underwent rigorous screening for PAINS activity leveraging the SwissADME web service, with the results indicating the absence of any lead compounds belonging to the PAINS class (Table 2), thereby affirming their likelihood to exhibit specific molecular interactions.

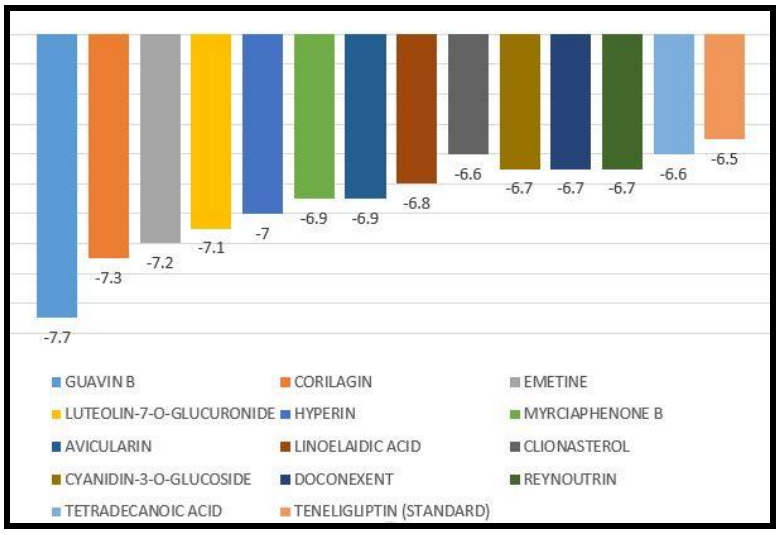


Figure 2: The top 14 phytochemicals docked against Human Dipeptidyl Peptidase (DPPIV).

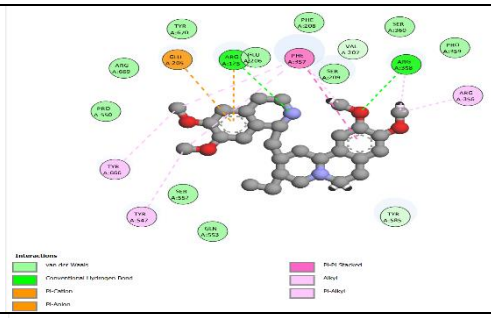
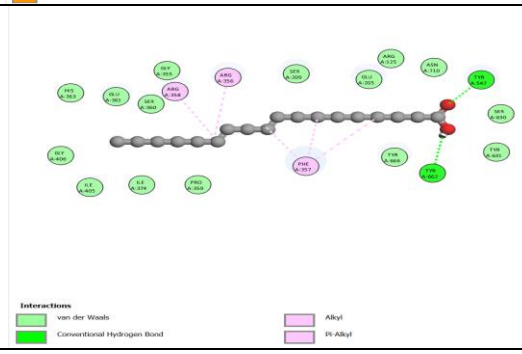
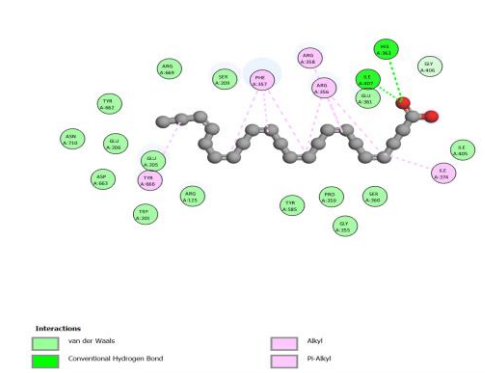
Subsequently, the binding interaction of these promising inhibitors were analyzed. Intriguingly, all were found to bind resolutely to the active residues of DPP-IV. Emetine, boasting a binding score of -7.2 kcal/mol, with two conventional hydrogen bonds (ARG 125, ARG 358) while also manifesting hydrophobic interactions (TYR666, TYR547, ARG356, PHE 357, ARG 358) with the targeted protein. Linoelaidic acid, with a binding score of -6.8 kcal/mol, showcased two conventional hydrogen bonds (TYR547, TYR666), alongside

hydrophobic interactions (ARG358, ARG356, PHE357) with the receptor (Figure 3). Doconexent, boasting a binding score of -6.7 kcal/mol, exhibited commendable binding affinities, characterized by two conventional hydrogen bonds (ILE407, HIS363), coupled with hydrophobic interactions (TYR666, PHE357, ARG358, ARG356, ARG357). Lastly, Clionasterol, with a binding score of -6.6 kcal/mol, engaged in hydrophobic/other interactions (PHE357, ILE374, ARG356) with the targeted protein (Fig. 3). Notably, it has been reported.[29,30] that an augmented number of hydrogen bonds in the interaction profile of a Protein-Ligand complex is indicative of a more stable complex. Hence, it can be inferred from the molecular docking results that Emetine emerges as the best among the selected ligands, followed by linoelaidic acid surpassing Teneligliptin, with a binding affinity of -6.5 kcal/mol and one conventional hydrogen bond (GLU 205). Additionally, it was discerned that the lead compounds exhibit a similar of mode of action to Teneligliptin, as they interact with their targets via akin amino acid residues.

3.2. Pharmacokinetics Analysis

The thorough evaluation of ADMET properties is paramount for discerning the pharmacokinetic and toxicological attributes of compounds.[31] Advanced webservers such as admetSAR, ProTox-II, and pkCSM are instrumental in delving into absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiling. We meticulously delved into the pharmacokinetic properties of the most promising lead compounds and the standard (Table 3). It's noteworthy that all compounds, with the exception of emetine and linoelaidic acid, showcase no permeability to the blood–brain barrier (BBB) (Figure 4). While emetine, linoelaidic acid, and clionasterol exhibited positive results for Caco-2 cell line permeability under adsorption and dispersion conditions, docohexent and teneligliptin displayed unfavorable outcomes. In addition, emetine demonstrate substrate and inhibitory effects on P-glycoprotein.

Table 1: 2D Interaction and list of interacting amino acids of lead compounds and the standard drug.

Compounds	Binding score	2D Interaction	H-bond Interaction	Hydrophobic Interaction	Other interaction
Emetine	-7.2		ARG125, ARG358	TYR666, TYR547, ARG356, PHE 357, ARG 358	Electrostatic Pi bond: GLU 205, ARG125
Linoelaidic	-6.8		TYR547, TYR666	ARG358, ARG356, PHE357	
Doconexent	-6.7		ILE407, HIS363	TYR666,PHE357, ARG358, ARG356, ARG357	

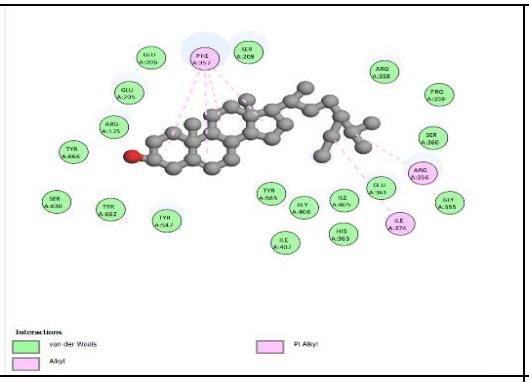
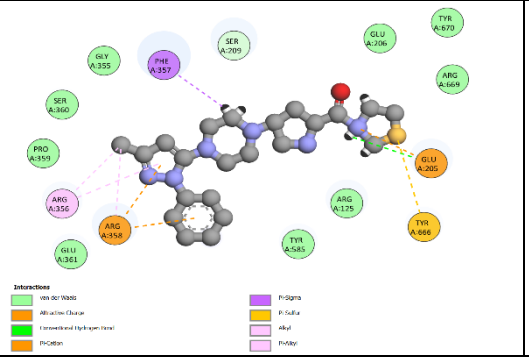
Clionasterol	-6.6		-------	PHE357, ILE374, ARG356	
Teneligliptin (Standard)	-6.5		GLU205	ARG356,PHE357	Electrostatic Pi Bond: GLU205, ARG358 Pi Sulfur: TYR666.

Table 2: Drug-likeness (Rule of 5) evaluation and physiochemical properties of selected compounds.

S. No	LIGANDS	Binding Score	Pubchem ID	MW	HBD	HBA	LOG P	LIPINSKI RULE	TPSA	PAINS
1	**Emetine**	-7.2	10219	480.64	1	6	4.19	Yes	52.19	0
2	**Linoelaidic acid**	-6.8	5282457	280.2	1	2	6.6	Yes	37.3	0
3	Doconexent	-6.7	445580	328.2	1	2	6.6	Yes	37.3	0
4	Clionasterol	-6.6	457801	414.3	1	1	7.6	Yes	20.2	0
5	Teneligliptin (Standard)	-6.5	11949652	426.6	1	7	1.76	Yes	81.94	0

In humans, an array of cytochrome P450 enzymes play pivotal roles in the metabolism of compounds. We meticulously scrutinized the inhibitory potential of the selected compounds and Teneligliptin on these enzymes, with a keen focus on their impacts, particularly on the CYP3A4 and CYP2D6 isoforms, which can significantly influence drug metabolism. According to our findings, none of the lead compounds inhibit CYP2C19 and CYP2C9 enzymes. However, linoelaidic acid and docohexent showcase inhibitory effects on the CYP1A2 enzyme class. Conversely, emetine, clionasterol, and tenegliptin emerge as non-inhibitors. When it comes to substrates, all compounds, except emetine, exhibit non-substrates of CYP2D6.

Interestingly, linoelaidic acid and docohexent surface as substrates of CYP2C9 and inhibitors of CYP1A2. On the other hand, teneligliptin, clionasterol, and emetine are identified as substrates of CYP3A4. Furthermore, none of the compounds exhibit inhibitory effects on crucial isoforms, suggesting a favorable overall metabolism profile. The efficient elimination of compounds and their metabolites via excretion is pivotal to curtail accumulation and eventual cytotoxicity. Our assessment of half-life and clearance descriptors revealed high clearance rates for emetine, doconexent, and clionasterol, contrasting with the low clearance rates observed for linoelaidic acid and the standard (teneligliptin). Notably, linoelaidic acid and doconexent showcase a higher probability of a prolonged half-life, while clionasterol exhibits a markedly low probability, and emetine and tenegliptin demonstrate moderate values.

Table 3: ADMET properties of the 4 lead compounds and the standard (Teneligliptin).

	Emetine	Linoelaidic acid	Doconexent	Clionasterol	Teneligliptin
Human Intestinal Absorption					
Caco-2 permeability					
P-glycoprotein I inhibitor					
P-glycoprotein II inhibitor					
P-glycoprotein substrate					

BBB Permeability					
CYP1A2 Inhibitor					
CYP2C19 Inhibitor					
CYP2C9 Inhibitor					
CYP2C9 Substrate					
CYP2D6 Substrate					
CYP2D6 Inhibitor					
CYP3A4 Substrate					
CYP3A4 Inhibitor					
Clearance					
Half-life ($T_{1/2}$)	0.4	0.600.60	0.6	0.03	0.4
Herg I Inhibitor					
Herg II Inhibitor					
AMES Toxicity					
Carcinogenicity (binary)					
Acute Oral Toxicity	III	IV	IV	I	III
Skin Sensitisation					
Hepatotoxicity					

Moreover, all compounds exhibit non-toxicity concerning the Ames toxicity parameter. Also, all the compounds and teneligliptin exhibit similar safety profile and acceptable level profile hERG-I (human ether-a-go-go-related gene I). It's also intriguing to note that all compounds exhibit non-carcinogenic properties. Regarding liver toxicity, only emetine and doconexent are non-hepatotoxic. Categorizing compounds based on acute oral toxicity reveals predictions where clionasterol belongs to class I, emetine and teneligliptin fall into Category III, and linoelaidic acid and docohexent are predicted to fall into class IV. These detailed insights shed light on the multifaceted pharmacokinetic and toxicological profiles of the compounds, offering valuable guidance for their potential therapeutic applications.

3.3. MMGBSA Calculation Analysis

MMGBSA (Molecular Mechanics with Generalized Born Surface Area) calculations represent a widely utilized approach in computational biology for predicting in silico drug-target interactions.[22,23] These calculations involve the summation of different energy interactions, also known as force fields (FFs), to evaluate the overall binding free energy of ligand-protein complexes. Our MMGBSA calculations including binding energy contribution from hydrogen bonding (MMGBSAΔG Bind Hbond), Van der Waals interactions (MMGBSAΔG Bind vdW), solvation (MMGBSAΔG Bind Solv), and total binding free energy (MMGBSAΔG Bind) were conducted to assess the binding free energy (Table 4) and the contributing factors to the total binding energy of various ligand-protein complexes. Among the evaluated compounds, including Emetine, Linoelaidic Acid, Clionasterol, Doconexent, and Teneligliptin, notable findings have emerged. Emetine demonstrates a robust total binding energy, primarily driven by the Van der Waals (vdW) interaction component. The compound exhibits favorable binding affinity with a binding free energy of -41.6889 kJ/mol, suggesting strong interactions with the protein target. Moreover, Emetine displays a significant contribution from hydrogen bonding (-0.72487), indicating stable protein-ligand interactions. Linoelaidic Acid exhibits a moderate total binding energy, primarily attributed to the Van der Waals interaction component. However, the compound demonstrates a relatively lower binding affinity compared to Emetine, with a binding free energy of -15.4533 kJ/mol (Figure 4).

Table 4: MMGBSA analysis results of the lead compounds and the standard (Teneligliptin).

Compound	MMGBSAΔG Bind	MMGBSAΔG Bind Hbond	MMGBSAΔG Bind vdW	MMGBSAΔG Bind Solv
Emetine	-41.6889	-0.72487	-43.7913	**41.91**
Linoelaidic Acid	-15.4533	-1.62641	-37.212	**-40.85**
Clionasterol	-26.3642	-0.89723	-37.3121	**35.98**
Doconexent	-12.7929	-2.41086	-35.9598	**-39.62**
Teneligliptin	-31.9778	-0.51904	-49.0269	**37.77**

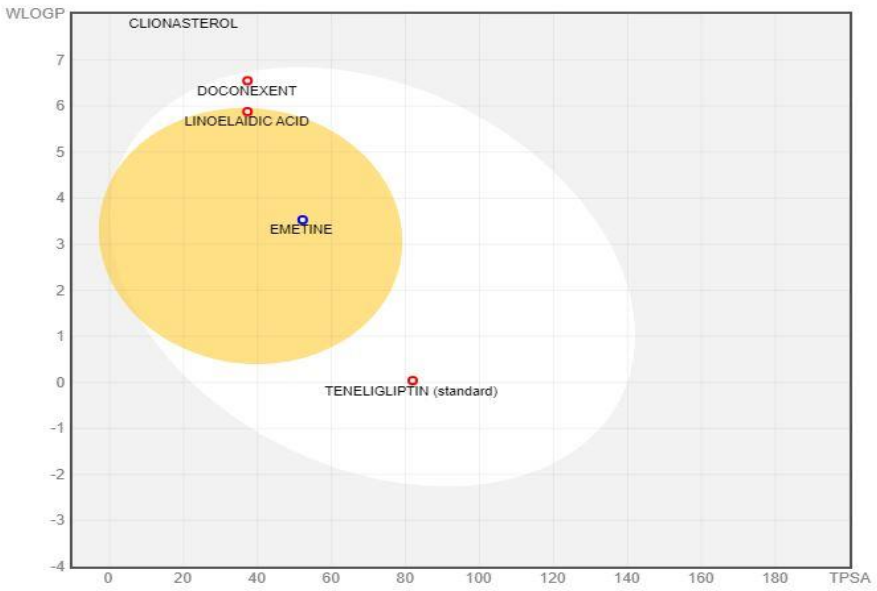
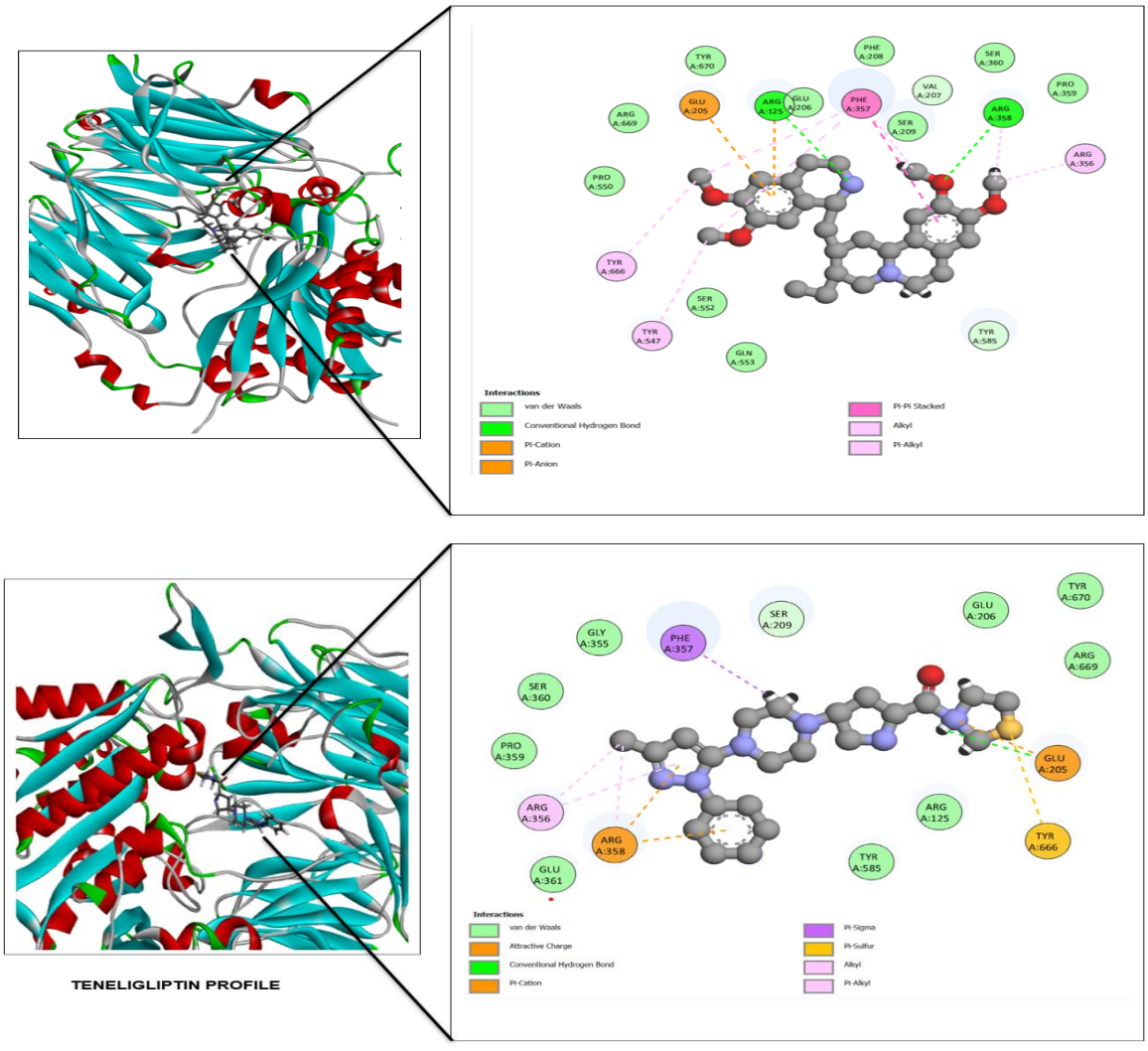


Figure 3: The boiled-egg analysis from swissADME for the 4 lead compounds and the standard drug. The yellow and white region represent the blood brain barriers and human intestinal absorption respectively.



TENELIGLIPTIN PROFILE

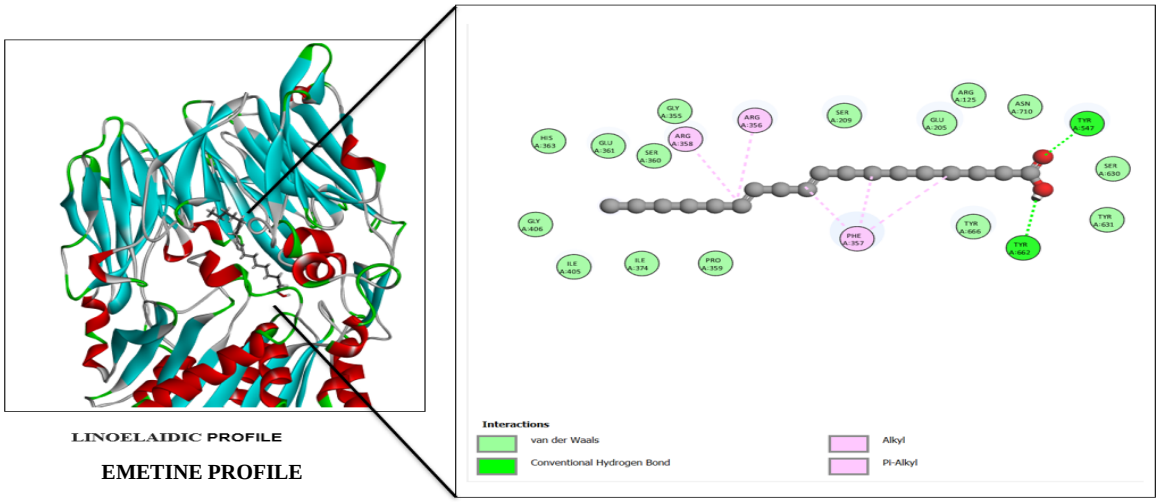


Figure 4: 3D structure of the lead compounds in DPPIV protein pocket and the 2D interaction of Emetine, Teneligliptin and Linoelaidic acid with conventional hydrogen Bonds, Pi-alkyl in green, purple and orange respectively.

Clionasterol displays a moderate total binding energy, predominantly driven by the Van der Waals interaction component. The compound exhibits reasonable binding affinity with a binding free energy of -26.3642 kJ/mol. Doconexent demonstrates a relatively lower total binding energy, with a substantial contribution from hydrogen bonding. The compound exhibits a binding free energy of -12.7929 kJ/mol. Teneligliptin shows a considerable total binding energy, primarily attributed to the Van der Waals interaction. The compound demonstrates favorable binding affinity with a binding free energy of -31.9778 kJ/mol. Based on the MMGBSA analysis results (Figure 5.), Emetine emerges as a particularly promising inhibitor, displaying a robust total binding energy and substantial contributions from both Van der Waals interactions and hydrogen bonding. These findings highlight Emetine's potential as an effective inhibitor compared to the other evaluated compounds.

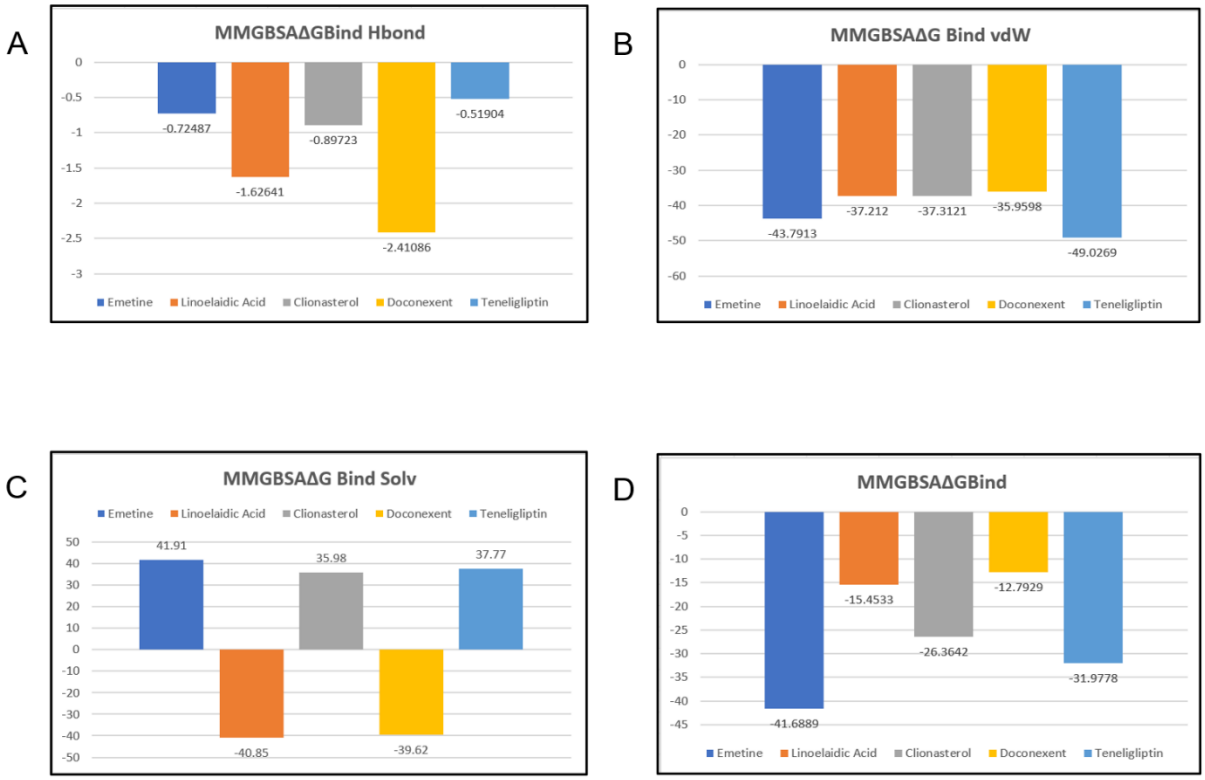


Figure 5: MMGBSA calculation analysis chart of the four lead compounds, depicting (A) MMGBSAΔG Bind Hbond (B) MMGBSAΔG Bind vdW, (C) MMGBSAΔG Bind Solv, and (D) MMGBSAΔG Bind.

3.5. Molecular dynamics simulation of the complexes

Molecular dynamics (MD) simulation has undergone significant evolution in recent decades due to advancements in quantum physics and computational chemistry. It has emerged as an indispensable tool for investigating the behavior of macromolecules, including membrane proteins and protein-ligand complexes.[32] MD simulations enable researchers to explore the structural and functional dynamics of biomolecules at atomistic resolution. With current computational resources and methodologies, MD simulations can accurately replicate biological systems over timescales ranging from nanoseconds to milliseconds for each molecular complex.[33] This level of detail allows for the study of intricate molecular interactions and conformational changes that occur within biomolecular systems. In this study, MD simulations were conducted for the protein target Dipeptidyl Peptidase-IV (DPP-IV), the standard drug Teneligliptin, and two promising DPP-IV inhibitors (Emetine and Linoelaidic acid) identified through docking screening. The simulations were performed for a duration of 50,000 picoseconds (ps), during which various parameters such as root-mean-square deviation (RMSD), root-mean-square fluctuations (RMSF), and molecular interactions were analyzed. GROMACS software was utilized for the simulations, allowing for detailed atomistic analysis of the dynamic behavior of the protein-ligand complexes.

3.4.1. RMSD

The Root Mean Square Deviation (RMSD) measures the conformational variations in specific ligand-protein complexes over time. The provided RMSD values offer valuable insights into the stability and structural alignment of the complexes formed between the compounds and the protein target (DPP-IV) before and during the simulation.[34,35]

As observed with the average RMSD value in Table 5, Emetine demonstrates the lowest average RMSD value (0.140nm) among the compounds analyzed. This suggests that the Emetine-protein complex maintains a relatively stable and closely aligned structure throughout the simulation period. The low RMSD value indicates minimal deviation from the initial conformation, indicating a strong interaction between Emetine and the protein target (DPP-IV) (Figure 4). On the other hand, Linoelaidic Acid exhibits a slightly higher average RMSD value (0.154nm) compared to Emetine but remains within an acceptable range. This suggests that the Linoelaidic Acid-DPP-IV complex maintains structural stability during the simulation, though with slightly more variability compared to Emetine. The relatively low RMSD value indicates a favorable interaction between Linoelaidic Acid and the protein target.

Teneligliptin, as the standard drug, displays a marginally higher average RMSD value (0.164nm) compared to both Emetine and Linoelaidic Acid. Despite this, the RMSD value remains within an acceptable range, suggesting that the Teneligliptin-protein complex maintains structural stability during the simulation (Figure 5). However, the slightly higher.

Table 5: Average values of RMSD, RMSF, and SASA of all simulated complexes.

Average RMSD values (nm)	
Emetine Complex	0.140
Linoelaidic acid Complex	0.154
Teneligliptin Complex (Standard)	0.164
Unbound Protein (DPPIV)	0.156
Average RMSF values (nm)	
Emetine Complex	0.069
Linoelaidic acid Complex	0.060
Teneligliptin Complex (Standard)	0.064
Unbound Protein (DPPIV)	0.064
Average SASA values (nm^2)	
Emetine Complex	303.19
Linoelaidic acid Complex	303.25
Teneligliptin Complex (Standard)	303.28
Unbound Protein (DPPIV)	304.42

RMSD value may indicate some degree of deviation from the initial conformation.[36] The Protein Target (DPP-IV) exhibits an average RMSD value comparable to Emetine and Linoelaidic Acid when complexed with the compounds (Table 5). This suggests that the DPP-IV-protein complexes maintain relatively stable structures during the simulation, with minimal deviation from the initial conformation.

Finally, the RMSD values provide evidence of the stability and structural alignment of the compounds when complexed with the protein target (DPP-IV) during the simulation. Emetine emerges as the compound with the lowest average RMSD value, indicating a stable and closely aligned complex with the protein target. Linoelaidic Acid follows closely, while Teneligliptin, despite displaying a slightly higher RMSD value, still maintains structural stability within an acceptable range.

These findings suggest that Emetine and Linoelaidic Acid may hold promise in pharmacological applications due to their favorable interaction with the protein target. Interestingly, this agrees with the analysis of molecular docking where the ligand showed better binding affinity and drug-likeness than all other docked complexes (Figure 1).

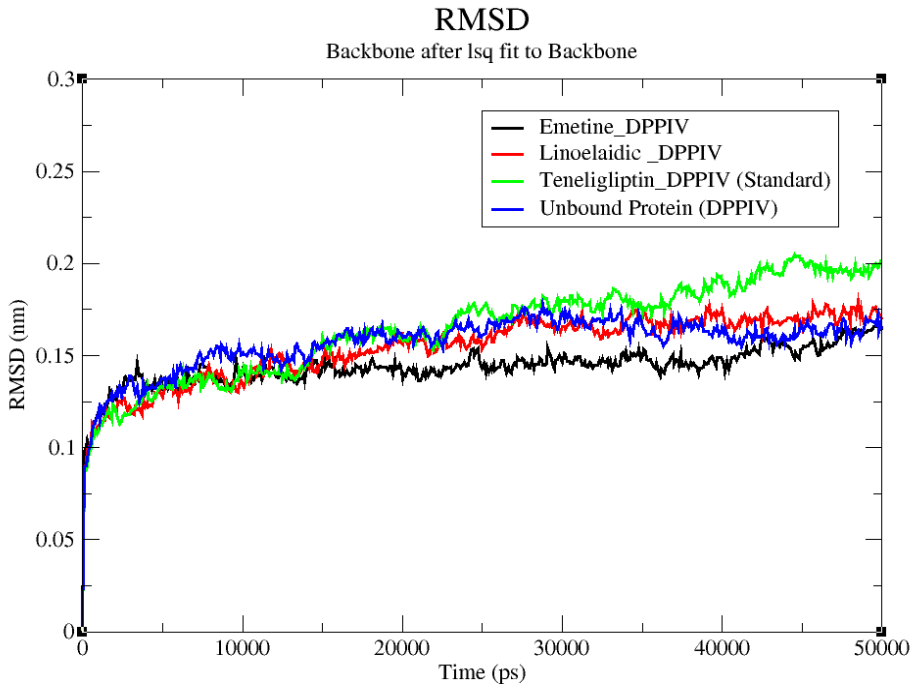


Figure 6 The Superimposed RMSD graph spectrum of the unbound protein (DPP-IV), the referenced compound (Teneligliptin) and the 2 promising compounds (Emetine and Linoelaidic acid) in complex with KRAS G12D.

3.4.2. RMSF

RMSF (Root Mean Square Fluctuation) analysis enables the identification of crucial residues engaged in the most significant interactions with a specific ligand. During MD simulations, higher RMSF values reflect increased atomic mobility of the protein's Cα atoms.[37] The graph in Fig. 5 overlays the RMSF spectra of the simulated entities. As indicated in Table 5, the average RMSF values for the Emetine and Linoelaidic acid complexes are 0.069 nm and 0.060 nm respectively.

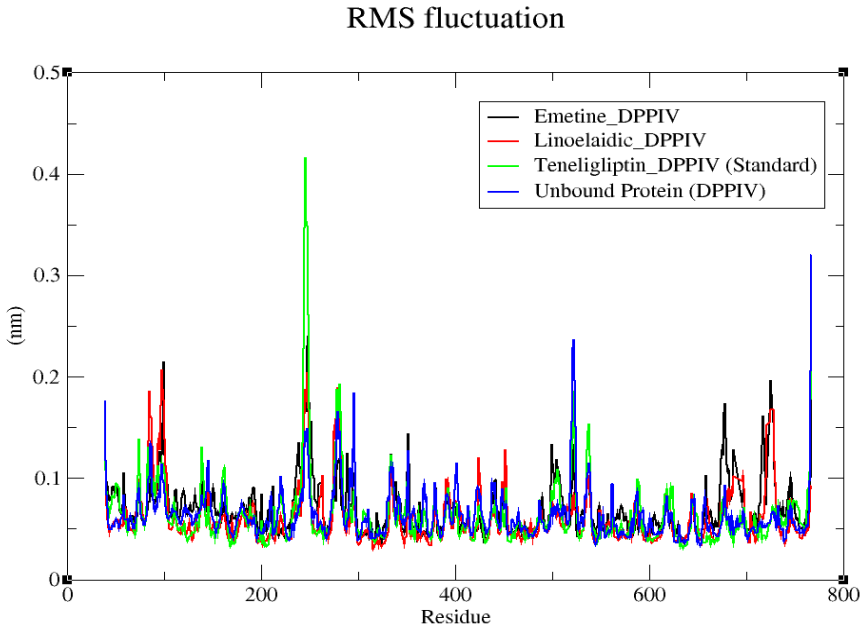


Figure 7: The Superimposed RMSF graph spectrum of the unbound protein (DPP-IV), the referenced compound (Teneligliptin) and the 2 promising compounds (Emetine and Linoelaidic acid) in complex with DPPIV.

The RMSF value for the Emetine complex indicates relatively low atomic mobility within the complex suggesting a stable interaction between Emetine and the protein target (DPPIV). This stability implies that Emetine may exhibit favorable binding affinity and structural integrity when complexed with DPPIV, making it a promising candidate for further pharmacological investigation. Interestingly, the RMSF value for the Linoelaidic Acid complex is even lower than that of Emetine, indicating minimal atomic mobility and fluctuation within the complex. This suggests a highly stable interaction between Linoelaidic Acid and DPPIV, with strong binding affinity and structural integrity. The exceptionally low RMSF value underscores the potential of Linoelaidic Acid as a promising drug candidate for targeting DPPIV. The RMSF value for the unbound DPPIV reflects inherent atomic fluctuations within the protein structure in the absence of ligand binding. This serves as a baseline for comparison with the RMSF values of the ligand-protein complexes. Teneligliptin Complex is comparable to that of the unbound DPPIV, indicating minimal atomic fluctuations within the complex (Figure 6). This suggests a stable binding interaction between Teneligliptin and DPPIV, consistent with its status as a standard drug. The low RMSF value reinforces the potential pharmacological relevance of Teneligliptin in targeting DPPIV. The observed RMSF values provide valuable insights into the stability and dynamic behavior of the ligand-protein complexes. While Emetine and Teneligliptin complexes exhibit low RMSF values, indicating stable interactions with DPPIV, the exceptionally low RMSF value for the Linoelaidic Acid complex highlights its remarkable stability and strong binding affinity. In summary, this interpretation reflects the significance of the exceptionally low RMSF value for Linoelaidic Acid Complex and its implications for drug candidacy, while also acknowledging the stable interactions observed for Emetine and Teneligliptin complexes.

3.4.3. SASA

SASA analysis plays a pivotal role in studying protein-ligand interactions, where it can aid in assessing the binding affinity, stability, and conformational changes of complexes.[38] Ligands with lower SASA values when bound to their target proteins often indicate tighter binding and enhanced structural integrity, making them promising candidates for drug development.[39,40] As shown in Table 5, emetine emerges as a compelling candidate when compared to the standard drug Teneligliptin, as indicated by its favorable Solvent Accessible Surface Area (SASA) value. While both compounds exhibit comparable degrees of solvent exposure, Emetine's slightly lower SASA value suggests a trend towards greater compactness and stability in its complex with the protein target (DPPIV) compared to Teneligliptin (Figure 7). The lower SASA value of Emetine implies reduced solvent accessibility, which may indicate a more tightly bound complex with DPPIV compared to Teneligliptin. This characteristic suggests that Emetine could potentially form stronger interactions with the protein, leading to enhanced binding affinity and stability. Furthermore, the comparison with the standard drug underscores Emetine's potential superiority in terms of its structural dynamics and stability. While Teneligliptin is an established standard, Emetine's ability to exhibit comparable or even better characteristics in terms of protein-ligand interactions is promising for its candidacy as a drug candidate. However, it's crucial to emphasize that the assessment of Emetine's superiority over Teneligliptin should be further validated through comprehensive studies, including assessments of binding affinity, structural integrity, pharmacokinetic properties, and biological activity. In conclusion, Emetine's favorable SASA value suggests that it holds promise as a potential alternative or improvement over the standard drug Teneligliptin. Further investigation is warranted to fully elucidate its pharmacological potential and establish its suitability for therapeutic applications as compared to the standard treatment.

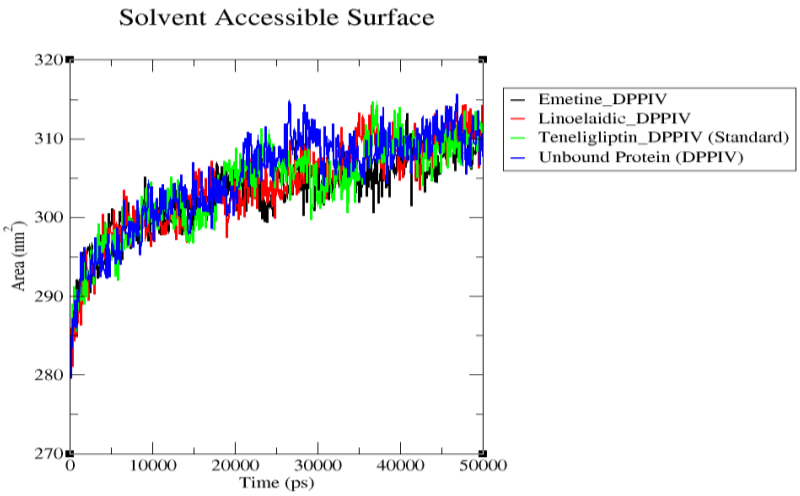


Figure 8: The Superimposed SASA graph spectrum of the unbound protein (DPP-IV), the referenced compound (Teneligliptin) and the 2 promising compounds (Emetine and Linoelaidic acid) in complex with KRAS G12D.

4. CONCLUSION

Our study highlights the promising pharmacological potential of Psidii Guajavae Folium bioactive compounds as DPP-IV inhibitors for Type 2 diabetes intervention. Through molecular docking, drug-likeness and pharmacokinetic analysis, emetine and linoelaidic acid demonstrated superior binding affinity, drug-likeness, and favorable ADMET properties compared to the standard teneligliptin. Furthermore, molecular dynamics simulations provided valuable insights into the stability and dynamic behavior of these compounds in complex with DPP-IV, highlighting the emerging stability, strong interactions of emetine and linoelaidic acid. MMGBSA calculations further supported Emetine's candidacy as a potent inhibitor, exhibiting robust total binding energy and favorable interaction profiles. These findings suggest the potential of PGF-derived compounds as novel therapeutics for managing Type 2 diabetes mellitus. Future experimental studies are warranted to validate the efficacy and safety of these compounds in vivo, paving the way for their clinical translation and eventual incorporation into diabetes management strategies.

ACKNOWLEDGEMENT

We acknowledge the contributions and support from Ehigie's Biochemistry and Biocomputational Laborarotory (EBBL).

REFERENCES

1. Galicia-Garcia U. Pathophysiology of Type 2 Diabetes Mellitus. *International Journal of Molecular Sciences*, 2020; 21(17): 1-34.
2. Goyal R, Jialal I, Singhal M. Diabetes mellitus type 2. National Center for Biotechnology Information. Published June 23, 2023. https://www.ncbi.nlm.nih.gov/books/NBK513253/
3. Marín-Peñalver JJ, Martín-Timón I, Sevillano-Collantes C, Cañizo-Gómez FJ del. Update on the treatment of type 2 diabetes mellitus. *World Journal of Diabetes*, 2016; 7(17): 354. doi:https://doi.org/10.4239/wjd.v7.i17.354
4. Díaz-de-Cerio E, Verardo V, Gómez-Caravaca AM, Fernández-Gutiérrez A, Segura-Carretero A. Health Effects of Psidium guajava L. Leaves: An Overview of the Last Decade. *International Journal of Molecular Sciences*, 2017; 18(4). doi:https://doi.org/10.3390/ijms18040897
5. Kumar M, Tomar M, Amarowicz R, et al. Guava (Psidium guajava L.) Leaves: Nutritional Composition, Phytochemical Profile, and Health-Promoting Bioactivities. *Foods*, 2021; 10(4). doi:https://doi.org/10.3390/foods10040752
6. Shabbir H, Kausar T, Noreen S, et al. In Vivo Screening and Antidiabetic Potential of Polyphenol Extracts from Guava Pulp, Seeds and Leaves. *Animals*, 2020; 10(9): 1714. doi:https://doi.org/10.3390/ani10091714
7. Chu S, Zhang F, Wang H, et al. Aqueous Extract of Guava (Psidium guajava L.) Leaf Ameliorates Hyperglycemia by Promoting Hepatic Glycogen Synthesis and Modulating Gut Microbiota. *Frontiers in Pharmacology*, 2022; 13. doi:https://doi.org/10.3389/fphar.2022.907702
8. Langley AK, Suffoletta TJ, Jennings HR. Dipeptidyl Peptidase IV Inhibitors and the Incretin System in Type 2 Diabetes Mellitus. *Pharmacotherapy*, 2007; 27(8): 1163-1180. doi:https://doi.org/10.1592/phco.27.8.1163
9. Makrilakis K. The Role of DPP-4 Inhibitors in the Treatment Algorithm of Type 2 Diabetes Mellitus: When to Select, What to Expect. *International Journal of Environmental Research and Public Health*, 2019; 16(15): 2720. doi:https://doi.org/10.3390/ijerph16152720
10. Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: a powerful approach for structure-based drug discovery. *Current computer-aided drug design*, 2011; 7(2): 146-157. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3151162/
11. Opeyemi Iwaloye, Paul O, Femi Olawale, et al. Computer-aided drug design in anti-cancer drug discovery: What have we learnt and what is the way forward? *Informatics in Medicine Unlocked*, 2023; 41: 101332-101332. doi:https://doi.org/10.1016/j.imu.2023.101332
12. Odunitan TT, Saibu OA, Apanisile BT, et al. Integrating biocomputational techniques for Breast cancer drug discovery via the HER-2, BCRA, VEGF and ER protein targets. *Computers in Biology and Medicine*, 2024; 168: 107737-107737. doi:https://doi.org/10.1016/j.compbiomed.2023.107737
13. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, 2001; 46(1-3): 3-26. doi:https://doi.org/10.1016/s0169-409x(00)00129-0
14. Röhrborn D. DPP4 in diabetes. *Frontiers in Immunology*, 2015; 6. doi:https://doi.org/10.3389/fimmu.2015.00386
15. Molecular Operating Environment (MOE), 2022.02 Chemical Computing Group ULC, 910-1010 Sherbrooke St. W., Montreal, QC H3A 2R7, Canada, 2024.
16. Gerber P, Klaus-Robert Müller. MAB, a generally applicable molecular force field for structure modelling in medicinal chemistry. *Journal of Computer-aided Molecular Design*, 1995; 9(3): 251-268. doi:https://doi.org/10.1007/bf00124456
17. Maier JA, Martinez C, Kasavajhala K, Wickstrom L, Hauser KE, Simmerling C. ff14SB: Improving the Accuracy of Protein Side Chain and Backbone Parameters from ff99SB. *Journal of Chemical Theory and Computation*, 2015; 11(8): 3696-3713. doi:https://doi.org/10.1021/acs.jctc.5b00255
18. Kumar YN, Kumar PS, Sowjenya G, et al. Comparison and correlation of binding mode of

ATP in the kinase domains of Hexokinase family. *Bioinformation*, 2012; 8(12): 543-547. doi:https://doi.org/10.6026/97320630008543
19. Mishra S, Dahima R. IN VITRO ADME STUDIES OF TUG-891, A GPR-120 INHIBITOR USING SWISS ADME PREDICTOR. *Journal of Drug Delivery and Therapeutics*, 2019; 9(2-s): 366-369. doi:https://doi.org/10.22270/jddt.v9i2-s.2710
20. Pires, D. E. V., Blundell, T. L., & Ascher, D. B. pkCSM: Predicting Small Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. *Journal of Medicinal Chemistry*, 2015; *58*(9): 4066–4072. https://doi.org/10.1021/acs.jmedchem.5b00104 Yu
21. Genheden S, Ryde U. The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities. *Expert Opinion on Drug Discovery*, 2015; 10(5): 449-461. doi:https://doi.org/10.1517/17460441.2015.1032936
22. Virtanen SI, Niinivehmas SP, Pentikäinen OT. Case-specific performance of MM-PBSA, MM-GBSA, and SIE in virtual screening. *Journal of Molecular Graphics and Modelling*, 2015; 62: 303-318. doi:https://doi.org/10.1016/j.jmgm.2015.10.012
23. Wang, 2019 (Binding Free Energy Calculation) | PDF | Drug Design | Solvation. Scribd. Accessed February 7, 2024. https://www.scribd.com/document/595063304/wang-2019-Binding-Free-Energy-Calculation
24. Van Der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE, Berendsen HJC. GROMACS: Fast, flexible, and free. *Journal of Computational Chemistry*, 2005; 26(16): 1701-1718. doi:https://doi.org/10.1002/jcc.20291
25. Huang J, Rauscher S, Nawrocki G, et al. CHARMM36m: an improved force field for folded and intrinsically disordered proteins. *Nature Methods*, 2016; 14(1): 71-73. doi:https://doi.org/10.1038/nmeth.4067
26. Vanommeslaeghe K, Hatcher E, Acharya C, et al. CHARMM general force field: A force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields. *Journal of Computational Chemistry*, 2010; 31(4): 671-690. doi:https://doi.org/10.1002/jcc.21367
27. Yu W, He X, Vanommeslaeghe K, MacKerell AD. Extension of the CHARMM General Force Field to sulfonyl-containing compounds and its utility in biomolecular simulations. *Journal of Computational Chemistry*, 2012; 33(31): 2451-2468. doi:https://doi.org/10.1002/jcc.23067
28. Nelson KM, Dahlin JL, Bisson J, Graham J, Pauli GF, Walters MA. The Essential Medicinal Chemistry of Curcumin. *Journal of medicinal chemistry*, 2017; 60(5): 1620-1637. doi:https://doi.org/10.1021/acs.jmedchem.6b00975
29. Chen D, Oezguen N, Urvil P, Ferguson C, Dann SM, Savidge TC. Regulation of protein-ligand binding affinity by hydrogen bond pairing. *Science Advances*, 2016; 2(3). doi:https://doi.org/10.1126/sciadv.1501240
30. María J. R. Yunta. It Is Important to Compute Intramolecular Hydrogen Bonding in Drug Design? *American journal of modeling and optimization*, 2017; 5(1): 24-57. doi:https://doi.org/10.12691/ajmo-5-1-3
31. Cao D, Wang J, Zhou R, Li Y, Yu H, Hou T. ADMET Evaluation in Drug Discovery. 11. PharmacoKinetics Knowledge Base (PKKB): A Comprehensive Database of Pharmacokinetic and Toxic Properties for Drugs. *Journal of Chemical Information and Modeling*, 2012; 52(5): 1132-1137. doi:https://doi.org/10.1021/ci300112j
32. Feng T, Li M, Zhou J, et al. Application of molecular dynamics simulation in food carbohydrate research—a review. *Innovative Food Science & Emerging Technologies*, 2015; 31: 1-13. doi:https://doi.org/10.1016/j.ifset.2015.06.015
33. Hollingsworth SA, Dror RO. Molecular Dynamics Simulation for All. *Neuron*, 2018; 99(6): 1129-1143. doi:https://doi.org/10.1016/j.neuron.2018.08.011
34. Kufareva I, Abagyan R. Methods of protein structure comparison. *Methods in molecular biology (Clifton, NJ)*, 2012; 857: 231-257. doi:https://doi.org/10.1007/978-1-61779-588-6_10
35. Fatai Oladunni Balogun, Naidoo K, Jamiu Olaseni Aribisala, Pillay C, Saheed Sabiu. Cheminformatics Identification and Validation of Dipeptidyl Peptidase-IV Modulators from Shikimate Pathway-Derived Phenolic Acids towards Interventive Type-2 Diabetes Therapy. *Metabolites*, 2022; 12(10): 937-937. doi:https://doi.org/10.3390/metabo12100937
36. Md. Rimon Parves, Solares MJ, Dearnaley WJ, Kelly DF. Elucidating structural variability in p53 conformers using combinatorial refinement strategies and molecular dynamics. *Cancer Biology & Therapy*, 2023; 25(1). doi:https://doi.org/10.1080/15384047.2023.2290732
37. Paul SK, Saddam Md, Rahaman KA, Choi JG, Lee SS, Hasan M. Molecular modeling, molecular dynamics simulation, and essential dynamics analysis of grancalcin: An upregulated biomarker in experimental autoimmune encephalomyelitis mice. *Heliyon*, 2022; 8(10): e11232. doi:https://doi.org/10.1016/j.heliyon.2022.e11232
38. Bora K, Sarma M, Shankar Prasad Kanaujia, Vikash Kumar Dubey. Dual-target drugs against Leishmania donovani for potential novel therapeutics. *Scientific Reports*, 2023; 13(1). doi:https://doi.org/10.1038/s41598-023-45448-x
39. ScienceDirect.com | Science, health and medical journals, full text articles and books. www.sciencedirect.com. Accessed February 7, 2024. https://www.sciencedirect.com/science/article/am/pii/S1359644618303738
40. Singh E, Rajat Kumar Jha, Khan R, et al. A computational essential dynamics approach to investigate structural influences of ligand binding on

Papain like protease from SARS-CoV-2, 2022; 99: 107721-107721. doi:https://doi.org/10.1016/j.compbiolchem.2022.107721