

Structure-Based *in Silico* Insights of Sitagliptin Analogs against Dipeptidyl Peptidase-4

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Dipeptidyl peptidase-4 (DPP-4) inhibition has remained one of the most established ways in treating type 2 diabetes mellitus. Therefore, the development of improved DPP-4 inhibitors is considered an essential strategy in the design of antidiabetic drugs. In the present study, a structure-based virtual approach was considered to design and evaluate new sitagliptin analogues with improved binding stability and pharmacokinetic properties. A virtual library consisting of 15,034 different compounds was created and then screened using a hierarchical Glide docking protocol (HTVS, SP, and XP). Using integrated computational analyses, the top-ranked compounds were further assessed. Based on ADMET predictions, MM-GBSA binding free energy calculations, the results revealed that out of all the screened compounds, only 28 candidates had better docking scores than sitagliptin and compounds 14 and 22 were the most promising screened analogues. Compared to other tested analogues, compound 14 showed stable binding profile, low MM-GBSA binding free energy, stable ionic interactions between the catalytic residues Glu205 and Glu206, stable hydrophobic interactions within the S1 pocket. In addition, based on ADME simulation, compound 14 presented acceptable drug-like properties which indicates good oral bioavailability and lower predicted CNS penetration compared to sitagliptin. Overall, further experimental validation and development is required to evaluate compound 14 which exhibits an increased electrostatic complementarity, dynamically stable properties, and acceptable pharmacokinetic properties making it a promising candidate as a DPP-4 inhibitor.

Keywords: ADME; DPP-4 inhibitors; *In silico* design; MM-GBSA; Sitagliptin analogues.

Insulin resistance, combined with the gradual failure of β -cells, defines type 2 diabetes mellitus (T2DM), a long-term metabolic disorder that disrupts glucose balance. The increasing prevalence of age-related metabolic disorders gives rise to an urgent need for targeted therapies to promote healthy aging among older adults. Since T2DM comprises one of the most prevalent age-associated metabolic disorders worldwide, further comprehension of its molecular mechanisms and therapeutic targets is necessary to develop safer

and more effective interventions. Glucagon-like peptide-1 (GLP-1) and Glucose-dependent Insulinotropic Peptide (GIP) are two examples of the incretin hormones that are crucial for controlling blood sugar levels after meals. They help increase insulin release and decrease glucagon secretion in response to glucose.^{1,2}

The serine protease dipeptidyl peptidase-4 (DPP-4) is primarily present in immune and endothelial cells. Its primary function is that it quickly breaks down these incretins.³ As a result,

the long-term therapeutic effectiveness of GLP-1 and GIP is significantly limited due to their short lifetime. Inhibiting DPP-4 extends the action of the incretins, which in turn supports insulin release and improves blood sugar control.⁴ This principle provides a basis for using “DPP-4 inhibitors gliptins”, a well-known class of oral diabetes drugs.⁵

The first DPP-4 inhibitor to be clinically effective was sitagliptin, which was approved in 2006 and has been a crucial model for the development of more recent medications.⁶ The triazolopiperazine structure shows a strong attraction to the DPP-4 active site, interacting with the S1 and S2 binding pockets. Later generations of DPP-4 inhibitors can produce a variety of analogues because of this interaction.^{7,8} The understanding of this binding architecture has facilitated the investigation of modified chemicals necessary for better potency and selectivity. Over the past ten years, computer-based methods have become crucial tools for developing and improving DPP-4 inhibitors. Researchers can accurately predict binding affinity and assess large collections of chemicals using methods like molecular docking, structure-based virtual screening, and molecular dynamics simulations.⁹ These methods have uncovered new structures and derivatives from natural sources that show strong inhibition against DPP-4.^{10,11}

Quantitative Structure-Activity Relationship QSAR and Structure-Activity Relationship SAR modeling assist medicinal chemists in identifying significant molecular features that immensely influence both effectiveness and selectivity.¹² Recently, to enhance the prediction performance, researchers have investigated the integration of machine learning ML and artificial intelligence AI with conventional docking techniques. Such integration assists in reducing time and experimental cost by allowing automated hit prioritization and data-driven analogue generation. Neuro-symbolic and deep learning approaches have shown high accuracy in predicting active DPP-4 inhibitors, thus lowering the experimental workload.^{13,12}

The successful implementation of computer-based simulations, commonly known as *in silico* techniques, has demonstrated that it can play a pivotal role in all drug discovery

stages from hit finding to SAR optimization. Researchers can systematically and rapidly develop compounds such as sitagliptin.^{15,16} As a result, these techniques provide a fundamental strategy necessary for designing sitagliptin-derived scaffolds with enhanced pharmacodynamic and pharmacokinetic properties. However, despite the beneficial therapeutical effect of the first-generation of gliptins such as sitagliptin, significant limitations still exist. Post-marketing and clinical data have reported several rare but dangerous side effects, such as severe joint pain, acute pancreatitis, and bullous pemphigoid related to immunological mechanisms.^{17,18,19} Sitagliptin has a limited metabolism and is primarily eliminated unaltered through urine (roughly 79–87% of an oral dose). This fact restricts its utilization for patients with severe renal impairment and thereby dose adjustment is required for those who have kidney functioning problems.²⁰ Furthermore, its broad distribution may reduce tissue selectivity and lead to negative effects on the off-target regions such as the skin and musculoskeletal system.²¹

In recent years, there has been significant progress in the structural optimization of DPP-4 inhibitors through computational chemistry techniques. Various studies have shown that the application of docking and molecular dynamics methods together enhances the accuracy of DPP-4 inhibitory potency and selectivity.^{22,23} In addition, artificial intelligence-assisted screening platforms have accelerated hit identification while reducing synthetic burden. These technological advancements facilitate the transition from empirical drug development toward molecularly derived design techniques.^{24,25}

Clinical safety studies and pharmacovigilance approaches have also shown that there is a need to optimize pharmacokinetics and immunology while developing second-generation gliptins.^{26,27} All these studies together emphasize the need to design rationally active sitagliptin analogs with high binding efficiency and better peripheral selectivity, is highlighted by these safety and pharmacokinetic concerns.

The aim of the current investigation is to design and simulate new sitagliptin-based analogues using SAR-guided *in silico* techniques, particularly multistep docking, ADMET predictions, and MM-GBSA free energy calculations. The purpose of the

designed compounds is to increase tissue selectivity and minimize off-target actions. The chemical structure of the suggested compounds is illustrated in Figure 1.

MATERIALS AND METHODS

Computational Method

In this section, the computational methods that were used to design and simulate new sitagliptin-based analogues will be presented. Molecular docking studies were conducted using Schrodinger suite tools on Maestro (version 14.5.160, 2025-3; Schrodinger LLC, New York, NY, 2025), including Glide, Desmond, and Qikprop. The computing facility was provided by the University of Baghdad's College of Pharmacy's Department of Pharmaceutical Chemistry.

Molecular Docking

Ligand structures were prepared using the LigPrep module. The Protein Preparation Wizard was employed to process the crystal structures for DPP-4/CD26 (PDB ID: 2P8S), which was obtained from the Protein Data Bank.²⁸

ADME Study

To predict the drug likeness characteristics of the proposed molecules, the ADMET properties including absorption, distribution, metabolism, and excretion of the proposed ligands were evaluated using the QikProp program. Molecular weight (MW), number of hydrogen-bond donors (donorHB), number of hydrogen-bond acceptors (accprHP), predicted apparent Caco-2 cell permeability (QPPCaco), and predicted human oral absorption (oralAbs) as suggested in.²⁹

MM/GBSA Study

MM-GBSA study was performed on the complexes of the protein–ligand that were produced by molecular docking against DPP-4/CD26 (PDB 2P8S). In the investigations, the total binding free energy (ΔG_{bind} , kcal mol⁻¹) of each ligand and its decomposed energy components van der Waals (vdW), Coulombic (electrostatic), and generalized-Born solvation (GB) were computed. The calculated ΔG_{bind} provides a trustworthy internal ranking based on the balance of hydrophobic packing, electrostatics, and desolvation penalties due to the identical utilization of the receptor and grid for all ligands.³⁰

Molecular Dynamics Simulation

To assess the dynamic stability of the complexes, the top-ranked molecule from the docking studies was further investigated using the Desmond module.^{31,32} Both Root Mean Square Fluctuation (RMSF) and Root Mean Square Deviation (RMSD) values were computed of the backbone atoms in the receptor protein and the protein-ligand complex.³³

RESULTS

Molecular Docking analysis

In the present investigation, a library comped of 15034 compounds was firstly screened against DPP-4 (CD26) enzyme using high throughput virtual screening (HTVS) molecular docking. After that, these compounds were initially re-docked with Standard Precision (SP) followed by Extra Precision (XP) to optimize screening accuracy and obtain more reliable predictions. The workflow that was followed in the molecular modeling study is illustrated in Figure 2. Because of their low docking energies compared to the sitagliptin, 16 compounds obtained from XP screening were chosen for further analysis, as demonstrated in Table 1.

ADME Study

Because of the crucial role of Pharmacokinetic in predicting absorption, distribution, and metabolism in drug design, the therapeutic effectiveness of the proposed compounds was investigated. The Pharmacokinetic properties of the top-ranking molecules were predicted using the QikProp module that is available in Schrödinger Suite. The simulation data are summarized in Table 2.

The results showed that the molecular weights of all designed analogues varied between 290 and 315 Da, which are far below the Lipinski's

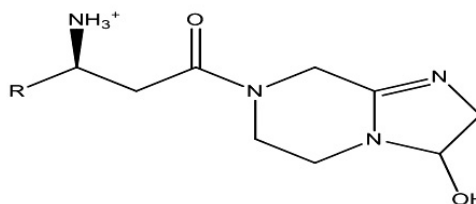


Fig. 1. The chemical structure of the proposed compounds.

Rule of Five threshold. All the compounds showed good human oral absorption and acceptable HBD and HBA counts. This indicates that the proposed compounds support Rules of Three and Five. In addition, the results showed that most of the molecules had low-medium to high QPPCaco. This indicates good intestinal permeability and supports the potential for oral delivery. Furthermore, most compounds were expected to be non-CNS active (CNS from “2 to 0), indicating low potential for central side effects.

Among analyzed derivatives, compound 14 exhibited the most equally balanced pharmacokinetic profile, with QPPCaco of 23.66 nm/s, no rule violation and with CNS = “2 moderate permeability, good oral absorption and peripheral selectivity. Similarly, compound 22 also presented a favorable absorption (QPPCaco = 35.65 nm/s) and it was in accordance with drug-likeness rules; with slight difference in its neutral CNS score (CNS = 0). Although, compound 6

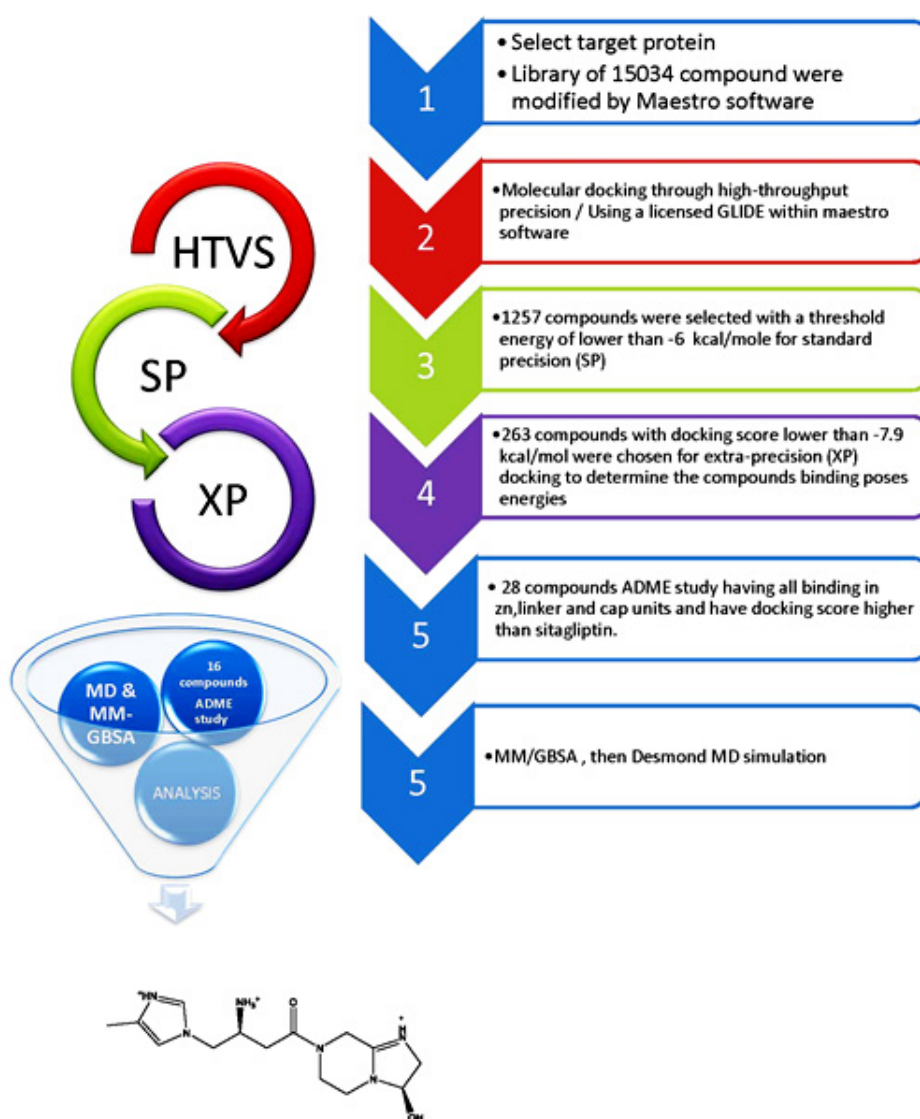
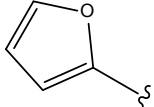
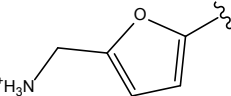
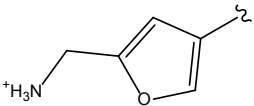
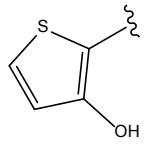
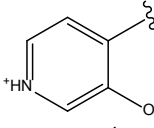
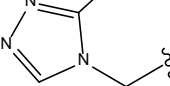
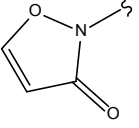
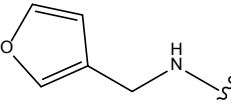
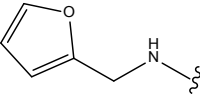
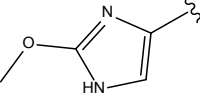
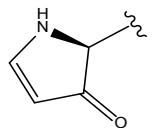
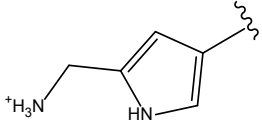
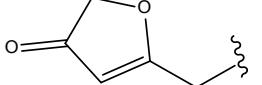
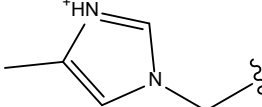
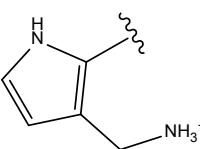
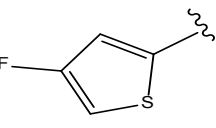
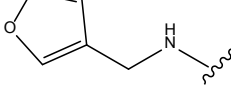
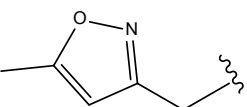
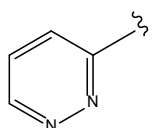
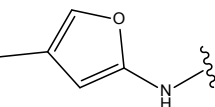
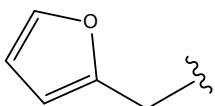
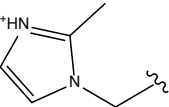
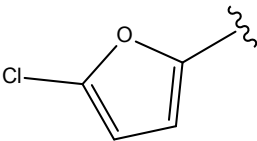
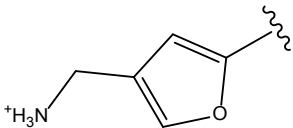
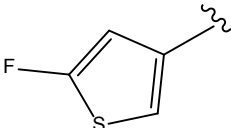
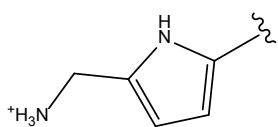
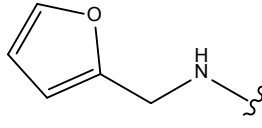
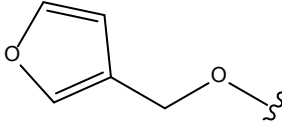


Fig. 2. The steps for molecular modeling studies.

Table 1. Docking scores of the proposed compounds on the protein (2P8S)

Compound ID	Structure	Docking score (xp) Kcal/mole (2P8S)
1		-10.642
2		-10.426
3		-10.351
4		-10.349
5		-10.281
6		-10.271
7		-10.207
8		-10.137
9		-10.089
10		-10.063
11		-9.995
12		-9.943
13		-9.925
14		-9.908
15		-9.888
16		-9.861
17		-9.843
18		-9.833
19		-9.819
20		-9.807
21		-9.775
22		-9.751

23		-9.750
24		-9.677
25		-9.672
26		-9.652
27		-9.650
28		-9.641
Reference (sitagliptin) PubChem CID 4369359		

showed a desirable profile (QPPCaco = 26.41 nm/s) and complete rule compliance in the analysis, it slightly provides more predicted metabolic routes, which might affect stability as well. On the other hand, the results showed that compound 7 showed comparable permeability (28.65 nm/s) but weaker binding kinetics, making it a less potent albeit pharmacokinetically acceptable lead. In contrast, compound 16 exhibited a higher permeability (QPPCaco = 84.81 nm/s) yet positive CNS activity which could be used as an indicator of being a potentially CNS-penetrant molecule with off-target effects. The reference drug sitagliptin (QPPCaco of 60.05 nm/s and CNS = +1) validated the high permeability of the molecule with a potential to penetrate blood–brain barrier as an observation of its systemic effects were reported. From the

ADME results one can notice that of these analogs, compounds 14 and 22 showed improved PK and safety profiles as compared to sitagliptin, which confirm their potential as better DPP-4 inhibitors. Therefore, these two compounds were chosen for further assessment using *in silico* and *in vitro* studies.

MM/GBSA Study

The MM-GBSA (Molecular Mechanics–Generalized Born Surface Area) was used to post-process the results of docking and to estimate the binding free energies for selected compounds with promising activities, i.e. Compounds 14, 22, 6, 16, 7, and reference sitagliptin. Binding free energy and its decomposition of the calculated parameters are listed in Table 3, which consists of the total binding free energy (ΔG_{bind}) and decomposed energetic terms (i.e. van der Waals, Coulombic, lipophilic intermolecular contacts and solvent contribution).

The analysis revealed that all the designed compounds showed lower binding free energies than the effective compound sitagliptin ($-50.69 \text{ kcal mol}^{-1}$), which confirmed stronger predicted affinities toward the DPP-4 active site. Of these tested compounds, compounds 14 and 22 with total binding free energy ($\Delta G = -76.48 \text{ kcal mol}^{-1}$) and ($\Delta G = -76.46 \text{ kcal mol}^{-1}$), respectively exhibited the most stable binding characterized by optimal van der Waals and electrostatic contributions while being penalized with less desolvation penalty. This interaction was also observed for compounds 6 and 16 but with larger solvation resistance, and relatively weakly in the case of compound 7. It can be said that compound 14 is the energetically most favored ligand based on MM-GBSA calculations followed closely by compound 22 and the commercial compound sitagliptin in terms of predicted binding affinity and ligand efficiency.

Molecular Dynamic Simulation Study

Molecular dynamics (MD) simulations were used to assess dynamic aspects of the ligand–target interactions. Both Sitagliptin and the most active ligand (Compound 14) were considered for MD simulations to investigate the time dependent variation in their binding affinities towards receptor. These simulations explore how the ligands bind with critical protein residues in the binding pocket and so influence ligand occupancy and biological activity.

Table 2. Pharmacokinetic properties for the top-ranking compounds.

Comp. ID	mol MW	CNS	donorHB	accptHB	Human Oral Absorption	QPPCaco	#metab	Rule of Three
1	307.35	1	2.000	7.700	2	7.475	8	2
2	307.35	-2	4.000	8.200	1	2.650	10	2
3	307.35	-2	4.000	8.200	1	3.594	10	2
4	310.37	-1	3.000	7.450	2	40.950	8	1
5	305.33	-2	3.000	8.950	2	19.141	9	2
6	307.35	-2	2.000	8.700	2	26.406	6	0
7	295.29	-2	2.000	9.700	2	28.648	5	0
8	307.35	1	2.000	7.700	2	10.227	8	2
9	307.35	1	2.000	7.700	2	10.335	8	2
10	308.33	-2	3.000	8.200	2	24.862	7	1
11	293.32	-1	3.000	10.200	2	5.958	6	1
12	306.36	-2	5.000	7.700	1	2.149	9	2
13	308.33	-2	2.000	9.450	2	18.316	7	2
14	306.36	-2	2.000	8.700	2	23.661	6	0
15	306.367	-1	5.000	7.700	2	7.596	9	2
16	312.361	1	2.000	6.700	2	84.811	7	1
17	307.352	1	2.000	7.700	2	13.421	8	2
18	307.352	0	2.000	8.200	2	50.171	7	1
19	290.324	-2	2.000	9.200	2	31.096	8	1
20	307.352	0	2.000	6.700	2	40.494	7	1
21	292.337	1	2.000	7.200	2	90.126	7	1
22	306.367	0	2.000	8.200	2	35.655	6	0
23	312.755	1	2.000	7.200	2	90.551	7	1
24	307.352	-1	4.000	8.200	1	4.633	10	2
25	312.361	1	2.000	6.700	2	87.113	7	1
26	306.367	-2	5.000	7.700	1	2.592	9	2
27	307.352	1	2.000	7.700	2	12.422	8	2
28	308.336	0	2.000	8.900	2	44.944	8	1
Sitagliptin	407.318	1	1.000	5.000	3	60.049	5	0

Table 3. MM/GBSA Related Parameters for sitagliptin, compounds 14, 22, 6, 16, and 7

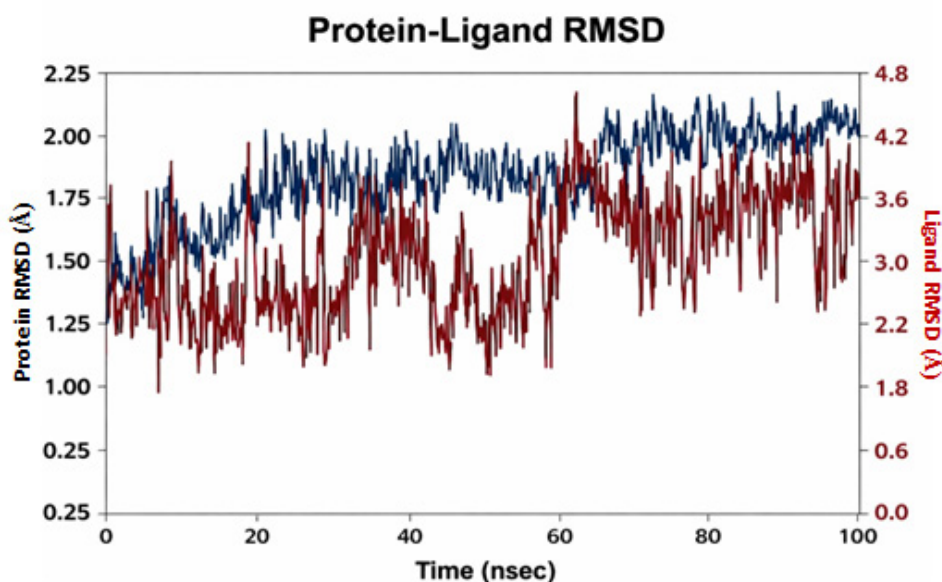
Compound	ΔG_{bind} (kcal/mol)	vdW	Coulomb	Lipo. intermolecular contacts	SolvGB	Ligand Efficiency
14	-76.48	-36.27	-66.16	-16.70	+42.76	-3.48
22	-76.46	-19.29	-93.56	-14.14	+46.23	-3.47
6	-68.39	-28.05	-101.87	-15.72	+75.76	-3.10
16	-67.20	-28.08	-82.14	-15.85	+69.80	-3.09
7	-53.95	-25.61	-91.10	-12.80	+71.99	-2.57
Sitagliptin	-50.69	-33.83	-56.07	-19.38	+62.11	-1.81

ΔG_{bind} : Binding Free Energy; vdW: van der Waals Energy; Coulomb: Electrostatic (Coulombic) Energy; SolvGB: Generalized-Born Solvation Energy; pKi (from ΔG): Negative Log of the Inhibition Constant.

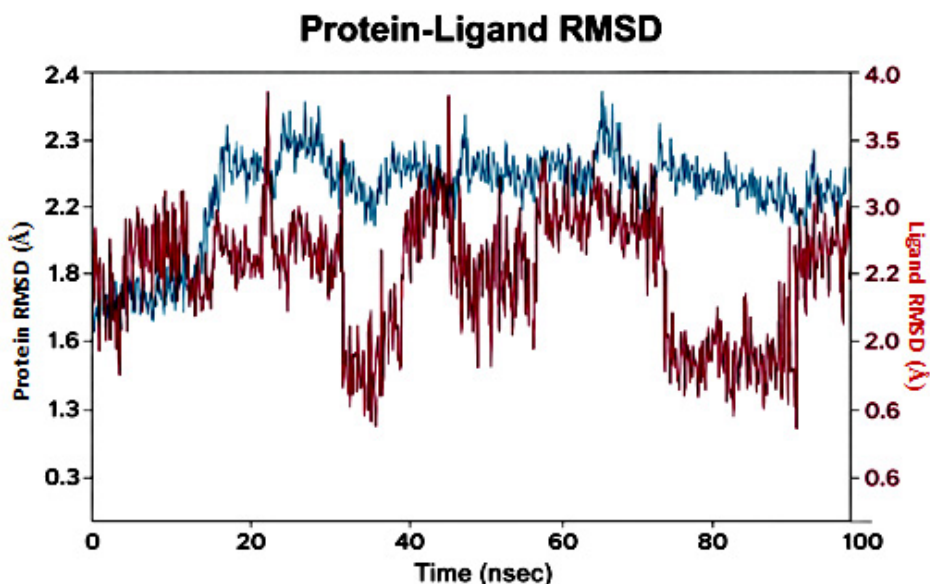
Protein-ligand RMSD value (PL-RMSD)

In this section, the protein ligand RMSD values are investigated, as can be seen in Figure 3. The complexes of Sitagliptin and compound 14 achieved equilibration over 100

ns, as evidenced by the plateau values in their RMSD plots and sustained fluctuations at 1–3 Å (structural stabilization), which indicates a well-packed globular protein. Blue and red colors stand for receptor and ligand respectively.



(a)



(b)

Fig. 3. The RMSD values for (a) compound 14 and (b) sitagliptin.

Sitagliptin protein RMSD saturated at 2.0 Å and ligand RMSD at 1.0 Å, indicating tight and stable binding, as illustrated Figure 3. On the other hand, compound 14 presented a similar profile with the protein RMSD 2.2 Å and ligand RMSD of 1.3 Å, suggesting a slight increased mobility while keeping bound tight. One can also notice that in

both tested cases, ligand RMSD was less than protein RMSD. Therefore, no ligand drifted from the pocket occurred and both complexes reached stable equilibrium. However, Sitagliptin showed slightly overall rigidity compared with compound 14.

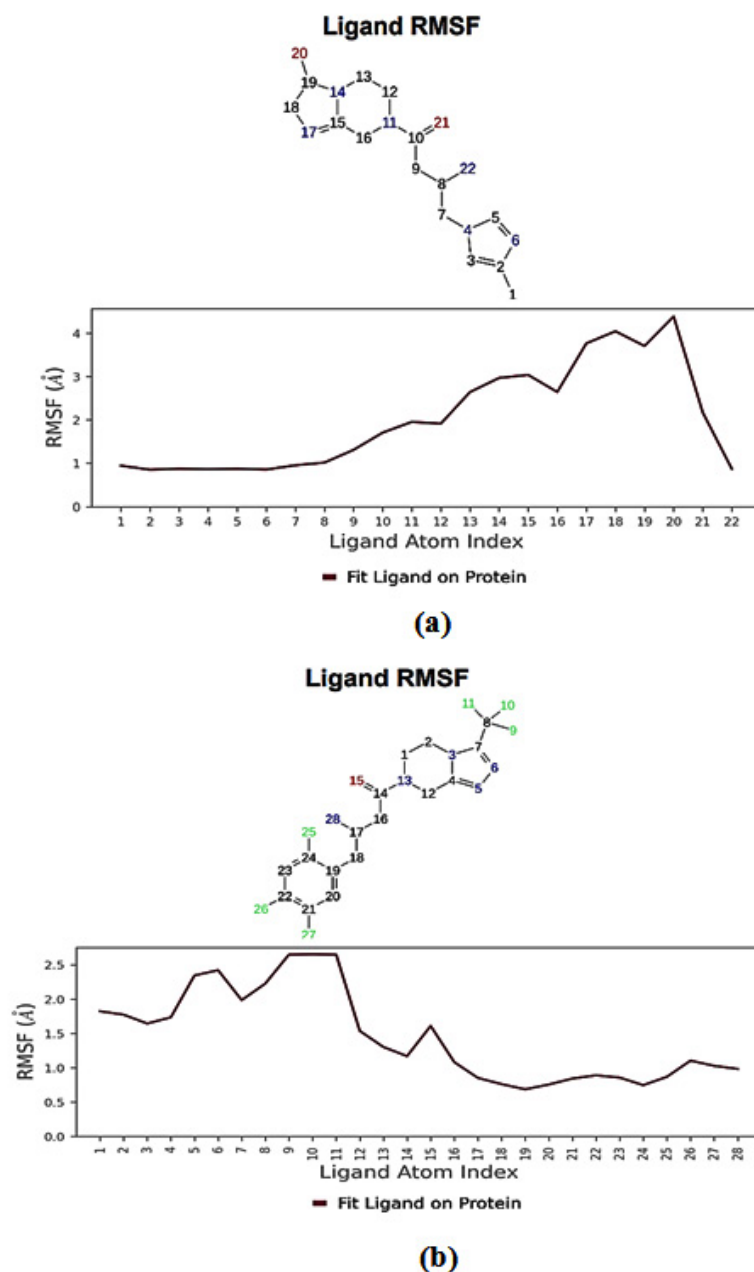


Fig. 4. The MS simulation L-RMSF value for (a) Compound 14 and (b) Sitagliptin.

Ligand RMSF (L-RMSF)

The ligand-RMSF gives detailed information about fluctuation level of the atom of ligand. This can be seen in the upper panel as it has a 2-dimensional structural illustration. The examination of L-RMSF further contributes to a clear elucidation in which ligand fragments engage in the interaction with protein and provide entropic contributions upon binding. In the present studies, a ligand predominantly showed L-RMSF values below 1.5 Å for DPP-4/CD26, suggesting solid stability inside binding sites.

In the result of the current L-RMSF analysis showed that in both ligands, the atomic displacements were low, which indicates a stable

binding in the active site. In addition, sitagliptin presented an average RMSF 0.5 Å, indicating subtle atomic motion particularly in the protonated amine and rigid bicyclic platform, and slight movement (< 1 Å) of the trifluorophenyl ring as well as side chains as shown in Figure 4. From Figure 4, compound 14 has a slightly higher RMSF (0.7 Å), which is an indication of moderately more flexible behavior. This can be attributed to the availability of multiple charged centers and rotatable bonds in compound 14. Both ligands were quite stable in the binding site, with Sitagliptin being better anchored and having lower internal reorganization than CPD-14.

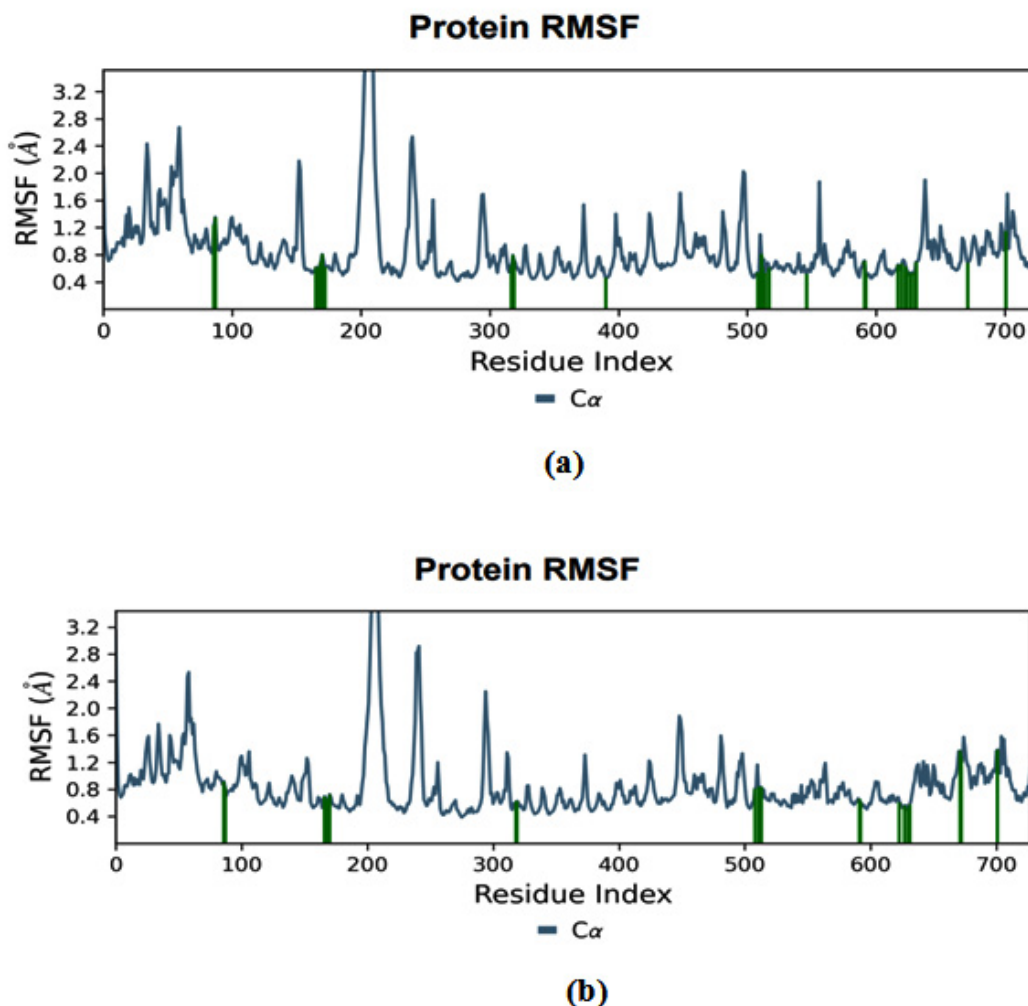


Fig. 5. The MD simulation P-RMSF value for (a) Compound 14 and (b) Sitagliptin.

Protein-RMSF (P-RMSF)

The Protein- RMSF (P-RMSF) provided results from the molecular dynamics simulation in a quantitative manner. It describes how much more rigid or flexible specific regions of the protein

(residues) are relative to other parts over time. Well-defined regions such as alpha helices and beta strands in general are less mobile than unstructured ones like loops. Both Sitagliptin and CPD-14 complexes presented very similar RMSF profiles,

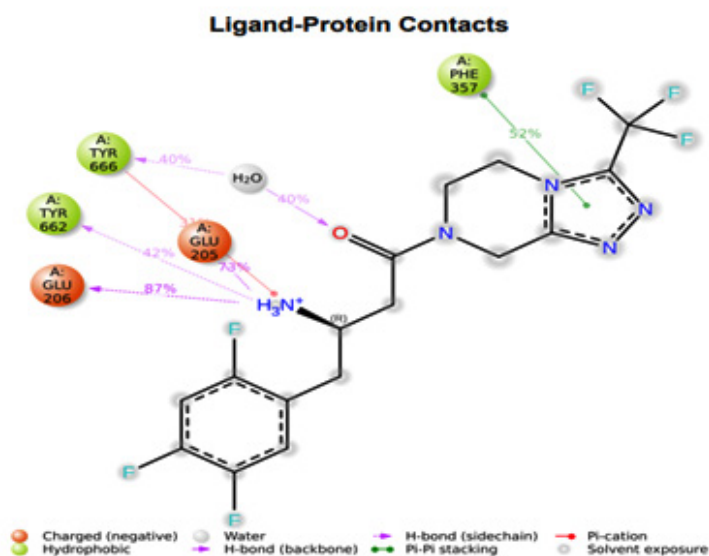
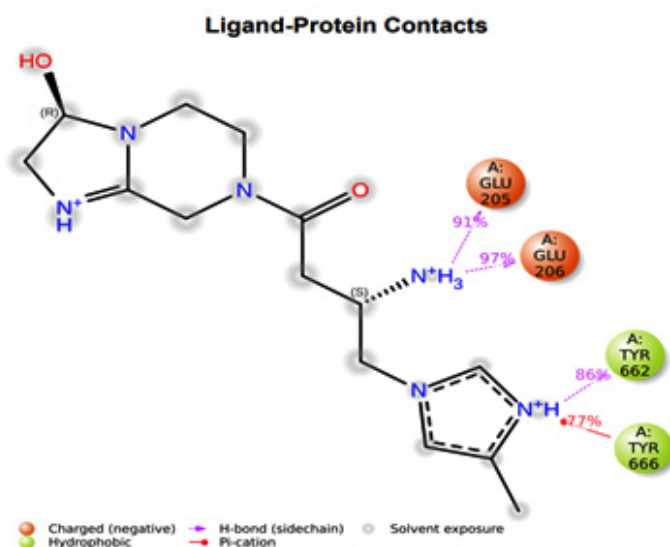


Fig. 6. The interaction probability of (a) Compound 14 and (b) Sitagliptin.

as illustrated in Figure 5. From Figure 5, it can be noticed that compound 14 has an average residue fluctuation of ca ~ 1.0 Å, which is an indication of the general stability of the protein. In addition, Low RMSF values within the α -helices and α -strands reflect relatively rigid secondary structures with higher peaks (> 1.5 Å) mainly observed at the N-terminus, C-terminus, and surface loops. Moreover, the active-site residues contacting each ligand and were consistently rigid for both complexes, indicating no disruption due to binding. The nonhomogeneous behavior is consistent with the local flexibility of compound 14 binding site. In general, it was found that both ligands were equally well bounded to the DPP-4 protein with similar stabilized DPP-4 dynamic profiles during the simulation.

Protein-Ligand Contacts

Both Sitagliptin and compound 14 share two primary interactions to the DPP-4 active site ionic anchoring via their primary amine and aromatic pocket stabilization. Primary Amine (Ionic) Interactions: The NH_3^+ group of each ligand makes strong salt bridges and hydrogen bonds with Glu205 and Glu206, the catalytic pocket's critical acidic residues. Unlike Sitagliptin, which consistently maintains these contacts, compound 14 has a stronger, higher occupancy interaction with the ionic partner, presumably due to its higher positive charge (+3) and potential for making multivalent salt bridges. This stronger ionic binding contributes to a higher proportion of simulation frames in which the compound 14 is anchored to, thus it increases the overall electrostatic stability afforded by the pocket.

Aromatic Ring Interactions: the aromatic ring of both ligands is in the active site pocket, interacting with Phe357 and Tyr662 through δ - δ interaction and hydrophobic contacts. The trifluoro phenyl ring of sitagliptin is capped by strong hydrophobic packing, whereas the compound 14 heteroaromatic ring forms a similarly stable interaction which could be reinforced by δ -cation interactions with Tyr662, as presented in Figure 6. In conclusion, both ligands have a stable binding, but compound 14 has a tighter ionic network with Glu205/206 an ionic contacts. However, Sitagliptin shows equal hydrophobic interaction to slightly favorable aromatic ring interaction stabilization.

DISCUSSION

A comprehensive structure-based computational analysis has led to identification of several proposed improved analogs of sitagliptin that will interact at the active site of DPP4. The data obtained via docking prior to any energetics/dynamics analyses is indicative of potential improved binding for several of the analogs; however, further energetic and dynamic analyses were required to identify if improvements in binding were a representative of true binding stability.

For compounds 14 and 22, binding scores consistently exceeded binding of sitagliptin through several tiers of analysis. In addition, with respect to improved scores associated with docking, MM-GBSA and free binding energies were also significantly lower for these compounds when compared to sitagliptin. Additionally, the differences in free binding energies (ΔG_{bind}) were primarily attributable to greater electrostatic contributions as well as improved van der Waals interactions between the compounds and the catalytic pocket of DPP4, thereby providing for superior complementarity within the catalytic pocket of DPP4.

The primary mechanistic result from this study was that compound 14 formed a stronger ionic binding to Glu205 and Glu206 than did sitagliptin. Both compounds form stable salt bridges with these residues. However, in contrast to sitagliptin, compound 14 exhibited increased ionic persistence through molecular dynamics simulation, supporting improved charge distribution and enhancing stability of the active region. The increased occupancy of these ionic interactions likely facilitated the greatly improved free energy profile associated with these compounds.

The role of an aromatic chemical system in hydrophobic stabilization of the S1 pocket has been shown to be significant. For example, the 4,6-di(3,5-dimethyl-phenyl)-pyrimidin-2-amine series (compound 14) was found to provide stable, consistent δ - δ (aromatic) and hydrophobic interactions with Phe357 and Tyr662 that were comparable to or greater than those found with the dipeptidyl peptidase-4 (DPP-4) inhibitor

sitagliptin. This stability was attributed to the combination of stable electrostatic anchoring and stable hydrophobic packing of compound 14.

The pharmacokinetic predictions indicate that compound 14 has the optimal balance of permeability versus peripheral selectivity relative to sitagliptin. The negative central nervous system (CNS) score of compound 14 indicates less penetration of the blood brain barrier relative to sitagliptin, and therefore implies fewer central nervous system adverse effects. In contrast, compound 16, though predicted to be highly permeable, had a predicted CNS activity that may limit its safety profile.

In contrast to optimizing compounds through structural similarity-based methodology, our design approach has been to improve dynamic stability and minimize desolvation penalties. The reduction of the solvation energy penalty associated with compound 14 confirms greater relative binding efficiency.

Overall, the results from docking studies, MM-GBSA calculations, ADMET predictions and molecular dynamics modeling provide overlapping evidence that supports enhanced electrostatic complementarity, sustained interaction stability and improved predicted pharmacokinetic selectivity of compound 14 relative to sitagliptin.

CONCLUSION

The aim of the current investigation is to design new sitagliptin-based analogues using in silico simulation techniques. These simulations included multistep docking, ADMET predictions, MM-GBSA free energy calculations and 100 ns MD simulations. A library of 15034 compounds was initially screened against DPP-4 (CD26) enzyme. The results revealed that among the screened sitagliptin analogs, compound 14 displayed the best overall profile. It showed the most favorable free binding energy and consistently formed ionic interactions with Glu205 and Glu206. In addition, there were stable hydrophobic contacts within the S1 pocket and reasonable predicted pharmacokinetics with reduced CNS penetration. On the other hand, Compound 22 showed promising characteristics as well. However, it exhibited lower energetic stability compared to Compound 14. Therefore, compound 14 can

be considered as a lead compound for further investigation in vitro and in vivo experiments to prove its potency, selectivity and safety be carried out for their biological activity.

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Conflicts of Interest

There are no conflicts of interest for the author.

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This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Author Contributions

The sole author was responsible for the conceptualization, methodology, data collection, analysis, writing, and final approval of the manuscript.

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