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Molecular Dynamics Simulations of Ethyl-4-[(α-L-rhamnosyloxy)-benzyl]carbamate from *Moringa oleifera* Lam. as a Dipeptidyl Peptidase-4 Inhibitor

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ABSTRACT

Diabetes mellitus is a global health problem that requires innovative solutions. Ethyl-4-[(α-L-rhamnosyloxy)-benzyl]carbamate (ERBC) compound contained in *Moringa oleifera* Lam. showed potential as a potent dipeptidyl peptidase-4 (DPP4) inhibitor, with an IC_{50} value of 0.798 μM. Molecular dynamics simulations indicated that ERBC interacts specifically with the active site of DPP4, providing a mechanistic basis for its inhibitory activity. The research utilized the latest technique developed by previously published plug-ins. The molecular docking simulations were performed 100 times. Then, the poses were clustered to sample the probable poses which were then subjected as the inputs in molecular dynamics simulations. Molecular dynamics simulations have shown that the ERBC compound interacts with the DPP4 protein at two possible poses. PyPLIF HIPPOS analysis demonstrated that ERBC, during its second replication, interacts with Glu205 and Glu206, two key amino acids involved in DPP4 activity.

INTRODUCTION

Type 2 diabetes mellitus is a chronic illness described as a glycemic, metabolic condition involving improperly elevated blood glucose (WHO, 2024). According to the International Diabetes Federation, the 10-year forecast is the prevalence of diabetes will grow from 10.5% in 2021 and rise to 11.3% in 2030. In 2040, it is also expected to further increase to 12.2% (Ye *et al*., 2023). In 2045, it is estimated that diabetes cases will escalate again by around 15%, totaling 700 million people in the world. Currently, there are 463 million people with diabetes aged 40-59 years and 374 million with pre-diabetes (Saeedi *et al*., 2019). Glucagon-like peptide-1 (GLP-1) and incretins are a group of hormones that can enhance insulin secretion from the pancreas and suppress glucagon secretion, a hormone antagonistic to insulin. However, both peptides are highly unstable in vivo and are quickly degraded and activated through dipeptidyl peptidase-4 (DPP4) inhibitors (Saini *et al*., 2023).

Indonesia has become the world's center of plant diversity, which has tremendous value for local communities and worldwide (Cahyaningsih *et al*., 2021). *Moringa oleifera* Lam. (MO) has been used traditionally for treating diabetes (Abdelazim *et al*., 2024). Chemical compounds contained in these plants are purported to have the potential to act as DPP4 inhibitors (Zanzabil *et al*., 2023). The existing DPP4 inhibitors exhibited slow inhibition kinetics and robust affinity and are proven to be reversible competitive inhibitors of DPP4 (Saini *et al*., 2023). In experimental animals, leaf extract from MO is known to lower blood glucose levels at a dose of 100-300 mg/kg for 2-8 weeks (Mthiyane *et al*., 2022). Furthermore, in vitro studies of ethyl-4-[(α-L-rhamnosyloxy)-benzyl]carbamate (ERBC) components in MO inhibited DPP4 activity with an IC_{50} value of

0.798 μM and vildagliptin as a positive control with an IC_{50} value of 0.528 μM. Therefore, using MO as part of diabetes therapy can become one of the treatment alternatives (Yang *et al*., 2020).

The drug discovery pipeline requires ±15 years and costs over $800 million until it reaches the commercial stage of development (Oliveira *et al*., 2023). Computational approaches that combine molecular docking and molecular dynamics are essential for predicting protein-ligand interactions, drug potency, and selectivity (Li *et al*., 2019). Protein-ligand interactions are pivotal for biological processes and signaling mechanisms (Payghan *et al*., 2018; Torres *et al*., 2019). Molecular docking contributes to the analysis of binding poses (Naqvi *et al*., 2018), prediction of atomic-level interactions (Pieroni *et al*., 2023), and an understanding of binding interactions (Kausar, 2022). Molecular dynamics simulations contributing to predicting the binding, affinity, and stability of protein-ligand interactions (Singh *et al*., 2022), movement of atomic interactions (Istyastono *et al*., 2023), atom positions change each time, protein-ligand interaction energy (Ahmed *et al*., 2022), and prediction of interaction modes (Kausar, 2022). This research also used the well-established software to identify protein-ligand interactions from protein-ligand complexes, PyPLIF HIPPOS, which successfully identified the interactions during molecular dynamics simulations (Istyastono *et al*., 2023). We utilized molecular dynamics approaches enhanced with recent techniques to quickly and accurately obtain insights into the mechanism of action at the sub-nano level.

METHODS

Materials and Instrumentations

The primary tool in this research consists of a cloud protein simulator (CPS) with specifications of CPU cores, 4 GB RAM, and 80 GB storage size—furthermore, YASARA-Structure software version 23.9.29. The materials used in this research consisted of a crystal structure model of DPP4 complex (PDB ID: 3G0B) retrieved from https://www.rcsb.org and ligand test SMILES code from ERBC compound (PubChem CID: 101942512) retrieved from https://pubchem.ncbi.nlm.nih.gov/ (accessed on June 29, 2024).

Procedures

Molecular docking procedure, molecular dynamics simulation, and identification of protein-ligand interactions followed previously published procedures (Istyastono and Gani, 2021; Istyastono *et al*., 2023). Modifying the procedure conducted in this research was based on a ligand test, specifically the ERBC compound contained in MO plants. Retrospective validation was conducted using a similar approach to the previous study (Istyastono *et al*., 2023) however, using a different active compound. The selection of ERBC compound was verified in in vitro studies compared to previously conducted research (Yang *et al*., 2020).

RESULTS AND DISCUSSION

Structure two-dimensionally ethyl-4-[(α-L-rhamnosyloxy)-benzyl]carbamate (ERBC) has an alkyl group consisting of two carbon atoms (C2H5). This benzyl is a phenyl group or benzene ring attached to one carbon atom. The 4-(rhamnosyl) indicates that in the fourth position of the benzene ring, a simple sugar called rhamnose is bound. Carbamate is a functional group consisting of carbonyl (C=O) attached to nitrogen (N), and this nitrogen is also attached to an alkyl or aryl group.

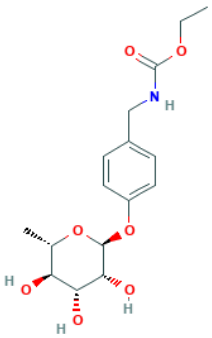

Figure 1. 2D Structure of ethyl 4-(rhamnosyloxy)benzylcarbamate (ERBC). (https://pubchem.ncbi.nlm.nih.gov/compound/101942512; accessed on June 29, 2024)

Table 1. ΔRMSD LigMove values of ERBC respectively 5 ns interval from 50 ns production run

Simulation Time (ns)	ΔRMSD LigMove (Å)		
	R1	R2	R3
0-5	3.306	2.603	3.629
5-10	2.800	3.458	4.035
10-15	3.781	0.825	1.815
15-20	1.620	1.583	4.796
20-25	0.956	2.652	4.858
25-30	0.812	2.927	5.865
30-35	0.562	2.524	12.674
35-40	0.936	0.901	11.241
40-45	1.730	1.065	11.991
45-50	0.787	2.149	8.759

Note: R1: Replication 1; R2: Replication 2; R3: Replication 3.

Before performing ERBC molecular docking simulations, redocking of the DPP4 native ligand was carried out to confirm the accuracy of the molecular docking configurations. Our findings showed that 100 redocking simulations of each protein native ligands had a Root Mean Square Deviation, (RMSD) value ≤2.000 Å, indicating that the redocking configuration was dependable and might be used for the molecular docking of the suggested chemical, or ERBC in the DPP4 active site (Waskitha *et al*., 2023). Molecular docking simulations of ERBC on the DPP4 active site showed that the best ERBC pose resulted in RMSD values ≤2.000 Å and >2.000 Å with a maximum value of 2.447 Å, which suggests that there is more than one docking pose. In addition, it was found that the best-docked ERBC was stable enough to interact with the DPP4 active site with binding energy values of 72.820 kcal/mol to 74.760 kcal/mol. It is necessary to perform molecular dynamics simulations to evaluate the molecular docking results and interaction stability between protein and ERBC molecules with the highest binding energy. This simulation aims to verify that the ERBC molecule does have the most robust and stable interaction with protein (Istyastono *et al*., 2023).

Based on the stability evaluation of molecular dynamics simulations referring to previous studies (Liu *et al*., 2017), the alogliptin-DPP4 complex has reached equilibrium after the last five ns of the total simulation time of 10 ns. This is characterized by the RMSD value of protein backbone (ΔRMSDBb) and RMSD of ligand motion (ΔRMSDLigMove), which are consistent and less than 2 Å threshold during the last five ns period of the total simulation time of 10 ns. These results indicate that the simulation has reached convergence and can be used to analyze the molecular interaction between alogliptin and DPP4. The deviation or difference between the atomic coordinates of a reference structure and the trajectory of structures acquired during an MD simulation is measured by the RMSD, which is especially helpful when evaluating how a system's or molecule's atom positions evolve throughout the simulation (Windah and Istyastono, 2024).

The stability of ERBC compounds on the DPP4 active site was evaluated by analyzing the RMSD of protein backbone atoms and ligand movement. Based on the previously conducted docking analysis, two main groups of poses were identified, and the pose with the best docking score was selected as the starting point of the molecular dynamic simulation. Simulation for 50 ns production run with the final result shows that the ΔRMSDBb value remains stable throughout the simulation. However, the ΔRMSDLigMove value of ERBC never reached below 2.000 Å, indicating that ERBC interacts unstably at the DPP4 active site.

Simulation analysis showed that in the third replication, the ligand started to leave the binding site after the simulation time reached 35.7 nanoseconds, as indicated by the increase in RMSDLigMove and the loss of ligand visualization in Figure 2. This behavior aligns with the docking results, which showed that the third replication had the worst docking score compared to the other two replications. Thus, the

first and second replicates, with better docking scores, can be considered more probable in describing the interaction between ligand and protein. The third replicate showed that the ligand could not maintain its position in the protein binding pocket, thus dissociating from the complex. This ligand dissociation event contradicts the results of in vitro experiments that show an interaction between the protein and ligand. The failure of the docking method in predicting this stable interaction suggests that the computational model used needs to be improved or the simulation parameters need to be adjusted to obtain more accurate results with experimental data. However, to overcome these problems, an analysis was performed every five ns interval at the beginning of the simulation when the ERBC ligand reached its most stable position (Istyastono and Prasasty, 2020). The analysis was performed on the part where the ΔRMSDLigMove value was less than 2.000 Å. This ensures that the ligand has not moved much and is considered relatively fixed, so it is likely already stable in the protein-ligand complex.

Based on the analysis of Table 1, the first replicate showed the most prolonged stability of the ERBC ligand, which was from 15 ns to 50 ns or 35 ns. The second and third replicates each had shorter stability periods. These findings support the conclusion that the docking method successfully identified three different stable poses for the ERBC ligand in the protein-ligand complex.

As reported by previous research, the active site of DPP4 consists of Tyr666, Tyr662, Tyr631, Tyr547, Phe357, Glu205, Glu206, and Arg125 (Istyastono *et al*., 2023). Tables 2 and 3 tabulate the amino acid residues that interact with ERBC for each snapshot generated from PyPLIF HIPPOS. Based on Table 2 and Table 3, the results of the second replication are fascinating. The ERBC molecule is still firmly bound to two specific amino acids, namely Glu205 and Glu206. The targeted DPP4 protein has a relatively sizeable binding pocket. Within this pocket, there

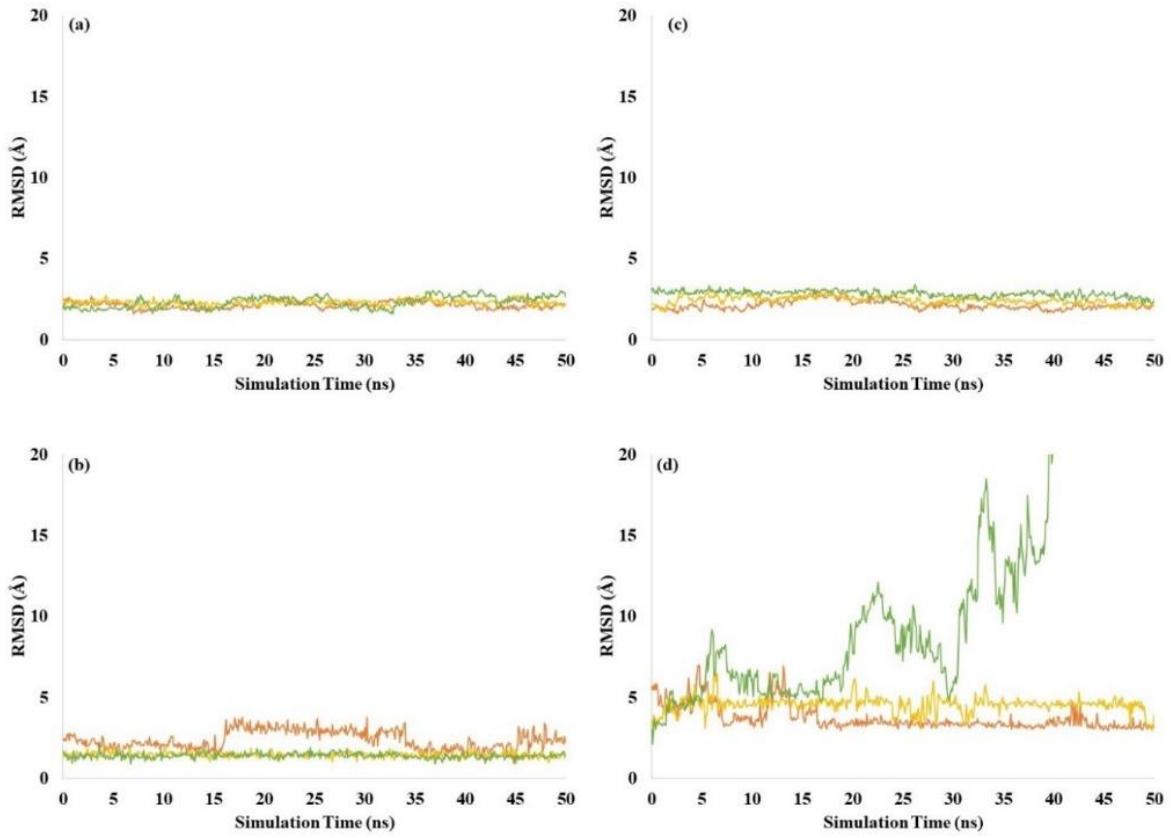

Figure 2. Transformation of RMSD of DPP4 backbone atoms complexed with ERBC and RMSD of ERBC ligand movement during 50-ns simulation. (a) RMSDBb of Alogliptin, (b) RMSD LigMove of Alogliptin, (c) RMSDBb of ERBC, (d) RMSD LigMove of ERBC. Replication 1 (Red), Replication 2 (Orange), Replication 3 (Green).

are several smaller pockets or sub-pockets. The amino acid residues Glu206 and Glu205 are critical because they are located in the binding site and the catalytic site of the DPP4 protein. These two amino acids are essential to the protein's enzymatic activity. The table shows that during simulations, ERBC interacts non-hydrophobically with amino acid residues Tyr547 (100%), Asp663 and Glu205 (100%), and Arg560 (82%), respectively, in each replicate predominantly. The study also found that the amino acid Tyr547 formed hydrophobic interactions predominantly across the three replicates.

Docking and molecular dynamics (MD) analysis showed that the DPP4 enzyme has several binding sites. Based on the results of the simulations, the ERBC molecule can bind to two mains, i.e., replication one and replication two. Although possible, replication three tends to be less stable and is not the main focus of this interaction. The binding energy calculation results show the relatively weak interaction between ERBC and DPP4 in the most stable dynamics duration. This weak interaction is reinforced by the MD simulation results, which show that the ERBC molecule detaches from the DPP4 enzyme binding site at the 35.7 nanosecond simulation time. This indicates that in the third replication identified pose by docking simulations, the bond between ERBC and DPP4 is transient and not strong enough to maintain a stable complex for an extended period.

Table 2. Hydrophobic interactions hotspots resulted from 10-15 ns molecular dynamics simulations of DPP4 interacting with ERBC

Interacting Residue	Interaction Type	Interaction Percentage		
		Replication 1	Replication 2	Replication 3
Trp201	Hydrophobic	-	2%	-
Glu205	Hydrophobic	-	100%	-
Glu206	Hydrophobic	-	72%	-
Phe357	Hydrophobic	34%	2%	-
Tyr547	Hydrophobic	100%	100%	98%
Pro550	Hydrophobic	94%	2%	-
Cys551	Hydrophobic	26%	28%	-
Gln553	Hydrophobic	-	18%	-
Lys554	Hydrophobic	30%	-	-
Arg560	Hydrophobic	30%	-	-
Tyr585	Hydrophobic	-	6%	-
Tyr631	Hydrophobic	82%	-	-
Tyr662	Hydrophobic	-	100%	-
Asp663	Hydrophobic	-	60%	-
Val665	Hydrophobic	-	2%	-
Tyr666	Hydrophobic	44%	80%	-
Tyr670	Hydrophobic	4%	-	-
Asn710	Hydrophobic	-	8%	-
His740	Hydrophobic	-	-	2%

Table 3. Non-hydrophobic interactions hotspots resulted from 10-15 ns molecular dynamics simulations of DPP4 interacting with ERBC

Interacting Residue	Interaction Type	Interaction Percentage		
		Replication 1	Replication 2	Replication 3
Arg125	Ionic as the cation	72%	-	-
Arg125	H-bond donor	-	14%	-
Glu205	Ionic as the anion	-	100%	-
Glu205	H-bond acceptor	-	2%	-
Glu206	Ionic as the anion	-	28%	-
Tyr547	Aromatic edge-to-face	100%	-	52%
Tyr547	Aromatic face-to-face	-	-	46%
Tyr547	H-bond acceptor	24%	-	-
Tyr547	Aromatic face-to-face	26%	-	-
Arg550	Ionic as the cation	44%	-	-
Lys554	Ionic as the cation	36%	-	-
Asp556	Ionic as the anion	2%	-	2%
Arg560	Ionic as the cation	-	-	82%
Ser630	H-bond acceptor	10%	-	-
Tyr631	Aromatic edge-to-face	36%	-	-
Asp663	Ionic as the anion	-	100%	-
Tyr666	Aromatic face-to-face	28%	-	-
Tyr666	Aromatic edge-to-face	-	6%	-
Tyr666	Aromatic edge-to-face	20%	24%	-

CONCLUSIONS

Molecular dynamics (MD) simulation is a computationally effective method to investigate the stability of ethyl-4-[(α-L-rhamnosyloxy)-benzyl]carbamate (ERBC) as a potent dipeptidyl peptidase-4 (DPP4) inhibitor. From the docking and molecular dynamics (MD) simulations results, it is known that ERBC binds to two possible poses, namely in replication one and replication two. Through PyPLIF HIPPOS, this study shows that ERBC in replication two can bind to Glu205 and Glu206, an essential amino acid in the DPP4 binding site. These findings provide further insights for researchers to establish appropriate MD protocols to determine the active poses of bioactive natural products, specifically, in this case, the active compound under study, ethyl-4-[(α-L-rhamnosyloxy)-benzyl]carbamate or ERBC.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

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