IN SILICO PREDICTION OF NOVEL BINDING INTERACTION OF CEBULACTAM A1 WITH *AEDES AEGYPTI* ARYLALKYLAMINE N-ACYLTRANSFERASE

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(Received: May 9, 2024; Accepted: September 12, 2024)

ABSTRACT

Unprecedented outbreaks of dengue transmitted by the mosquito *Aedes aegypti* have occurred recently in Southeast Asia. Insecticides remain the most effective control approach for the *Aedes* mosquito; however, there is an increasing prevalence of insecticide resistance in these countries. To preserve their usefulness in mosquito vector control, new modes of action of insecticides need to be studied. In this study, the crystal structure of *Ae. aegypti* arylalkylamine N-acyltransferase (aaNAT) was utilized for the molecular docking of selected polyene macrolactams retrieved from the Natural Products Atlas database. Molecular dynamics simulations and free energy calculations confirmed that cebulactam A1 binds strongly to *Ae. aegypti* aaNAT. The hotspot amino acid residues His55, Phe109, and Leu112 were found to be essential for the strong binding of cebulactam A1 to the receptor binding site. Taken together, the results of this study revealed, for the first time, a novel binding mechanism that will drive further investigation to design cebulactam A1 as a potential inhibitor of aaNAT for dengue mosquito control. This study was conducted from October 2022 to February 2023 at the Microbial Insecticides Laboratory, BIOTECH UP Los Baños.

Key words: insecticidal activity, molecular docking**,** molecular dynamics, polyene macrolactams

INTRODUCTION

Aedes aegypti (Diptera: Culicidae) is a dangerous insect vector for dengue in many parts of the world (Aliaga-Samanez et al. 2021). Dengue is a human viral disease that often leads to hemorrhagic fever in serious cases (Leowattana et al. 2021). The associated losses in economic productivity and human suffering due to this disease are considerable (Edillo et al. 2015; Onuh et al. 2016). Recently, unprecedented dengue outbreaks have been reported in many countries in Southeast Asia (Yek et al. 2021). Insecticides remain the most effective control approach for *Aedes* mosquitoes (Gan et al. 2021). The increasing prevalence of insecticide resistance in these countries should be addressed to preserve its usefulness as an important mosquito vector control tool (Hassan et al. 2021; Zulfa et al. 2022).

Expanding further on the rising cases of insecticide resistance in *Aedes* mosquito, novel molecular targets could be identified for development of new mosquitocidal compounds to replace current commercial insecticides that are no longer effective. For instance, insect arylalkylamine N-acetyltransferase (iaaNAT) is a promising target for the development of novel insecticides. It is responsible for the

regulation of essential physiological functions such as cuticle morphology and neural signaling. Insect cuticle is an extracellular substance secreted by the epidermis. It is mostly comprised of proteins and the carbohydrate chitin which covers the entire insect body surface. The cuticle therefore shapes the exterior morphology of insects which protects insects from harm, facilitates mobility, and serves as an interaction interface with the environment (Tajiri 2017). On the other hand, insect neural signaling involves a network of specialized cells called neurons that serve as an "information highway" within the insect's body (Robertson et al. 2020). These neurons generate electrical impulses (known as action potentials) that travel as waves of depolarization along the cell membrane (Spong et al. 2016). Inactivation of iaaNAT would result in compromised insect survival, resulting not only from disrupted neural signaling but also from the severe consequences of impaired cuticle development (O'Flynn et al. 2018). Compared to mammals, insects are particularly susceptible to iaaNAT inactivation because they do not possess the monoamine oxidase enzyme that inactivates arylalkylamines in mammals (Ganguly et al. 2002). To date, the Insecticide Resistance Action Committee (IRAC) mode of action classification has defined 32 classes of insecticides (Sparks et al. 2020). The classification scheme does not include iaaNAT inhibitors because this mode of action has not been utilized to develop any insecticide.

Microbial natural products that could potentially be used to target iAANAT has not been studied. For instance, polyene macrolactams (PMLs) are an underexplored group of natural products that have only been found in actinobacteria (Skellam et al. 2013). PMLs are a fascinating class of natural products characterized by a 16–34-membered lactam ring that bears two isolated/separated polyene fragments. These polyene fragments often undergo intramolecular cyclization, resulting in complex polycyclic scaffolds (Zhao et al. 2022). However, the insecticidal activity of these natural products has not been investigated. Thus, they could potentially provide a new mode of action insecticide for *Aedes* mosquito control.

The traditional high-throughput screening for bioactivity of vast libraries of small molecules including natural products presents many experimental problems (Adedeji et al. 2022; Lynch et al. 2024). Responding to these challenges, *in silico* insecticide design has been adopted by agrichemical industries as an effective tool to discover and develop novel insecticides (Speck-Planche et al. 2012). *In silico* methods include molecular docking and molecular dynamics simulations which offer faster and cheaper way to screen for potential inhibitors of a target protein receptor (Sohraby et al. 2019). Molecular docking is an established computational method to predict the preferred orientation of a small molecule when it binds to a receptor (usually a protein) to form a stable complex (Pinzi and Rastelli 2019). Molecular docking is also accepted as a first-tier method for the discovery of bioactive molecules from natural product databases (Ma et al. 2011). However, in view of the limitations inherent in molecular docking (Saikia and Bordoloi 2019), it is strongly recommended to validate the docking results using molecular dynamics (MD) simulations. MD simulation is a computer-based method for studying the dynamic behavior of atoms and molecules (Schlick 2010). It allows the exploration of molecular motion on an atomic scale, providing a virtual laboratory for understanding complex systems (Badar et al. 2022). In this study, molecular docking and molecular dynamics simulations (MDS) were used to identify polyene macrolactam as a potential inhibitor of *Ae. aegypti* aaNAT.

MATERIALS AND METHODS

This study was conducted from October 2022 to February 2023 at the Microbial Insecticides Laboratory, BIOTECH UP Los Baños.

Data preparation. The 3D structure of the receptor protein from *Ae. aegypti's* arylalkylamine N-acyltransferase, known as PDB code 4FD5, was obtained from the Protein Data Bank. This structure was detailed enough to show a resolution of 1.64 Å. The receptor protein was made up of 222 amino acids and weighed 26.43 kDa (https://www.rcsb.org). To prepare the receptor for further analysis, ions and water molecules were stripped away from the file. After this, the receptor's 3D structure was

uploaded to the PREFMD server for additional refinement. PREFMD is a method for refining protein structures using molecular dynamics (Heo and Feig 2018). Additionally, the position of the allosteric site within the receptor was estimated using the AlloSite webserver (Song et al. 2017). An allosteric site, also known as a regulatory site, is a distinct location on an enzyme or receptor where effector molecules can bind. Unlike the active site, which directly interacts with substrates, the allosteric site induces a conformational change in the protein (Guarnera and Berezovsky 2016). This alteration can either enhance or inhibit the enzyme's function. Thus, the allosteric site plays a crucial role in insecticide design by producing unique mode of action and in broadening the range of insecticide target (Samurkas et al. 2020).

Ten polyene macrolactam compounds were retrieved as sdf files from Cluster 878 of the Natural Products Atlas database. In addition, cebulactam A1 was retrieved as an sdf file from Cluster 1565 of the same database. The sdf is a widely used industry standard for representing chemical structures. It's a text-based file format that adheres to a strict structure for storing multiple chemical records along with associated data fields. A sdf file include**s** information about atoms, bonds, coordinates, charges, and other properties (Richard et al. 2002). The Natural Products Atlas is a database of microbially derived natural products (van Santen et al. 2019). Information on the molecular size and source of each compound, referred to as the ligand, is presented in Table 1. Afterward, all the ligand sdf files were converted to the pdb file format and finally geometry-optimized with Avogadro V.1.2.0 software (Hanwell et al. 2012). The protein databank (pdb) file format is primarily used for protein and macromolecule structures. It contains information about the 3D coordinates of atoms, bonds, secondary structures, ligands, and solvent molecules (Abriata 2017). PDB files are essential for studying protein structures, docking simulations, and drug design. Thus, for the first phase of the study, the 3D structures of both the receptor and ligands were used for virtual screening on a MacBook Pro computer with four Intel core i5 CPUs running at 2.3 GHz.

Table 1. Information on the screening compounds used for molecular docking.

Compound ID	Common name	Mol Wt (Da)	Origin*
NPA 023874	Dracolactam B	485.621	Unknown bacterium
NPA 024588	FW05328-1	483.649	*Micromonospora* sp.
NPA 001511	Lobosamide C	467.650	*Micromonospora* sp.
NPA 020343	Micromonolactam	469.622	*Micromonospora* sp.
NPA 018089	Mirilactam A	455.595	*Actinosynnema mirum*
NPA 017261	Mirilactam B	439.596	*Actinosynnema mirum*
NPA 028031	Mirilactam D	471.594	*Actinosynnema mirum*
NPA 028032	Mirilactam E	471.594	*Actinosynnema mirum*
NPA 026151	Pretilactam	437.580	*Actinosynnema pretiosum*
NPA 014064	Salinilactam	469.622	*Salinispora tropica*
NPA 008754	Cebulactam A1	345.395	*Saccharopolyspora cebuensis*

*Reference: Natural Products Atlas, https://npatlas.org

The second phase of the study involved MDS, wherein trajectories generated by simulation of unbound and bound receptors were analyzed to determine the stability of ligand binding. A total of 20,000 frames for each trajectory were generated from the MDS of unbound and bound receptors. The MDS

of each complex was performed in a high-performance computing cluster containing 3,168 CPU cores hosted at the Advanced Science and Technology Institute (ASTI) of the Department of Science and Technology (DOST), Diliman, Philippines.

Virtual ligand screening. Structure-based ligand-receptor docking was implemented using AMDock software to determine ligand binding poses, inhibition constant (K_i), and ligand efficiency. The software has built-in external programs such as Open Babel, PDB2PQR, AutoLigand, and ADT scripts to prepare input molecular structures and define the docking search space in the target receptor (Valdes-Tresanco et al. 2020). Blind docking was used to scan the entire receptor surface to identify possible allosteric sites (Adams et al. 2021). The top-scoring ligand-receptor complex, with at least a ligand efficiency score greater than −0.30 was further subjected to molecular dynamics simulation to determine binding stability. The highest scoring binding positions for each NP in the active site of *Ae. aegypti* aaNAT were visualized using LigPlot+ V.2.2.4 (Wallace et al. 1995) and the UCSF Chimera V.1.14 software (Pettersen et al. 2004).

Molecular dynamics simulation (MDS). Each of the top-scoring bound complex from the molecular docking study was selected as the starting structure for MDS using the GROMACS version 2020.4 software (Abraham et al. 2015). GROMACS which stands for Groningen Machine for Chemical simulation is a free and open source software suite designed for high-performance MDS (Van der Spoel *et al*. 2005). It allows researchers to simulate the Newtonian equations of motion for systems containing hundreds to millions of particles, including proteins, lipids, and nucleic acid (https://gromacs.org). The preparation of ligand and protein topology files, system solvation, energy minimization, equilibration, and production runs were performed as described previously (Vanommeslaeghe et al. 2010). A production run of 200 ns was performed in triplicate for each of the unbound and bound complexes. The only difference between replicates was in the initial velocity assignments at the start of the simulation. The initial velocity determines the trajectory of a simulation run, so judging the stability of ligand binding in only one simulation is not advisable (Ng et al. 2014).

Analysis of trajectory files. Trajectory files produced by the MDS were analyzed using built-in GROMACS utilities. A trajectory file produced by MDS contains the coordinates of all atoms in a system over time. These files capture the dynamic motion of molecules as they evolve during the simulation. Prior to the analysis, the periodic boundary condition was removed from the system using the gmx trjconv tool. The PBC is a commonly used MDS technique to mimic an infinite system while simulating a finite portion of it (Tuckerman 2010). Afterward, the structural stability was analyzed using the gms rms command. The radius of gyration was calculated using gmx gyrate to determine the compactness of the system throughout the simulation. The number of hydrogen bonds (H-bonds) between the receptor and the ligand was calculated using gmx hbond. Data were plotted using xmgrace software version 5.1.25 (https://scicomp.ethz.ch). Five hundred frames taken from 1 ns to 200 ns of MD simulation of the bound complex were utilized for gmx_MMPBSA analysis to estimate the free energy of binding (ΔG) as a measure of binding stability and to decompose the energy term to individual hotspot residues in the receptor-binding pocket (Valdes-Tresanco et al. 2021). gmx_MMPBSA software is a new tool designed to perform end-state free energy calculations with GROMACS files. End-state free energy calculations are essential in understanding molecular interactions, protein-ligand binding, and conformational changes (Valdes-Tresanco et al. 2021).

Statistical analysis. The average root mean square deviation (RMSD) and percentage H-bond occupancy of the trajectory from each MDS run were calculated using Visual Molecular Dynamics version 1.9.3 software (Humphrey et al. 1996). Spearman rank correlation analysis was conducted to determine significant differences in RMSD between unbound and bound ligand-receptor structures.

RESULTS AND DISCUSSION

Identification of lead compound through structure-based virtual screening. In the field of insecticide discovery, a lead compound serves as the initial step for developing effective insecticides. This study conducted blind docking experiments on a subset of polyene macrolactams from the Natural Products Atlas database, and the results are summarized in Table 2. The focus was on ligand efficiency (LE) scores, which helped prioritize candidate ligands for further analysis. Among the compounds studied, two potential lead candidates stood out: cebulactam A1 and mirilactam A. Both compounds exhibited LE scores greater than −0.3, indicating favorable interactions with the target receptor (Valdes-Tresanco et al. 2020). Interestingly, there is no prior record of any bioactivity associated with cebulactam A1 (Berneaud-Kotz 2021). However, this study revealed a novel finding: cebulactam A1 binds favorably to the target receptor in the fall armyworm (FAW), suggesting insecticidal activity. Comparing closely related compounds, mirilactam A lacks antitrypanosomal activity due to its distinct methylation pattern (Schulze et al. 2015). Nevertheless, this structural deviation may have conferred novel insecticidal properties to mirilactam A. Notably, both cebulactam A1 and mirilactam A exhibited higher ligand efficiency values than other promising small molecule inhibitors of protein-protein interactions (Gowthaman et al. 2016).

Table 2. Summary results of blind docking natural products retrieved from the Natural Products Atlas database to dengue mosquito *Aedes aegypti* arylalkylamine N-acetyltransferase.

Natural Product	Binding Affinity (kcal/mol)	Estimated K_i	K_i units	Ligand Efficiency
Dracolactam	-8.7	419.63	nM	-0.25
FW05328-1	-8.2	975.81	nM	-0.23
Lobosamide C	-6.7	12.27	μM	-0.20
Micromonolactam	-10.2	33.37	nM	-0.30
Mirilactam A	-10.8	12.12	nM	-0.33
Mirilactam B	-8.0	1.37	μM	-0.25
Mirilactam D	-9.1	213.63	nM	-0.27
Mirilactam E	-8.8	354.46	nM	-0.26
Pretilactam	-8.4	696.25	nM	-0.26
Salinilactam	-7.3	4.46	μM	-0.21
Cebulactam A1	-7.9	1.62	μM	-0.32

Description of binding site interactions. This study explored how cebulactam A1 interacts with specific amino acid residues in a predicted binding site. The key findings are as follows: first, Figure 1 illustrates the binding interactions of cebulactam A1. The top-scoring binding pose involves hydrophobic contacts with 11 amino acid residues (Leu52, His55, Phe105, Ile108, Phe109, Leu112, Tyr113, Asn116, Glu132, Arg134, and Ile135). All these residues are located within the predicted allosteric binding site. The presence of an aromatic ring in cebulactam A1 (Dong et al. 2015) hints at the potential for π-cation interactions. Specifically, it could interact with a cationic amino acid residue (Arg) within the allosteric site (Infield et al. 2021). In another study, an allosteric site arginine was found to be

involved in π-cation interaction with a ligand inhibitor (Panday et al. 2023). Secondly, π-cation interactions play a crucial role in maintaining the overall stability of receptor structures (Infield et al. 2021). They also contribute to molecular recognition (Liang and Li 2018). Leveraging these interactions could help design more effective enzyme inhibitors, especially in terms of targeting specific insects and managing insecticide resistance. Third, it can be speculated that cebulactam A1's allosteric binding might disrupt the binding of acetyl coenzyme A. This disruption could lead to the inhibition of aaNAT (arylalkylamine N-acetyltransferase) activity in the mosquito species *Ae. aegypti*. Previous research has highlighted the importance of acetyl coenzyme A in regulating enzyme conformation and substrate binding (Wu et al. 2020). Lastly and interestingly, there is a contrasting binding mechanism of other ligands binding to the active site of *Ae. aegypti* aaNAT primarily relying on electrostatic interactions (Lourenco et al. 2015).

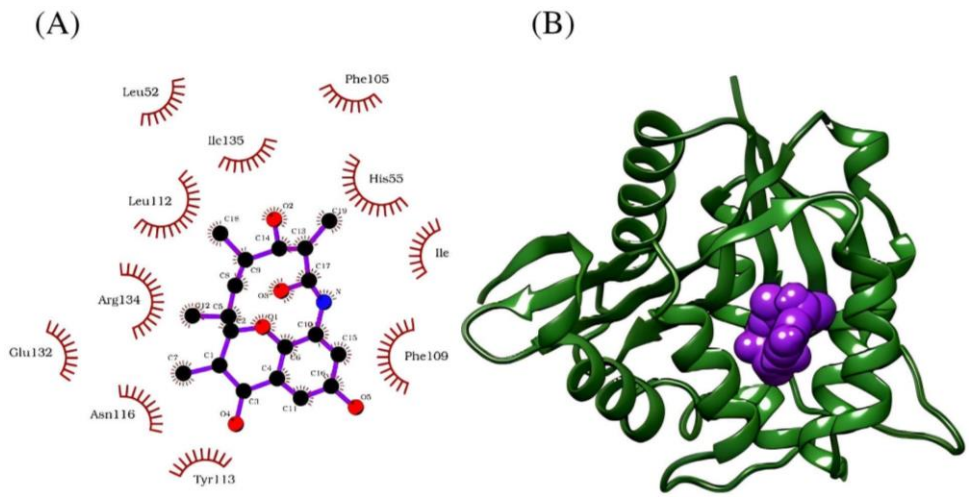

Figure 1. Receptor-ligand interaction analysis. A) 2D representation of cebulactam A1-*Aedes aegypti* aaNAT docked complex structure showing hydrophobic interactions as red arcs. B) 3D representation of cebulactam A1-*Aedes aegypti* aaNAT docked complex structure. The 2D- and 3D representations of docked complex structures were generated through LigPlus ver. 2.2.5 and UCSF Chimera software ver. 1.14, respectively.

Molecular dynamics for evaluating structural stability. MDS captures essential processes within biomolecules, such as how they bind to other molecules (like a lock and key) (Karplus and McCammon 2002). It also predicts how these biomolecules react when we add or remove something (like a ligand) (Hollingsworth and Dror 2018). In this study, MDS was used to double-check the binding stability of two compounds, mirilactam A and cebulactam A1, with a mosquito enzyme called *Ae. aegypti* aaNAT. The MDS results showed that mirilactam A binds weakly to the target receptor (−1.10 kcal/mol). This weak interaction was due to something called EDISPER (a fancy term for how non-polar interactions affect solvation energy). Since the binding was not strong, this study did not dive deeper into the MDS trajectory for mirilactam A. Previous research suggested that ligands with affinity values below −2.5 kcal/mol are weak binders (Araujo et al. 2020). The findings of this study highlight the importance of using accurate methods like MDS to evaluate ligand interactions. It helps separate real hits from false positives obtained during molecular docking studies (Sohraby et al. 2019).

Root mean square deviation (RMSD). This study wanted to understand how stable cebulactam A1 is when it interacts with *Ae. aegypti* aaNAT. The binding stability was monitored by using a tool called GROMACS gmx rms to track the stability of cebulactam A1 during a simulation. RMSD measures how much a group of atoms deviates from its initial shape. High RMSD values indicate significant

instability, suggesting changes in the molecule's shape. If a ligand (like cebulactam A1) has a high RMSD value, it might not fit well in the binding pocket during the simulation (Liu et al. 2017). Figure 2, specifically panels A1 to A3, shows how stable the structure was. Looking at values for both the ligand-receptor complex and the unbound receptor, the fluctuations in RMSD were below 2.5 Å (that's less than 0.25 nm). This level of variation is acceptable for stable interactions between ligands and proteins. The average RMSD for the unbound receptor ranged from 0.1154 nm to 0.1259 nm. For the bound receptor, the average RMSD ranged from 0.1225 to 0.1560 nm. Interestingly, there was no significant difference (p=0.76) between the RMSD values of unbound and bound receptor structures. In simpler terms, cebulactam A1 seems to fit well and stay stable within the mosquito enzyme's binding site.

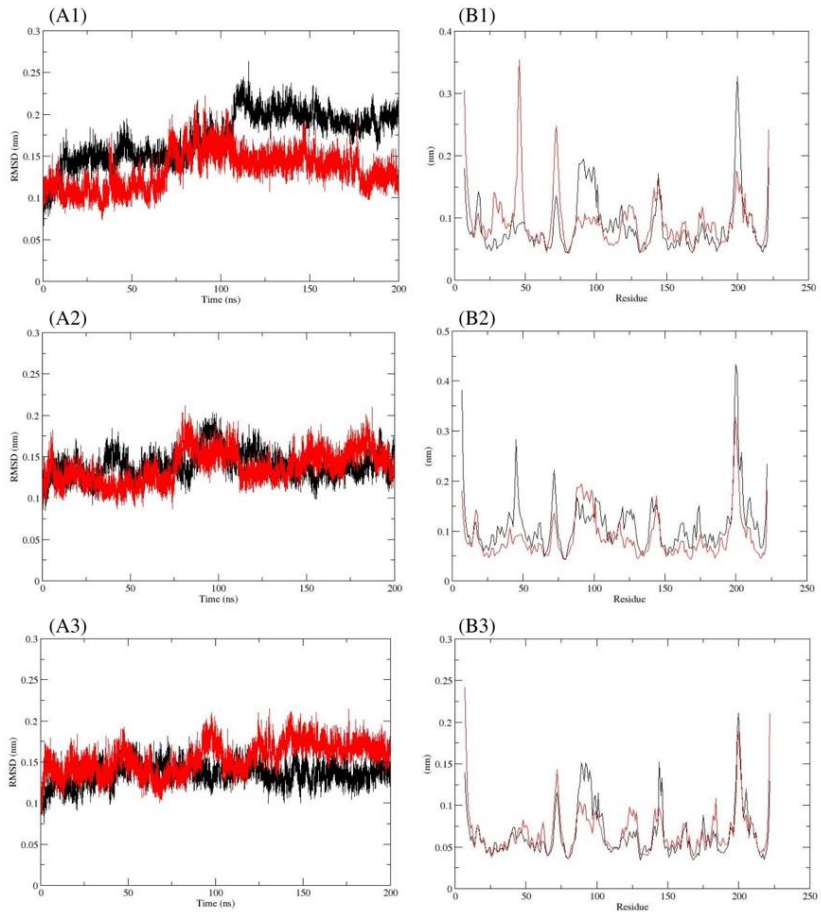

Figure 2. Molecular dynamics profile from three trials of cebulactam A1 (ligand) binding to *Aedes aegypti* arylalkylamine N-acyltransferase (receptor). Panel A1-A3, Time-dependent RMSD of unbound and bound receptors, Panel B1-B3, Time-dependent RMSF of the C-α backbone of unbound and bound receptors. The color codes are black (unbound receptor) and red (ligand-receptor complex).

Root mean square fluctuation (RMSF). This study looked at how cebulactam A1 affects the flexibility of individual parts of *Ae. aegypti* aaNAT enzyme. RMSF measures how much each part of the enzyme wiggles or moves during a simulation. The goal was to see how ligand binding influences this wiggling behavior. Three trials (Fig. 2B1-B3) were conducted and checked the flexibility of the enzyme's building blocks (called residues). When the ligand bound to the enzyme, some residues became more flexible (regions 25–75) (Figure 2, panels B1 and B2). The biggest wiggles occurred in residues

within regions 45–50 and 70–80 (Figure 2, panels B1 and B2). In one trial, there was a small increase in wiggling for the same regions (Figure 2, panel 3). However, other parts of the enzyme became less flexible (regions 80–115 and 190–210). Specifically, the area between residues 100 and 115, where the ligand binds, became less flexible. This reduction in flexibility suggests that the enzyme's function might be affected by the binding. Similar findings have been reported for other insect target receptors (Sakthivel et al. 2019).

Radius of gyration (R_g). This study also looked into "R_g" to understand how stable a receptor is. R_g measures how compact a receptor is. It is like checking how well a folded shirt maintains its shape. If the receptor stays folded (like a well-folded shirt), its R_g value remains steady. If the receptor unfolds, the R_g changes over time. During the 200 ns simulation, R_g values were tracked over time. In all the trials, the R_g fluctuations during the first 100 ns were very small (Fig. 3A1-A3). Even better, ligand binding remained stable throughout the last 100 ns of the simulation. The complex (with the ligand) was more stable than the unbound receptor in the final 50 ns. The R_g values for all complexes ranged from 1.68 nm to 1.76 nm. Other studies have also used R_g to show ligand-binding stability in different insect receptors (Sakthivel et al. 2019). In summary, R_g helps us understand how well the receptor holds its shape when interacting with a ligand.

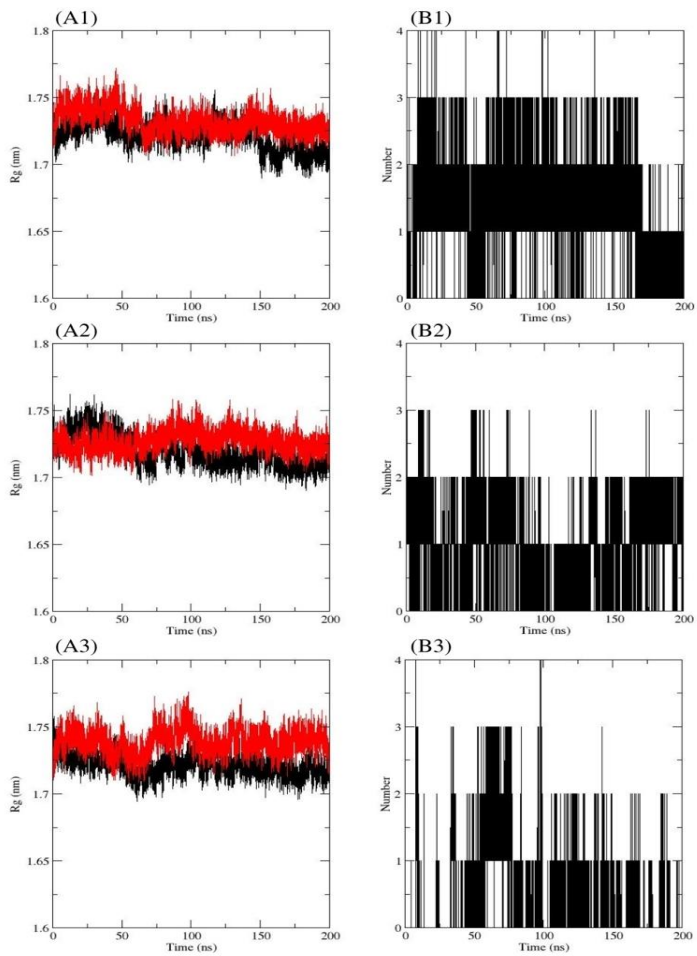

Figure 3. Molecular dynamics profile from three trials of cebulactam A1 (ligand) binding to *Aedes aegypti* arylalkylamine N-acyltransferase (receptor).) Panel A1-A3, Time-dependent radius of gyration

of unbound and bound receptors, Panel B1-B3, Dynamic behavior of hydrogen bonding between ligand and receptor. The color codes for Panel A are black (unbound receptor) and red (ligand-receptor complex).

Hydrogen bond analysis. The presence of hydrogen bond interactions is also important for the stability of the ligand-protein complex. The time evolution of the H-bonding is depicted in Figure 3, panels B1–B3. The H-bond analysis of each trajectory from the three trials showed a decreasing trend in H-bond formation. It was observed that a maximum of four H-bonds occurred during the first 100 ns of simulation, which decreased to one or two H-bonds at the end of the simulation. In a previous study, it was observed that three to four H-bonds occurring throughout the simulation were necessary for stable ligand binding to the target receptor (da Fonseca et al. 2022).

Further assessment of H-bond stability by the percentage of its presence during the simulation revealed that the decreasing trend in H-bond formation could be explained by the low percent occupancy from only two residues, His55 (3.29% to 9.47%) and Asp170 (11.82% to 59.51%), implying that the H-bonds did not last long during the simulation. H-bonds that exist for more than 60% of the entire trajectory are considered the residues that most strongly contribute to the binding free energy (Byun and Lee 2021).

MM-PBSA: Calculation and analysis of binding free energy. Binding free energy is the total of all non-bonded interactions between a ligand and protein receptor. It also refers to the energy change associated with the formation of a complex between a drug (or ligand) and its target protein. In the context of binding, it represents the energy required for the ligand to bind to the protein (Limongelli 2020). A lower free energy signifies a more energetically favorable interaction. The components of the binding free energy estimated for the ligand and receptor interactions from the three trials are listed in Table 3. The free energy of ligand binding (ΔG_{total}) to the receptor which ranged from −10.01 kcal/mol to −12.79 kcal/mol was mainly contributed by favorable ΔG gas (−35.98 kcal/mol to −43.63 kcal/mol) composed of van der Waals and electrostatic interactions which were sufficient to offset the unfavorable energy contributions of component interactions to ΔG solv (25.93 kcal/mol to 30.84 kcal/mol).

Similar findings have been previously reported where the estimated ΔG_{total} values ranged from -8.90 kcal/mol to -27.53 kcal/mol (Kim et al. 2024) to as high as -48.02 kcal/mol (Ramos et al. 2020) for different insecticidal ligand-receptor binding systems. ΔG_{total} values greater than -10 kcal/mol are considered to be an indication that a candidate insecticidal ligand is binding to its true target receptor (Araujo et al. 2020).

Other studies have also consistently shown the significant contribution of ΔG gas to the favorable binding of ligand to respective target receptor in European corn borer *Ostrinia furnacalis* (Liu et al. 2012) and thrips *Bemisia tabaci* (Mangat et al. 2022). The low energy values contributed by electrostatic interaction (EEL= −2.15 kcal/mol to −4.20 kcal/mol) to the total free energy of binding as observed in this study could be explained by the low percent occupancy of H-bonding as mentioned above.

The favorable binding interaction of cebulactam A1 as revealed in this study suggests that the structure of this compound could be used as a scaffold to guide in the design of new cebulactam A1 analogues containing aromatic systems that could promote the presence of strong π-cation interactions in the allosteric binding site.

Table 3. Estimated binding free energy and its components for cebulactam A1 and *Aedes aegypti* arylalkylamine N-acyltransferase complex calculated using the gmx_MMPBSA method.

	Trial I		Trial 2		Trial 3	
Energy component	**Average**	**Standard deviation**	**Average**	**Standard deviation**	**Average**	**Standard deviation**
VDWAALS	-39.43	2.71	-37.58	2.62	-33.83	2.40
EEL	-4.20	1.10	-4.02	1.35	-2.15	2.02
EPB	8.23	0.75	8.57	0.94	5.63	1.49
ENPOLAR	-29.15	0.82	-28.96	1.17	-25.20	1.28
EDISPER	51.75	1.03	51.98	1.38	45.51	1.55
ΔG gas	-43.63	2.50	-41.60	2.84	-35.98	3.13
ΔG solv	30.84	1.45	31.59	1.82	25.93	1.90
ΔG (total)	-12.79	2.66	-10.01	3.28	-10.04	2.75

Per-residue energy decomposition analysis. The contribution of specific amino acid residues within the receptor-binding pocket to the overall binding energy was revealed through per-residue energy decomposition analysis. Table 4 highlights six hotspot amino acid residues (His55, Phe105, Ile108, Phe109, Leu112, Arg134) detected in at least two trial molecular dynamics simulations (MDS). These residues likely play an essential role in ligand binding to the receptor site, surpassing the −1 kcal/mol threshold (Shulga et al., 2022). Notably, the strongest binding interactions occurred with Phe109 and Leu112, exhibiting average binding energies of −2.16±0.167 kcal/mol and −1.93±0.84 kcal/mol, respectively. Previous research emphasized the significance of Phe and Leu in the molecular recognition of bioactive ligands within the receptor-binding pocket (Manjula and Kumaradhas 2020).

Table 4. Per-residue energy contributions to the formation of cebulactam A1-dengue mosquito *Aedes aegypti* arylalkylamine N-acyltransferase complex.

Trial Number	Residues (kcal/mol±standard deviation)
I	Leu52 (-0.74±0.25), **His55** (-1.63±0.82), **Phe105** (-1.34±0.39), **Ile108** (-1.57±0.40), **Phe109** (-2.26±0.48), **Leu112** (-2.72±0.52), Tyr113 (-0.64±0.34), Asn116 (-0.79±0.61), Glu132 (1.30±0.66), **Arg134** (-1.44±0.53)
II	**Leu52** (-1.17±0.39), **His55** (-1.53±0.87), Phe105 (-0.75±0.58), **Ile108** (-1.49±0.47), **Phe109** (-1.97±0.46), **Leu112** (-2.02±0.48), Glu132 (0.63±0.36), **Arg134** (-1.38±0.47), Asp170 (0.91±0.65)
III	Leu52 (-0.69±0.42), **His55** (-1.81±0.69), Lys96 (-0.69±0.41), Phe105 (-0.75±029), **Phe109** (-2.26±0.51), **Leu112** (-1.05±0.32), Glu132 (0.53±0.30)

Note: Hot spot residues are shown in bold font.

CONCLUSION

This study employed several techniques—molecular docking, molecular dynamics simulations (MDS), MM-PBSA (Molecular Mechanics/Poisson-Boltzmann Surface Area), and per-residue energy decomposition—to investigate cebulactam A1 as a potential allosteric inhibitor targeting *Ae. aegypti* arylalkylamine N-acyltransferase. The significance of specific amino acid residues (His55, Phe105, Ile108, Phe109, Leu112, and Arg134) in recognizing cebulactam A1 at the binding site is highlighted.

To further develop cebulactam A1 as a novel bioinsecticide for dengue mosquito control, the following research directions are recommended: 1) Insecticide design to iteratively modify the chemical structure of cebulactam A1 to enhance absorption, potency, and selective toxicity against dengue mosquito vectors; 2) *In vivo* bioassay to conduct efficacy and dose-response assessments; 3) Delivery system development to explore microencapsulated or nanotechnology-based formulations for efficient field application; and 4) Ecological impact assessment to perform tier testing studies to evaluate the impact on non-target organisms, including beneficial insects and aquatic life.

ACKNOWLEDGEMENTS

The author wishes to thank the Advanced Science and Technology Institute of the Department of Science and Technology, Philippines for granting free access to the High Performance Computing facility and Elaine De Guzman for assistance in the preparation of the figures.

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