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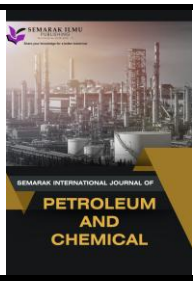

In Silico Studies of the Interactions of Chitin-Based Compounds and AeHKT Enzyme for the Development of Dengue Larvicides

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ABSTRACT

Waste shells from the seafood industry pose a significant environmental challenge due to their abundance. However, these shells are a valuable source of chitin, a polysaccharide that can be extracted and converted into chitosan. Chitosan has gained attention for its diverse applications, including its potential as a larvicide against *Aedes aegypti* mosquitoes. With dengue fever continuing to be a major public health threat, there is a pressing need for safer and more effective larvicides. This study investigates the potential of chitin-derived compounds as larvicides through *in silico* methods, focusing on their interaction with the Alanine Glyoxylate Aminotransferase (AeHKT) enzyme. The primary aim of this research is to evaluate the efficacy of chitin-based compounds as larvicides against *A. aegypti* via computational approaches. The study follows three main steps: ligand-based screening, solubility prediction, and structure-based screening using molecular docking. Initially, 500 compounds were retrieved from the ChEMBL database using the Rchemcpp web-based application. These compounds were ranked based on their similarity to N-Acetyl-beta-D-glucosamine, a chitin derivative, and the top ten were selected for further analysis. Among these, 2,4,6-tris(methylsulfanyl)pyrimidine emerged as the most promising compound, with a 98.28% structural similarity to N-Acetyl-beta-D-glucosamine, high solubility (greater than 0.06 mg/mL), and a favourable logP value (-2.58). Docking studies using AutoDock4 revealed a strong binding affinity (-5.17 kcal/mol) and low torsional energy (1.49 kcal/mol) of this compound when interacting with AeHKT, indicating its potential as an environmentally friendly larvicide. This study lays the groundwork for the development of new, environmentally sustainable larvicides for the control of dengue vectors, offering a promising approach to combat the spread of dengue fever.

1. Introduction

Dengue fever has emerged as one of the significant global health threats over the past two decades, with cases increasing from 500,000 to 5.2 million, and 2019 witnessing the highest number of reported cases across 129 countries [1]. However, between 2020 and 2022, dengue cases showed

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a slight decline, likely due to the COVID-19 pandemic. Despite this, the World Health Organization (WHO) [2] reported that by early 2023, ongoing transmission coupled with an unexpected surge in cases had led to over five million infections. The Americas accounted for about 4.5 million of these cases, with 2,300 deaths. Asia also saw significant case numbers, including Bangladesh (321,000), Malaysia (111,400), Thailand (150,000), and Vietnam (369,000).

Dengue fever is caused by the dengue virus (DENV), which is transmitted by *Aedes aegypti* mosquitoes and classified as an Arbovirus. The dengue virus belongs to the Flaviviridae family and the Flavivirus genus, with a positive-sense, non-segmented, single-stranded RNA genome [3]. The virus is divided into four serotypes: DENV1, DENV2, DENV3, and DENV4 [4]. These serotypes were first isolated from different regions; DENV1 and DENV2 were isolated in 1944 from Hawaii and New Guinea, respectively, while DENV3 and DENV4 were isolated from the Philippines in 1957 [4]. The virus can cause fever, rash, and vasculopathy, with severe infections potentially leading to dengue hemorrhagic fever [5].

Developing effective larvicides is a key strategy for combating dengue fever, offering a more sustainable and preventive approach compared to pharmaceutical treatments. While drugs target infected individuals, larvicides focus on mosquito larvae, preventing *A. aegypti* mosquitoes, the primary vectors of dengue from maturing and transmitting the virus. This method not only reduces the immediate mosquito population but also disrupts the transmission cycle of dengue, leading to a reduction in new infections. Chemical larvicides, such as Bacillus thuringiensis israelensis (Bti), Methoprene, and Temephos, have been widely used in mosquito control programs. However, they present significant limitations. Bti, while environmentally friendly, has a short residual effect and is susceptible to degradation by sunlight and microbial activity, reducing its long-term efficacy [6]. Methoprene, an insect growth regulator, and Temephos, a quick-acting larvicide, can accumulate in the environment, potentially harming non-target organisms, including fish and aquatic life. These chemical larvicides also contribute to the development of insecticide resistance, which further reduces their effectiveness. Pyriproxyfen, another insect growth regulator, offers long-lasting control but also poses environmental risks and comes at a higher cost [7]. These issues highlight the urgent need for alternative larvicides that are both effective and environmentally friendly.

Chitin-based compounds, such as chitosan have emerged as promising alternatives due to their biodegradability and environmental safety. Derived from the exoskeletons of crustaceans like shrimp and crab, chitin is a polysaccharide that has shown potential as a larvicide [8]. Recent research has demonstrated that chitin-based compounds can target the larvae of various insects, including mosquitoes, without harming other organisms in the ecosystem. These compounds are not only eco-friendly but also hold significant promise for reducing the ecological footprint of mosquito control efforts. Perez et al. [9] have highlighted chitosan's ability to inhibit mosquito larval growth and its potential to prevent resistance development, providing a sustainable solution to traditional chemical insecticides. Despite the promising potential of chitin-based larvicides, research specifically targeting key enzymes involved in larval development remains limited.

Among the enzymes involved in the larval development of *Aedes aegypti*, Alanine Glyoxylate Aminotransferase (AeHKT) stands out as a critical target for mosquito control. AeHKT plays a key role in the detoxification pathway, converting 3-hydroxykynurenine (3-HK) to xanthurenic acid (XA). This process is essential for maintaining metabolic balance, as 3-HK can accumulate to toxic levels if not adequately processed. Inhibition of AeHKT disrupts larval development by interfering with their metabolic pathways, leading to mortality [10]. Furthermore, AeHKT is highly conserved across insect species, making it an attractive target for broad-spectrum larvicides. While there has been substantial research on the antimicrobial properties of chitin-based compounds, their application as enzyme inhibitors for mosquito control has not been fully explored. However, recent studies by Zhang et al.

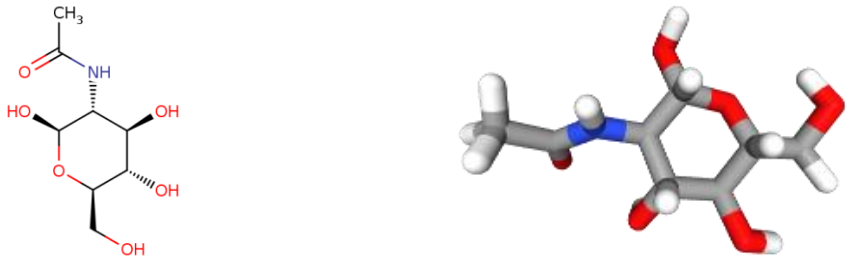
[11] have begun to explore the inhibition of metabolic enzymes as an effective strategy to combat insect pests, suggesting that targeting AeHKT could provide a novel approach for dengue control.

In this study, both ligand-based and structure-based methods were employed to identify potential inhibitors of the AeHKT enzyme using N-Acetyl-beta-D-glucosamine, a chitin derivative, as the reference molecule. The ligand-based screening aimed to identify similar compounds by comparing their structures to N-Acetyl-beta-D-glucosamine. Ten compounds with the closest structural similarities were selected for further analysis, including solubility predictions and structure-based screening. The solubility of these compounds was predicted using Chemaxon's solubility predictor, which calculates aqueous solubility and partition coefficient (logP). Structure-based screening involved molecular docking simulations between the compounds and AeHKT to predict optimal interactions and assess binding affinity using scoring algorithms [11]. By utilizing these computational approaches, this study aims to uncover new, environmentally sustainable larvicides that effectively target AeHKT, offering a novel strategy for long-term dengue control.

2. Methodology

2.1 Ligand-Based screening

In this study, N-Acetyl-beta-D-glucosamine, a chitin derivative was used as the query molecule for the initial ligand-based screening (Figure 1). The 3D structure of N-Acetyl-beta-D-glucosamine (PubChem CID: 24139, molecular formula C8H15N06) was downloaded in Structure Data File (SDF) format from PubChem (https://pubchem.ncbi.nlm.nih.gov/). This structure was then used to search for similar compounds using the Rchemcpp tool (Web Interface) available at http://shiny.bioinf.jku.at/Analoging/service-chembl/ [12]. A total of 500 compounds were retrieved, along with their ZINC IDs, from the Rchemcpp program, based on their similarities to the query molecule. The 2D structure and molecular weight of these compounds can be found in the ZINC database (http://zinc.docking.org/). The similarity of these compounds was ranked using a score range of 0 to 1, where a higher score indicates a closer structural similarity to the query molecule [13]. Based on the similarity scores, the top 10 most similar compounds to N-Acetyl-beta-D-glucosamine were selected for further solubility prediction.



2D 3D

Fig. 1. The 2-dimensional and 3-dimensional structures of N-Acetyl-beta-D-glucosamine (PubChem CID: 24139) utilized in the ligand-based virtual screening analysis. The grey, red, and blue color indicate carbon, oxygen, and nitrogen atoms, respectively

After selecting the top 10 compounds based on their similarity scores, solubility predictions were carried out using Chemaxon's solubility predictor tool (https://plugins.calculators.cxn.io/solubility/). The MOL or SD file formats of these compounds were imported into the software for solubility analysis. In addition to solubility, the logP values representing the logarithm of the partition coefficient between octanol and water were calculated to assess the compounds' solubility

characteristics. Solubility predictions were specifically made at a physiological pH of 7.4. The results included 2D structures of the compounds, solubility data, and graphical representations showing how solubility varies with changes in pH. These outputs provided valuable insights into the compounds' behaviour under physiological conditions.

2.2 Structure-Based Screening

Following the ligand-based screening, structure-based screening was conducted to evaluate the binding affinities and energies of the selected compounds capable of interacting with AeHKT. The process began with the preparation of the AeHKT enzyme (PDB ID: 2HUU), which was retrieved from the Protein Data Bank (https://www.rcsb.org/) to ensure the enzyme structure was accurate and suitable for docking analysis. Several optimization steps were performed on the enzyme structure, including the removal of water molecules, the addition of hydrogen atoms, and the assignment of partial charges to the atoms [14]. These steps were essential for ensuring accurate molecular interactions during the docking simulations.

The molecular docking simulations were performed using AutoDock4 (version 4.2.6), which employs a Lamarckian Genetic Algorithm (LGA) to simulate evolutionary processes and find the most favorable binding conformation of the ligand within the enzyme's active site. The grid parameter files (GPF) were generated to define the grid space and receptor properties, ensuring that the docking simulations accurately represented the enzyme's active site and the surrounding region. Dock parameter files (DPF) were then created to provide instructions for the docking process. The docking algorithm was configured with specific parameters, including 50,000 generations and a population size of 150. These settings were chosen to optimize the balance between search efficiency and accuracy. The number of generations refers to the number of iterations the genetic algorithm performs, while the population size determines the number of candidate solutions evaluated in each generation. Larger values for these parameters result in a more exhaustive search for the optimal binding pose, which is critical for ensuring the reliability of the docking results.

Next, a grid box was positioned around the enzyme's active site, specifically targeting the predicted catalytic residues to ensure the ligands interacted with the relevant areas of the enzyme. The dimensions of the grid box were set to 40 × 40 × 40 Å, and it was centered at the coordinates (x = 18.567; y = 21.342; z = 15.932). This grid box size was chosen to cover the active site and ensure accurate docking of the ligands within the most relevant region of AeHKT. The docking process generated two key output files: the grid log file (GLG), which recorded grid calculation details, and the docking log file (DLG), which contained the docking results, including binding energies and ligand-protein conformations. The results from the docking simulations were analyzed by examining the DLG files, particularly the Root Mean Square Deviation (RMSD) table, which provided information on the consistency of the docking poses. This analysis allowed for the evaluation of binding affinities, which indicate how tightly the compounds bind to the enzyme. The binding energy values were used to assess the strength of the interactions between the ligands and the active site of AeHKT. These results were essential for identifying potential inhibitors of AeHKT and provided insights into their binding mechanisms.

For post-docking analysis, PyMOL was used to visualize the docking results and examine the interactions between the ligands and the AeHKT enzyme. The 3D structures of the docked complexes were visualized, allowing for a detailed inspection of how the ligands fit into the enzyme's active site. Key interactions, such as hydrogen bonds, hydrophobic interactions, and electrostatic interactions, were identified and analyzed to understand the binding mechanisms. This post-docking analysis was

crucial for identifying the most promising compounds based on their binding affinity and interaction profiles with AeHKT, guiding further experimental investigation.

3. Results and Discussion

3.1 Ligand-Based Screening

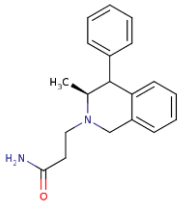
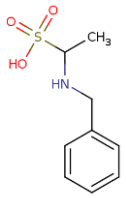
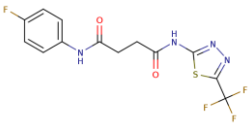
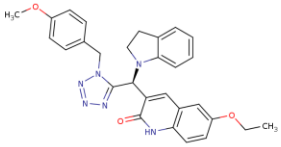
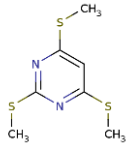
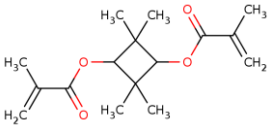
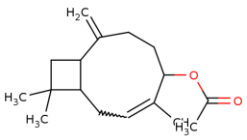
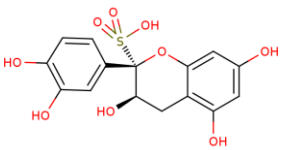
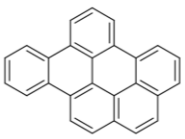
Ligand-based virtual screening is a powerful computational technique for identifying potential analogues of a target molecule. In this study, the Rchemcpp web-based application was used to screen the ChEMBL database for compounds structurally similar to N-Acetyl-beta-D-glucosamine. From this screening, 500 analogues were identified, with the top ten most promising candidates selected based on their similarity scores, solubility at pH 7.4, and logP values, as summarized in Table 1.

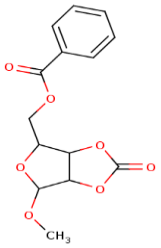
The similarity score is a quantitative measure of the structural resemblance between the query molecule and the identified analogues, ranging from 0 to 1. This score is derived using molecular kernels that assess shared chemical substructures [15]. Among the ten selected compounds, three compounds (ChEMBL ID: 39064, 240524, and 1414815) achieved a perfect similarity score of 1.0, indicating that these compounds have nearly identical molecular features to N-Acetyl-beta-D-glucosamine. These structural similarities suggest that these analogues may exhibit similar biological activities, particularly the potential to inhibit the AeHKT enzyme. The remaining compounds, with similarity scores greater than 0.98, also showed promise as alternative inhibitors [16]. The similarity score is an important criterion as it predicts the analogues' functional resemblance to N-Acetyl-beta-D-glucosamine, guiding the selection of the most promising candidates.

Solubility is a critical factor in determining a compound's bioavailability and effectiveness as a potential larvicide. Solubility at pH 7.4 represents the compound’s ability to dissolve in biological fluids, reflecting its behaviour under physiological conditions. All ten selected compounds exhibited solubility values greater than 0.06 mg/mL, with some compounds, such as ChEMBL ID: 1160333, showing solubility as high as 4.5 mg/mL. This is a significant improvement over chitin, which is poorly soluble in both aqueous and organic solvents. The enhanced solubility of these analogues overcomes chitin's limitations, ensuring better absorption, transport, and bioavailability in biological systems. Compounds with solubility values around or above 1 mg/mL are particularly advantageous, as they ensure sufficient concentrations at the target site, which is crucial for effective larvicidal action.

The partition coefficient (logP) measures a compound’s hydrophilicity or lipophilicity, indicating the balance between water and lipid solubility. A negative logP value indicates that the compound is hydrophilic and favours solubility in aqueous environments. In this study, all selected compounds showed negative logP values, ranging from -3.56 (ChEMBL ID: 39064 and 240524) to -2.58 (ChEMBL ID: 1414815), confirming their strong affinity for aqueous media. This hydrophilic nature is beneficial for drug absorption and transport in biological systems, as it facilitates dissolution in extracellular fluids. In contrast, compounds with high lipophilicity (positive logP values) often face challenges like poor bioavailability and non-specific binding to cellular membranes. The hydrophilic characteristics of these analogues make them ideal candidates for aqueous formulations, enhancing their potential as bioavailable and effective inhibitors of AeHKT.

Table 1
The list of chitin analogues generated from ChEMBL database

Chemical Formula	Chemical Structure	ChEMBL Compound ID	Similarity Score	Solubility at pH 7.4	logP
$C_{19}H_{99}N_2O$		39064	1	2.5	-3.56
$C_9H_{13}NO_3S$		240524	1	-0.16	-3.22
$C_{13}H_{10}F_4N_4O_2S$		1414815	1	3.7	-3.56
$C_{29}H_{28}N_6O_3$		1160333	0.9895	4.5	-3.04
$C_7H_{10}N_2S_3$		238755	0.9828	2.1	-2.58
$C_{16}H_{24}O_2$		104350	0.9827	4.2	-3.04
$C_{21}H_{20}N_2O_4S_2$		105755	0.9827	3.7	-3.56
$C_{15}H_{14}N_9S$		134270	0.9827	0.8	-3.22
$C_{14}H_{14}O_7$		136014	0.9827	1.7	-3.22

$C_{14}H_9ClF_3NO_2$		286575	0.9812	4.0	-3.22

Based on the integrated analysis, the top three candidates for AeHKT inhibition are ChEMBL ID: 238755, 1414815, and 105755. In particular, ChEMBL ID: 238755 (2,4,6 Tris(methylsulfanyl)pyrimidine) stands out with a similarity score of 0.9828, a solubility of 2.1 mg/mL at pH 7.4, and a logP of -2.58, demonstrating a strong structural resemblance to N-Acetyl-beta-D-glucosamine along with favorable solubility and hydrophilicity. Meanwhile, ChEMBL ID: 1414815 (N-(4-fluorophenyl)-N'-[(2E)-5-(trifluoromethyl)-1,3,4-thiadiazol-2(3H)-ylidene] butanediamide) is another strong candidate, achieving a perfect similarity score of 1.0, excellent solubility of 3.7 mg/mL, and a highly hydrophilic logP of -3.56, making it an ideal structural mimic of N-Acetyl-beta-D-glucosamine. Similarly, ChEMBL ID: 105755 (4,11,11-trimethyl-8-methylidene-5-bicyclo [7.2.0] undec-3-enyl) acetate), with a similarity score of 0.9827, solubility of 3.7 mg/mL, and a logP of -3.56, offers a balanced profile in terms of structural similarity and solubility. These compounds not only overcome chitin's solubility challenges but also retain its inhibitory potential, positioning them as promising candidates for the development of dengue larvicides.

3.2 Structure-Based Screening

Structure-based screening is a powerful approach for discovering and optimizing lead compounds for pesticidal applications. This computational method utilizes the three-dimensional structures of target enzymes or proteins to predict how small molecules will bind, aiding in the identification of promising candidates for pesticide development [17]. In this study, the focus was on the AeHKT enzyme (PDB ID: 2HUU), which is critical to the biology of *A.aegypti*, the primary vector of the dengue virus. Given its central role, AeHKT is an ideal target for developing dengue larvicides. After preparing the enzyme, docking studies were conducted on five chitin-based compounds, each docked into the catalytic site of AeHKT. To validate the docking results, redocking experiments were performed: known ligands were extracted from the protein-ligand complex, redocked into the protein, and their docking positions compared to the original conformations. Successful docking was confirmed if the RMSD (root-mean-square deviation) value from the redocking experiment was below 2 Å, indicating that the docked compound maintained a conformation similar to the original ligand [18].

The catalytic site of AeHKT was identified as containing several key residues critical for ligand binding, such as Ser-155, which plays a significant role in interactions with the ligands. The grid box used for docking was set to 40 × 40 × 40 Å, with center coordinates (x = 18.567; y = 21.342; z = 15.932) chosen to cover the catalytic site [19]. Five compounds were docked into this site, and their binding affinities and interactions with critical catalytic residues were analyzed. Binding energies were calculated for each compound, and the top-ranked compounds were selected based on their strong interactions and high binding affinities with key residues involved in the catalytic activity of AeHKT [20]. These top candidates show promise as potential dengue larvicides and warrant further experimental validation.

In this study, molecular docking simulations were performed to analyze the interactions between AeHKT and potential inhibitors, focusing on chain A of the protein. In particular, Ser155, a critical residue in the active site, was used as a key reference point to guide the docking process. The ligand, 2,4,6-Tris(methylsulfanyl)pyrimidine (ChEMBL ID: 238755), demonstrated significant binding interactions within the active site, highlighting its potential as an inhibitor.

Specifically, the ligand forms hydrogen bonds with the hydroxyl group of Ser155, which anchors the ligand and facilitates its proper orientation within the binding pocket. Additionally, Arg350, a positively charged residue, establishes further hydrogen bonding interactions with the ligand, enhancing binding affinity and specificity. Beyond these direct interactions, Llp206, (lysine pyridoxal phosphate), a covalently modified form of the amino acid lysine is another critical residue near the active site, plays an essential role in stabilizing the ligand-protein complex. Llp206 likely interacts *via* hydrophobic contacts or van der Waals forces, which help maintain the structural integrity of the binding pocket and ensure optimal positioning of the ligand [21]. This combination of hydrogen bonding and stabilizing interactions ensures a strong and specific association between AeHKT and the ligand, supporting the compound's potential as a dengue larvicide candidate. These findings provide valuable insights into the molecular basis of ligand binding, guiding future optimization efforts to enhance the efficacy of AeHKT inhibitors.

The molecular docking results, summarized in Table 2, provide valuable insights into the interactions between N-Acetyl-β-D-Glucosamine analogues and AeHKT, highlighting their potential as enzyme inhibitors. Among the compounds tested, ChEMBL ID: 238755(2,4,6-Tris(methylsulfanyl)pyrimidine) exhibited the highest binding affinity of -5.17 kcal/mol, indicating a strong and stable interaction with AeHKT, making it a particularly promising candidate for further investigation. The second most favorable binding affinity was observed for ChEMBL ID: 136014 (Dibenzo[b,pqr]perylene), with a value of -5.15 kcal/mol, closely followed by ChEMBL ID: 1414815 (N-(4-fluorophenyl)-N'-[(2E)-5-(trifluoromethyl)-1,3,4-thiadiazol-2(3H)-ylidene] butanediamide) at -4.24 kcal/mol. Both compounds showed relatively high binding affinities, suggesting they could also serve as potent inhibitors. ChEMBL ID: 105755(4,11,11-trimethyl-8-methylidene-5-bicyclo [7.2.0] undec-3-enyl) acetate) exhibited the weakest binding affinity at -4.17 kcal/mol, though this value remains within a reasonable range for inhibitory activity, indicating that it may interact with AeHKT, albeit weaker.

Table 2
Binding affinities and torsional energies of top five analogues of N-Acetyl-β-D-Glucosamine and AeHKT

ChEMBL Compound ID	Binding affinity (kcal/mol)	Torsional energy
1414815	-4.24	1.49
105755	-4.17	1.96
238755	**-5.17**	**1.49**
134270	-4.39	1.79
136014	-5.15	2.39

In terms of torsional energies, which reflect the flexibility of the ligands within the binding site, ChEMBL ID: 238755(2,4,6-Tris(methylsulfanyl)pyrimidine) and ChEMBL ID: 1414815(N-(4-fluorophenyl)-N'-[(2E)-5-(trifluoromethyl)-1,3,4-thiadiazol-2(3H)-ylidene] butanediamide) both exhibited the lowest torsional energy of 1.49 kcal/mol, suggesting that these compounds are relatively rigid and may adopt a stable binding conformation [22]. In contrast, ChEMBL ID: 136014(Dibenzo[b,pqr]perylene), showed the highest torsional energy of 2.39 kcal/mol, indicating

greater flexibility. While this could potentially affect the compound's ability to maintain a stable interaction with AeHKT, its high binding affinity suggests that flexibility does not significantly hinder its potential as an effective inhibitor. The torsional energies of the other compounds ranged from 1.49 kcal/mol to 1.96 kcal/mol, reflecting moderate flexibility that is unlikely to undermine their inhibitory potential.

Overall, ChEMBL ID: 238755 (2,4,6-Tris(methylsulfanyl)pyrimidine stands out as the most promising candidate, offering both a high binding affinity and low torsional energy, making it a strong contender for development as a dengue larvicide targeting AeHKT [23]. While ChEMBL ID: 1414815 (N-(4-fluorophenyl)-N'-[(2E)-5-(trifluoromethyl)-1,3,4-thiadiazol-2(3H)-ylidene] butanediamide) and ChEMBL ID: 134270 (Dibenzo[b,pqr]perylene) show in Figure 2 slightly lower binding affinities, their favorable interactions with the enzyme still present strong prospects for their role as inhibitors. Although ChEMBL ID: 105755(4,11,11-trimethyl-8-methylidene-5-bicyclo [7.2.0] undec-3-enyl) acetate) exhibited the weakest binding affinity, its inclusion in further testing is warranted, as its flexibility and moderate affinity may still support its function as an inhibitor. These results suggest that the compounds tested in this study hold significant promise for the development of novel dengue larvicides, with ChEMBL ID: 238755 (2,4,6-Tris(methylsulfanyl)pyrimidine) emerging as the top candidate for further experimental validation, including *in vitro* and *in vivo* assays to assess its efficacy and safety.

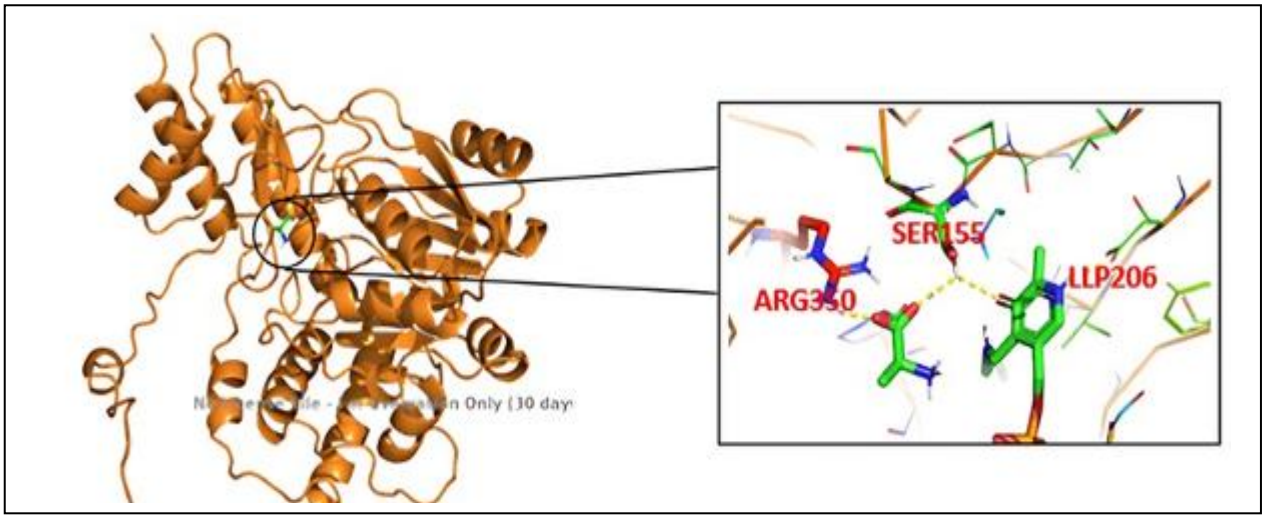


Fig. 2. The interactions between AeHKT (orange) and 2,4,6-Tris(methylsulfanyl)pyrimidine (green), highlighting key residues involved in the interaction: Ser155 and Arg350, and Llp206. The ligand ChEMBL ID: 238755 (2,4,6-Tris(methylsulfanyl)pyrimidine) is shown, forming hydrogen bonds with Ser155 and Arg350, while Llp206 plays a crucial role in stabilizing the complex.

3.3 Comparative Analysis

When it comes to mosquito control, the environmental impacts of chitin-based larvicides versus traditional chemical insecticides are crucial in deciding their long-term viability. Traditional chemical insecticides can persist in the environment for weeks or months, contaminating soil, water, and food chains [24]. Pyrethroids and organophosphates, for example, can accumulate in aquatic environments, leading to long-term contamination and potential bioaccumulation in wildlife [25]. This makes them less sustainable for long-term use in mosquito control. On the other hand, chitin-based larvicides are biodegradable, meaning they break down naturally into non-toxic by-products, reducing their long-term environmental footprint. In this study, compounds like ChEMBL ID: 238755

(2,4,6-Tris(methylsulfanyl)pyrimidine) showed high solubility in water and are expected to degrade rapidly, making them environmentally safer than chemical alternatives. Their biodegradability makes them a sustainable option for mosquito control, as they do not contribute to environmental pollution or persist in ecosystems.

Next, another challenge with chemical insecticides is the rapid development of resistance in mosquito populations. As mosquitoes evolve to survive exposure to insecticides, the effectiveness of these chemicals declines, leading to higher application rates and increased costs. Pyrethroid resistance in mosquitoes, for example, has become widespread, undermining the effectiveness of these chemicals in vector control programs [25]. Chitin-based larvicides, however, pose a lower risk of resistance. The compounds work by targeting the molting process or exoskeletons of mosquitoes, a biological function that is hard for mosquitoes to evolve resistance against. This mechanism of action makes chitin-based compounds a more stable solution in the long run. As demonstrated in this study, the high binding affinity of ChEMBL ID: 238755 towards the AeHKT enzyme indicates its potential to effectively disrupt mosquito larvae development, without triggering resistance.

Other than that, the effectiveness of a larvicide is determined not only by its ability to kill mosquitoes but also by its selectivity. Chemical insecticides often have non-specific effects, killing a wide range of organisms, including beneficial insects and aquatic life, which can harm ecosystems [26]. In contrast, chitin-based larvicides are more targeted in their action, as they specifically affect arthropod pests. For instance, compounds like ChEMBL ID: 238755 showed good solubility and hydrophilicity, allowing for effective delivery to mosquito larvae without affecting other species. This selectivity makes chitin-based compounds a safer and more effective option for controlling mosquito populations, especially in environmentally sensitive areas.

For regulatory agencies, public health officials, and pest control companies, adopting chitin-based larvicides offers significant benefits. While traditional chemical insecticides might initially appear more cost-effective, the environmental risks they pose along with the development of resistance and the need for repeated applications make them less sustainable in the long term. In contrast, chitin-based larvicides offer a more sustainable and eco-friendly solution for mosquito control. The results of this study, including ChEMBL ID: 238755, which showed excellent solubility, hydrophilicity, and high binding affinity for AeHKT, position these compounds as promising candidates for use in integrated pest management (IPM) systems. They represent a socially acceptable solution, with lower environmental impacts and a higher level of safety for non-target species.

3.4 Limitations and Future Directions

While this study provides valuable insights into the potential of chitin-based larvicides for mosquito control, several limitations should be acknowledged, and areas for future research have been identified to build on the findings. One limitation of this study is the reliance on *in silico* methods to predict the interactions between chitin analogues and the AeHKT enzyme. Although molecular docking provides useful insights into the binding affinities and mechanisms of action, the lack of experimental validation remains a significant gap. Hence, *in vitro* and *in vivo* studies are needed to confirm the effectiveness and toxicity of the selected chitin-based compounds in real-world applications. The bioavailability and stability of these compounds in biological systems also need to be tested to ensure that they perform as expected outside of computational models. Future research could focus on synthesizing the top candidates identified, such as ChEMBL ID: 238755, and conducting laboratory tests to evaluate their actual larvicidal activity and their potential toxicity to non-target organisms.

Another limitation lies in the solubility predictions used in this study. While the compounds were selected based on their solubility at physiological pH 7.4, this does not account for the complex environmental conditions in which mosquito larvae develop. Factors like salinity, temperature variations, and organic matter in aquatic environments could alter the solubility and effectiveness of these compounds. Therefore, future studies could explore the environmental stability of these compounds under different conditions and assess how factors like pH variations or the presence of organic pollutants affect their efficacy as larvicides.

Moreover, while AeHKT was identified as a promising target for inhibiting mosquito larvae development, future research could expand to other metabolic enzymes that may also play a critical role in the survival and development of *A.aegypti*. Studies could investigate additional targets within the mosquito's detoxification pathways or enzymes involved in molting and exoskeleton formation, which might offer new opportunities for developing multi-target larvicides. It is possible that combination therapies, targeting multiple enzymes or biological processes simultaneously, could be more effective in reducing resistance development and improving the longevity of the larvicides.

Finally, while the focus of this study was primarily on AeHKT and chitin-based compounds, there is significant potential for future research to explore the development of formulations for field applications. Investigating the practical challenges associated with the formulation, stability, and delivery systems of these compounds could lead to more efficient methods of applying these larvicides in mosquito breeding habitats. Additionally, the cost-effectiveness of chitin-based larvicides compared to traditional chemical insecticides should be examined to assess their economic feasibility for large-scale mosquito control programs, especially in regions with limited resources.

4. Conclusions

In conclusion, virtual screening analyses have proven to be an essential tool in the design of novel larvicides for inhibiting dengue larvae. By utilizing molecular docking, these techniques allow for the prediction of compound solubility and binding interactions, offering valuable insights that guide experimental testing of inhibitory activity. The results of this study highlight 2,4,6-tris(methylsulfanyl)pyrimidine as the most promising candidate, identified through ligand-based screening, solubility predictions, and molecular docking analysis. This compound exhibits a strong binding affinity of -5.17 kcal/mol with the AeHKT enzyme, suggesting stable interactions with critical catalytic residues, such as Ser-155, which are key to enzyme inhibition. Furthermore, 2,4,6-tris(methylsulfanyl)pyrimidine (ChEMBL ID: 238755) demonstrates a 98.28% structural similarity to chitin and shows high solubility in aqueous solutions, with a logP value of -2.58, reinforcing its potential as an effective larvicide. The findings not only contribute to the identification of promising chitin-based compounds but also lay the groundwork for future experimental validation. Further studies are necessary to confirm the inhibitory activity of these compounds and their potential as AeHKT inhibitors for the development of new dengue larvicides.

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