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COMPUTATIONAL SCREENING OF POTENTIAL AChE INHIBITORS IN *ANOPHELES GAMBIAE*: TOWARDS OVERCOMING INSECTICIDE RESISTANCE

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ABSTRACT

Background: Insecticide resistance in *Anopheles gambiae* poses a major challenge to malaria vector control. The G119S mutation in acetylcholinesterase (AChE) is associated with resistance to organophosphate and carbamate insecticides, while glutathione S-transferases (GSTs) can contribute to metabolic resistance. This study aimed to identify candidate AChE inhibitors using hierarchical virtual screening, with additional computational filtering against glutathione S-transferase delta (GSTD).

Methods: The AChE structure (PDB ID: 6ARY), containing the G119S variant, and the GSTD structure (PDB ID: 1PN9) were prepared using AutoDockTools 1.5.6. A library of five million commercially available compounds from Mcule was screened against AChE, and compounds with predicted binding scores below −6.5 kcal/mol were selected for subsequent docking against GSTD. Compounds with predicted GSTD binding affinities stronger than that of glutathione (−5.2 kcal/mol) were excluded. The remaining complexes were assessed using Discovery Studio Visualizer 4.5, and four selected candidates were redocked using AutoDock Vina 1.1.2 and compared with chlorpyrifos.

Results: Initial screening predicted 982 compounds with AChE binding scores below −6.5 kcal/mol. Sequential filtering based on docking and interaction with GSTD yielded four candidate compounds. All four were positioned within the AChE active-site gorge and exhibited several interactions involving Cys447. All four candidates had more favorable predicted docking scores than chlorpyrifos (−6.5 kcal/mol).

Conclusion: The virtual screening identified four commercially available compounds with favorable predicted binding to AChE and comparatively weak predicted binding to GSTD under the applied docking criteria. These compounds need further investigation through biochemical inhibition assays, metabolic stability studies, and bioassays against insecticide-resistant An. gambiae to establish their inhibitory activity and potential relevance to insecticide resistance control.

Keywords: *In silico*; Insecticide, AChE, Inhibitors; Molecular docking; Virtual screening

1 INTRODUCTION

Malaria is still a global health problem (particularly in sub-Saharan Africa where mosquito borne transmission causes most deaths from the disease). While malaria control has been achieved, insecticide use has led to resistance of major mosquitoes vectors (*Anopheles gambiae*) and other mosquito vectors (Jagannathan & Kakuru 2022; Takken *et al*. 2024).Two major mechanisms contribute to insecticide resistance in mosquitoes: target-site mutations and metabolic detoxification. Acetylcholinesterase (AChE; EC 3.1.1.7) is an important target of organophosphate and carbamate insecticides because it regulates nerve signalling by hydrolysing acetylcholine. In An. gambiae, the G119S mutation in the ace-1 gene reduces susceptibility to these insecticides by altering the enzyme's active-site environment (Weill *et al*., 2004; Djogbénou *et al*., 2010). Consequently, identifying compounds that can inhibit the resistant enzyme is an important step towards developing alternative insecticides.

Metabolic resistance provides an additional challenge. Glutathione S-transferases (GSTs) and cytochrome P450 enzymes contribute to the detoxification of several insecticides, reducing their effectiveness in resistant mosquito populations (Riveron et al., 2014; Adolfi *et al*., 2019). Therefore, compounds with strong predicted binding to AChE may not necessarily be suitable candidates if they also interact strongly with detoxification enzymes. Considering both target-site resistance and metabolic liability during compound selection may improve the identification of promising candidates.

Computer-aided screening offers a practical approach to evaluating large chemical libraries before experimental testing. Molecular docking and virtual screening can help identify compounds with favourable predicted interactions with specific enzymes, reducing the time and cost associated with initial compound selection (Lu et al., 2011; Ramos et al., 2020).

In this study, an *in silico* screening strategy was applied to identify inhibitors of the G119S variant of AChE from An. gambiae. A large library of commercially available organic compounds were screened against the resistant enzyme and subsequently screened against glutathione S-transferase delta (GSTD). Candidates with good predicted binding to AChE but poor binding to GSTD were chosen (compounds that may be less sensitive to metabolic interference). This approach provides a computational framework for selecting resistance breaking insecticide candidates for experimental validation.

2 MATERIALS AND METHODS

2.1 Target selection and library preparation

The Protein Data Bank (https://www.rcsb.org/) was employed to obtain the 3D structures of two enzymes found in *Anopheles gambiae*. These enzymes are Acetylcholinesterase (AChE), Glutathione S transferase delta (GSTD1-6), corresponding to PDB codes 6ary, and 1pn9, respectively. To prepare the structures for docking, we used AutoDockTools (ADT) version 1.5.6 (Morris *et al*., 2009). This software enabled the removal of unwanted ions and molecules. Additionally, ADT was used to add polar hydrogen atoms and assign partial charges, with the Gasteiger charge method (Lin *et al*., 2021) employed for these calculations. For the preparation of the compound library, we utilized the mcule server (Kiss *et al*., 2012), which contains a collection of five million organic compounds available for purchase (in stock).

2.2 Molecular docking-based virtual screening

Virtual screening was conducted using the mcule server (Kiss *et al*., 2012). This procedure targeted two enzymes, AChE and GSTD, in a sequential manner. For the docking step, the grid center coordinates for Acetylcholinesterase were set at -58.055for X, 54.416 for Y, and 17.155 for Z. Based on the obtained binding scores (<-6.5), 982 compounds were selected. This selection underwent further docking simulations targeting GSTD. Compounds with a binding affinity higher than that of the substrate molecule Glutathione (GSH) (-5.2 kcal/mol) were excluded. Consequently, only the five compounds with weaker binding affinities were chosen for additional evaluation. The highlighted complexes were visualized using Discovery Studio Visualizer (version 4.5) (Dassault Systèmes BIOVIA, 2017). Protein-ligand complexes lacking hydrogen bonds or showing improper positioning were excluded from further analysis. This selection process resulted in the identification of four compounds. The top four complexes were then re-evaluated through redocking using AutoDock Vina version 1.1.2 (Trott and Olson, 2010). It is noteworthy that all simulation parameters for the utilized software and tools were kept at their default settings.

3 RESULTS

3.1 Molecular docking-based virtual screening

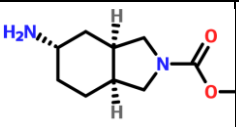
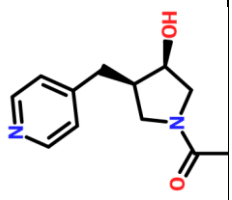
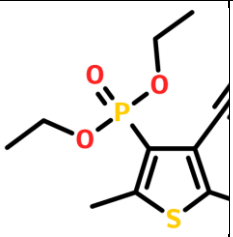
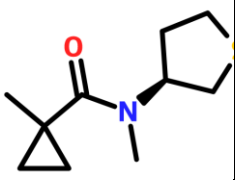
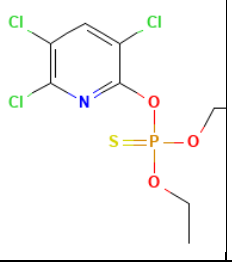
To identify potential inhibitors against *Anopheles gambiae*, we conducted molecular docking-based virtual screening. This involved testing a vast library of commercially available organic compounds against AChE, an enzyme of pivotal importance in the insect nervous system due to its critical role in the cholinergic system (Colović *et al*., 2013).

Although several commercially available insecticides, such as organophosphates and carbamates, target insect AChE, environmental concerns and insect resistance have posed significant challenges. It has been reported that one of the ways in which insecticide resistance is developed. is by metabolism of insecticide molecules by specific insect enzymes (Glutathione S-transferases (GSTs) GSTs). GSTD enzyme in particular; some insects have managed to counteract effects of insecticides by increasing expression of this enzyme as an adaptive response (Hossain et al., 2023). Therefore, we carried out *in silico* screening to find candidates that could effectively inhibit AChE while also avoiding insecticide resistance by evading enzymatic degradation by GSTD. First molecular docking was performed to screen a large compound library against the target enzyme AChE of *Anopheles gambiae*. Compounds with binding scores <-6.5 kcal/mol were selected (n = 982). Then they were filtered by molecular docking against GSTD: compounds which bound strongly to GSTD (binding >7 kcal/mol) could be enzymatic detoxified and compounds expected to bind strongly in the active site of GSTD (binding score compared to substrate (-5.2 kcal/mol)) were excluded from consideration since they were not expected to bind to AChE (Table 1). Thus only four compounds were considered as potential AChE inhibitors. The active site gorge of AChE in *Anopheles gambiae* comprises the following amino acids: Trp245, Gly278, Gly279, Ser280, Ser360, Trp441, Cys447, Phe449, Glu486, Tyr489, Tyr493, His600 (Chen *et al*., 2003). These residues are involved in binding interactions with both substrate and standard insecticide Chlorpyrifos. To understand and predict the effects of the screened compounds on the biological action of acetylcholinesterase we need to know what is going on within the enzyme's active site. As shown in Figure 1 all four compounds were inside the active site and multiple interactions occurred between them and various active residues.

The four selected compounds had multiple interactions with hotspot residues of AChE: conventional hydrogen bonds, Van der Waals forces, π-sigma interactions, π-sulfur, π-donor hydrogen bonds, π-π T-shaped interactions, alkyl interactions, π-alkyl interactions, carbon based hydrogen bonds, π-cation and π-anion interactions. Among the four ligands 1 had the highest number of interactions (15). This extensive interaction network might explain why it has a higher docking score (-7.5 kcal/mol) (Table 1).

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Table 1: Docking outcomes of the top four compounds targeting AChE following filtration and subsequent visualization.

No.	Structure	mcule ID	SMILES	Docking score from mcule (kcal/mol)	Docking score from autodock vina (kcal/mol)
1		MCULE-7406126763-0-3	O=C(OC(C)(C)C)N1C[C@@]2([H])[C@@](CC[C@@H](C2)N)([H])C1	-6.8	-7.5
2		MCULE-6332998141-0-3	N1(C[C@@H]([C@H](CC2C=CN=CC=2)C1)O)C(CSC)=O	-6.8	-7. 0
3		MCULE-4797740637-0-35	C1(=C(C)SC(N)=C1C#N)P(OCC)(OCC)=O	-6.8	-6.7
4		MCULE-9045455547-0-4	CN(C(C1(CC1)C)=O)[C@@H]1CS(=O)(=O)CC1	-6.8	-6.6
Chlorpyrifos (Standard)		MCULE-3208902318	CCOP(=S)(OCC)OC1=NC(=C(C=C1Cl)Cl)Cl	-6.4	-6.5

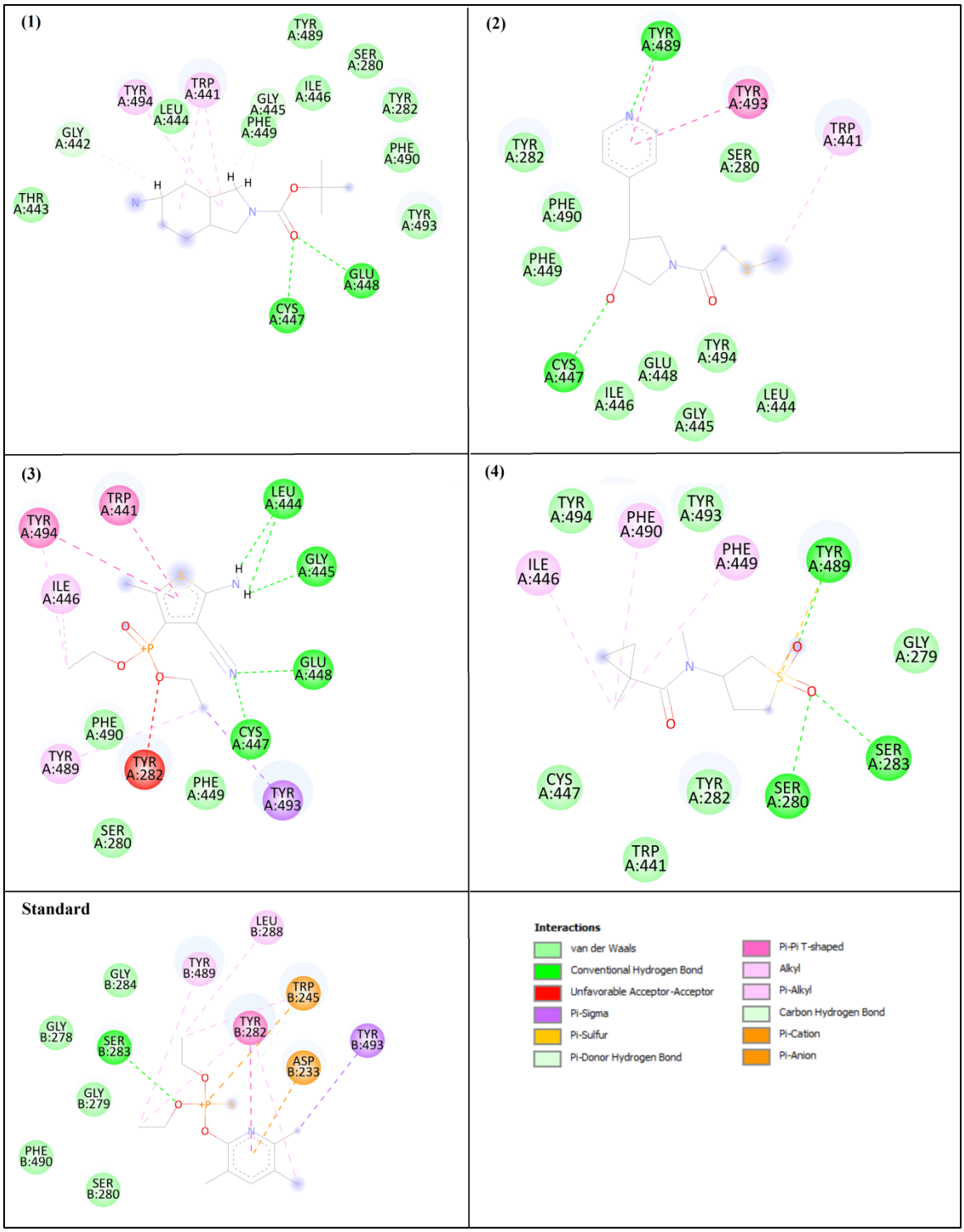


Figure 1: Visualizing the binding modes of the compounds 1-4 located inside the active site of Acetylcholinesterase (*Anopheles gambiae*).

4 DISCUSSION

In most insects, including *Anopheles*, there are two AChE genes: ace1 (AChE1) and ace2 (AChE2) (Knutsson *et al*., 2017). Notably, AChE1 serves as the predominant cholinesterase in the nervous system of many of these insects. Previous experimental evidence indicates that AChE1 is responsible for the hydrolysis of the majority of acetylcholine in *An. gambiae*. Consequently, AChE1 becomes the primary target for carbamates and organophosphates in *Anopheles* species (Zhao *et al*., 2013). This study specifically focuses on investigating the enzyme AChE1 due to its key role in acetylcholine hydrolysis and its susceptibility to targeted inhibition by certain chemical compounds.

The 3d structure of AChE (pdb: 6ary) was obtained from insecticide-resistant mosquito (*Anopheles Gambiae*), in this particular protein there is an amino acid change (G119S) which is contributed to the insects resistance.

The interactions observed following molecular docking of compounds 1-4 with specific amino acid residues (Trp245, Gly278, Gly279, Ser280, Ser360, Trp441, Cys447, Phe449, Glu486, Tyr489, Tyr493, His600) within the acetylcholinesterase enzyme are closely resemble those documented by Cheung *et al*., 2018. The critical residue Cys447, which is thought to play a distinguishing function in the binding with the substrate, was interacted with by all four compounds (Rants'o *et al*., 2022). This validates our selection as a promising inhibitor for the insect acetylcholinesterase enzyme.

Metabolic resistance, the predominant type of insecticide resistance, results from increased biodegradation of insecticides triggered by stress response pathways. This often involves upregulation of glutathione-S-transferases (GSTs), leading to insecticide detoxification (Hossain *et al*., 2023). Therefore, we conducted additional screenings of the identified compounds targeting AChE against GST Delta2. Compounds predicted to bind to this enzyme and potentially susceptible to detoxification were subsequently excluded from consideration, as a result the four candidates were predicted to overcome the insecticide degradation.

5 CONCLUSION

This study employed a hierarchical virtual screening strategy to identify four commercially available compounds with favorable predicted binding to the G119S acetylcholinesterase (AChE) of Anopheles gambiae and comparatively weak predicted binding to glutathione S-transferase delta (GSTD). The selection criteria considered target binding and GSTD interaction profiles in addition to AChE docking so that screening was not limited to docking alone. All four candidates were located within the AChE active-site gorge, had interactions with Cys447 and their predicted docking scores were higher than chlorpyrifos under the docking protocol used here. These results inform us which of the identified compounds should be prioritized for further study (biochemical inhibition assays, metabolic stability studies, bioassays against insecticide resistant mosquitoes) but biochemical inhibition assays, metabolic stability studies and bioassays against insecticide resistant mosquitoes are required to establish inhibitory activity and whether they overcome target site and metabolic resistance mechanisms respectively. Finally, this work illustrates the potential of integrating target-based screening with computational counter-screening to prioritize candidate compounds for insecticide discovery.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Data availability statement

All data and information are available in the manuscript.

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