



## Research Article

### *In Silico* Evaluation of *Nigella sativa* Phytochemicals as Inhibitors of Insecticide-Resistant Acetylcholinesterase in *Anopheles gambiae*

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#### ABSTRACT

This study explored essential oil-derived compounds from *Nigella sativa* as inhibitors of insecticide-resistant acetylcholinesterase (AChE1) from *Anopheles gambiae*, a key malaria vector. A library of 132 phytochemicals retrieved from the LOTUS database was subjected to screening for physicochemical properties, pharmacokinetics, and drug-likeness using SwissADME. Only four compounds met combined criteria for molecular weight, hydrogen bonding potential, topological polar surface area, and predicted gastrointestinal absorption, alongside minimal interference with cytochrome P450 enzymes. These candidates proceeded to molecular docking with the G119S-mutated AChE1 (PDB ID: 6ARX), linked to resistance against carbamate and organophosphate insecticides. Docking scores ranged from –6.90 kcal/mol to –9.60 kcal/mol. The strongest binder, CID:2005341, formed a hydrogen bond with Tyr282 and engaged multiple hydrophobic residues critical for active site stabilization. Other compounds showed varying combinations of hydrogen bonding and hydrophobic contacts with residues Ser283, Cys447, and Trp441. These results indicate the potential of selected *N. sativa* phytochemicals to inhibit the resistant AChE1 variant, which highlights them as promising candidates for developing plant-based agents to support vector control programs. Further work, including molecular dynamics simulations and biological assays, is recommended to confirm these findings and evaluate their suitability as biopesticide leads.

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## INTRODUCTION

Mosquitoes (*Diptera: Culicidae*) are vectors of some of the most debilitating and deadly diseases affecting humanity, including malaria, dengue fever, yellow fever, and Zika virus (WHO, 2022). These diseases contribute to significant morbidity and mortality, particularly in tropical and subtropical regions. During the last century alone, mosquito-transmitted diseases have killed over 300 million people, mostly infants and children, and it infected and debilitated hundreds of millions of others. Each year, about 700,000,000 people are infected by diseases carried by mosquitoes. In comparison, only 21 million people died in combat in World War I, World War II and the Korean War combined.

Mosquito-borne diseases pose a significant public health burden in Nigeria, with the country accounting for a substantial proportion of global malaria cases (WHO, 2020). In Jigawa State, although no mosquito-borne disease other than malaria has previously been reported, prevalence of malaria is particularly high, with a prevalence rate of 23% reported in 2018 alone (NMIS, 2019). Over the years, malaria has been a leading cause of mortality in Jigawa State, accounting for a significant proportion of under-five deaths (NBS, 2020). It contributes substantially to maternal mortality in the State, with an estimated 11% of maternal deaths attributed to the disease (MHW, 2020). The transmission of malaria in Jigawa State exhibits a seasonal pattern, peaking during the rainy season (Okoro *et al.*, 2020). Since 2014, outbreaks of dengue, malaria, chikungunya, yellow fever, and Zika have resulted in deaths and overwhelmed health systems in many countries. Climate change has led to the expansion of vector-borne diseases, especially malaria and dengue, with over half the world's population at risk. Longer hot seasons are enlarging the seasonal window for the spread of mosquito-borne diseases, leading to more frequent outbreaks. Droughts and floods linked to climate change can increase transmission of mosquito-borne viruses, with stored water providing additional mosquito breeding sites.

The control of mosquito populations is a critical component of reducing the transmission of these diseases. However, the widespread use of synthetic insecticides has led to the development of resistance in mosquito populations and raised environmental and health concerns (Hemingway *et al.*, 2016). The alarming rise in mosquito-borne diseases, coupled with the growing resistance of mosquito populations to synthetic insecticides (Şengül *et al.*, 2022), necessitates the exploration of novel, sustainable, and environmentally friendly mosquito control strategies.

Mosquito-borne diseases continue to pose a significant public health challenge globally, with *Anopheles gambiae* serving as the principal vector of malaria across sub-Saharan Africa. The increasing emergence of insecticide resistance—particularly to organophosphates and carbamates has rendered traditional vector control strategies less effective (Vidal-Albalat *et al.*, 2023). At the molecular level, this resistance is often attributed to mutations in the acetylcholinesterase (AChE1) enzyme, with the G119S substitution being a well-characterized resistance marker.

In the search for alternative, environmentally safe vector control agents, plant-derived essential oils and their bioactive compounds have garnered interest for their broad-spectrum insecticidal properties. *Nigella sativa* (black seed), in particular, has demonstrated potent bioactivity against various insect species, with its essential oil constituents such as carvacrol and thymoquinone, displaying larvicidal and neurotoxic effects through diverse molecular mechanisms (Zouirech *et al.*, 2023). These findings are reinforced by previous docking and in vitro studies that linked *N. sativa* components to significant inhibitory activity against insect AChE homologues. Advancements in structure-based virtual screening (SBVS) and molecular docking now allow for high-throughput assessment of plant-derived molecules against validated insect targets such as AChE. These computational approaches accelerate the identification of novel inhibitors by simulating ligand-receptor interactions, evaluating binding affinities, and predicting binding site conformations. When integrated with in silico ADME and toxicity profiling, these tools offer a powerful pipeline for screening natural product libraries with enhanced precision and pharmacological relevance (Omoniwa *et al.*, 2024). Given the need for new mosquitocidal agents that are both potent and environmentally benign, this study aims to screen, dock, and pharmacologically evaluate a curated library of essential oil-derived compounds from *Nigella sativa* against the insecticide-resistant AChE protein. By combining molecular docking, ADME-Tox profiling, and interaction analysis, this study contributes to the discovery of candidate leads for future vector-targeted biopesticides.

## MATERIALS AND METHODS

### Retrieval of Protein and Structure Preparation

The crystal structure of the insecticide-resistant acetylcholinesterase mutant from *Anopheles gambiae* (PDB ID: 6ARX) was obtained from the Protein Data Bank (<https://www.rcsb.org>). This enzyme, carrying the G119S mutation, is associated with resistance to organophosphate and carbamate insecticides (Pang,

2006). The structure was selected due to its high resolution (2.75 Å) and absence of bound ligands, which eliminates the need for homology modelling. Previous studies have confirmed its suitability for docking investigations (Gorecki *et al.*, 2019; Rants'o *et al.*, 2022).

Preparation of the protein was performed using AutoDockTools 1.5.6. All water molecules and non-standard ligands were removed. Hydrogen atoms were added, and side chains were protonated to reflect physiological pH (7.4). The active site was inspected, focusing on the region around Ser119, which is part of the catalytic triad in mosquito acetylcholinesterase (Verma, 2014).

### Virtual Screening of Ligands

Virtual screening was conducted to identify potential acetylcholinesterase mutant inhibitors from *Anopheles gambiae*, an enzyme implicated in insecticide resistance (Pang, 2006; Vidal-Albalat *et al.*, 2023). A dataset of 132 natural product-derived compounds was sourced from the LOTUS database, which provides curated structural and taxonomic profiles of phytochemicals (Rutz *et al.*, 2022).

All compounds were evaluated using SwissADME (Daina *et al.*, 2017) to predict their physicochemical properties, pharmacokinetic behaviour, and compliance with established drug-likeness guidelines. Specifically, the screening assessed molecular weight, topological polar surface area (TPSA), consensus LogP, number of hydrogen bond donors and acceptors, and rotatable bonds. Drug-likeness filters were applied based on Lipinski's rule of five, restricting molecular weight to  $\leq 500$  Da, LogP between -0.5 and 5, no more than five hydrogen bond donors, and no more than ten hydrogen bond acceptors (Lipinski *et al.*, 2001). Compounds were further screened for high predicted gastrointestinal absorption and absence of P-glycoprotein substrate characteristics. Predictions of blood-brain barrier permeation and cytochrome P450 (CYP) enzyme inhibition were also examined, focusing on the main metabolic isoforms: CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. Molecules identified as non-inhibitors across these enzymes without PAINS or Brenk structural alerts were prioritised for their favourable safety and pharmacokinetic profiles. This filtering process allowed the selection of candidates with optimal oral bioavailability potential and reduced likelihood of metabolic interactions.

### Molecular Docking Studies

Compounds exhibiting suitable physicochemical, pharmacokinetic, and drug-likeness profiles were

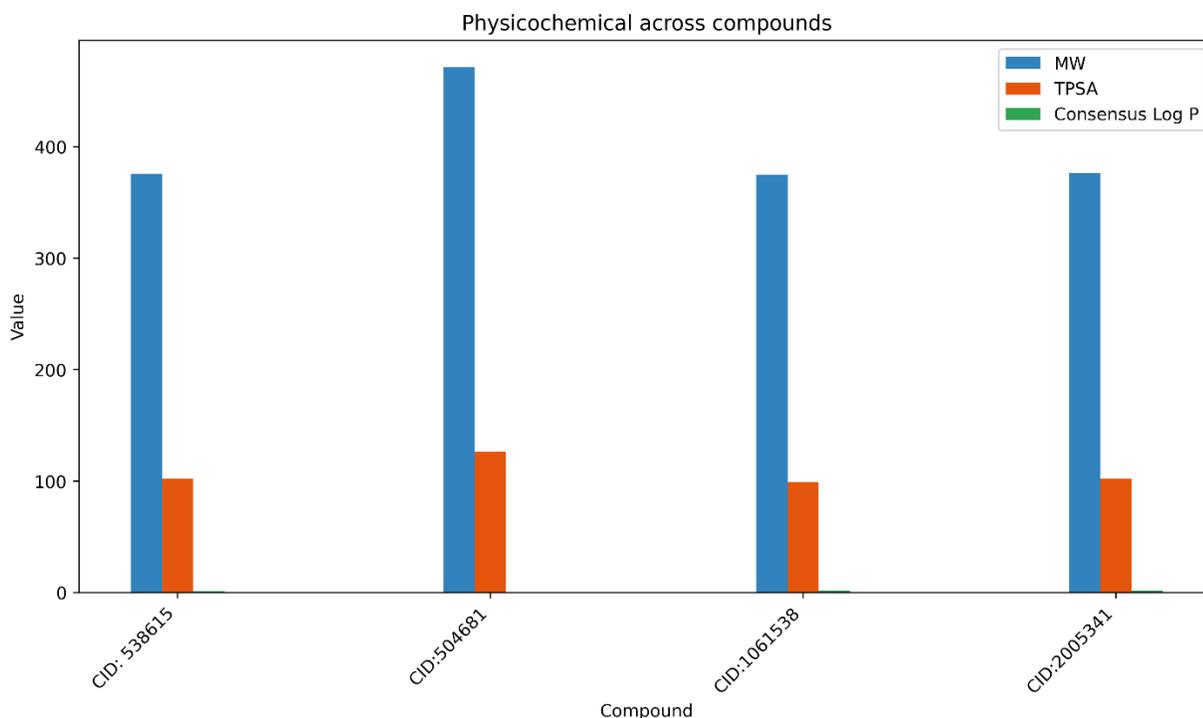
selected for docking studies. Docking was performed using AutoDock 4.2.6, applying the Lamarckian Genetic Algorithm to predict binding poses and estimate binding free energies (Morris *et al.*, 2009). The previously prepared structure of the acetylcholinesterase mutant (6ARX) was used as the receptor. Ligands were processed with Open Babel to generate energy-minimised PDBQT files with defined torsion parameters and Gasteiger charges (Gasteiger & Marsili, 1980). Docking grids were centred on the active site around Ser119 with dimensions of  $60 \times 60 \times 60$  and a spacing of 0.375 Å. Each docking run involved 100 genetic algorithm searches and 2.5 million energy evaluations to ensure adequate sampling. The resulting complexes were analysed in PyMOL for structural orientation and LigPlot+ to identify hydrogen bonding and hydrophobic contacts (Laskowski & Swindells, 2011).

## RESULTS AND DISCUSSION

### Physicochemical, Pharmacokinetics and Drug-Likeness Analysis

The virtual screening approach implemented in this study was designed to assess the potential of natural product-derived compounds to inhibit the acetylcholinesterase mutant from *Anopheles gambiae*, an enzyme linked to resistance against organophosphate and carbamate insecticides (Pang, 2006; Vidal-Albalat *et al.*, 2023). Only four met the combined physicochemical, pharmacokinetic, and drug-likeness criteria out of an initial collection of 132 structurally diverse phytochemicals retrieved from the LOTUS database (Rutz *et al.*, 2022). This marked reduction underscores both the selectivity of the screening protocol and the inherent challenge of identifying natural compounds that fit within established parameters for oral drug development (Lipinski *et al.*, 2001; Veber *et al.*, 2002).

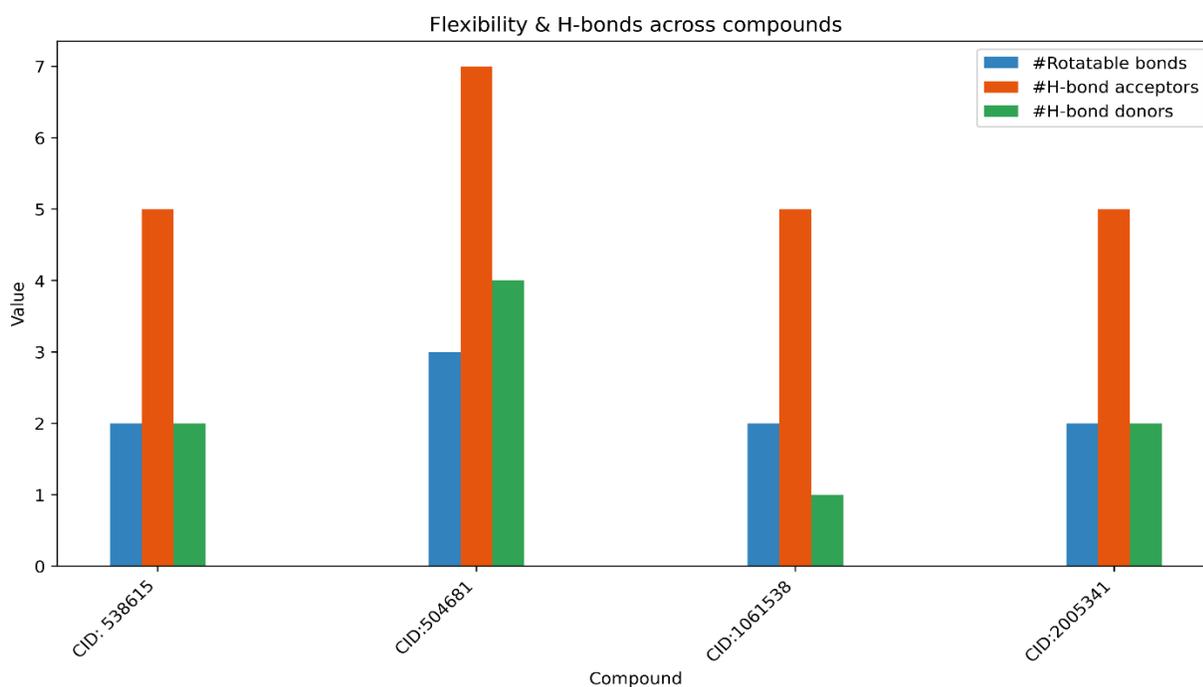
Analysis of physicochemical properties revealed that these four compounds exhibit features favourable for oral bioavailability. Their molecular weights ranged from approximately 374 to 472 Da, remaining below the commonly accepted threshold of 500 Da, which often diminishes permeability and absorption (Lipinski *et al.*, 2001). TPSA values, spanning 98 to 126 Å<sup>2</sup>, indicate sufficient polarity to maintain aqueous solubility while retaining lipophilicity necessary for effective membrane diffusion. Consensus LogP values ranged from roughly 0.3 to 2.1, suggesting a balanced hydrophilic-lipophilic profile supporting systemic exposure and transport across biological barriers (Daina *et al.*, 2017) (Figure 1)



**Figure 1:** Comparative Analysis of Molecular Weight, Topological Polar Surface Area, and Lipophilicity of Screened Phytochemicals

The compounds displayed low molecular flexibility, containing only two or three rotatable bonds. Such rigidity may favour stable binding conformations within the active site, potentially enhancing affinity by minimising entropic penalties upon complex

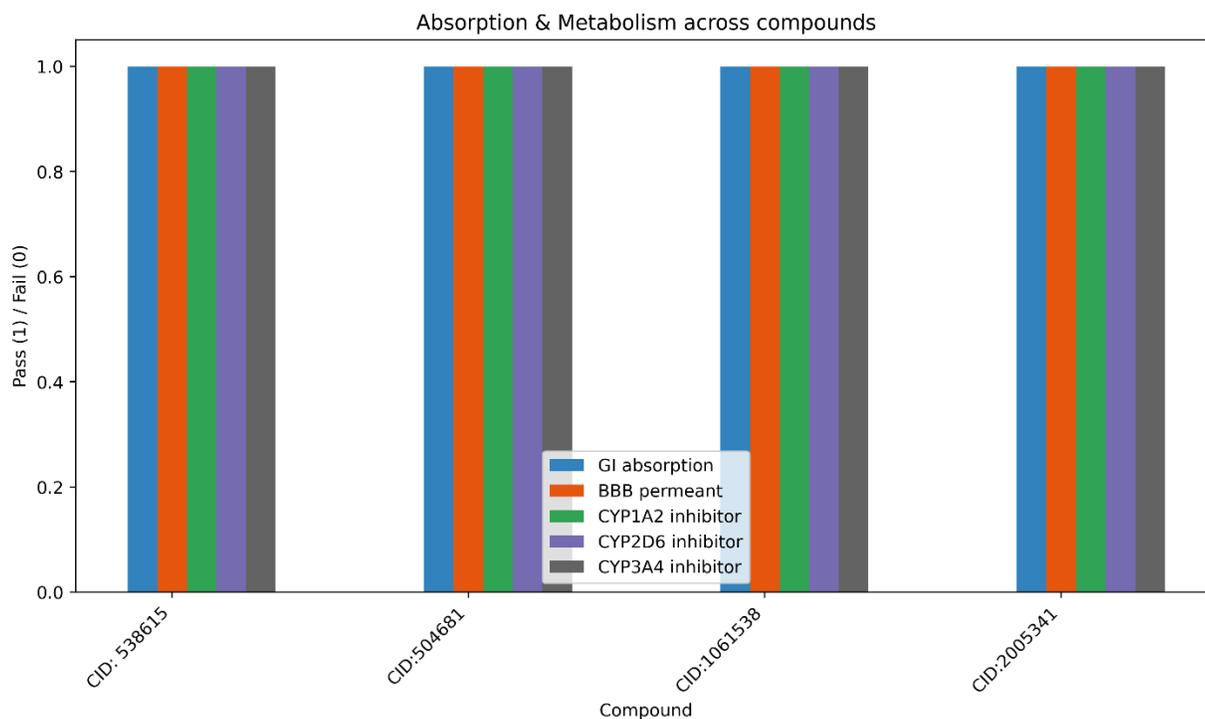
formation. Hydrogen bond acceptor counts ranged from five to seven, and donor counts from one to four, aligning with typical ranges that promote specific, stable interactions with catalytic residues in target proteins (Lipinski *et al.*, 2001) (Figure 2)



**Figure 2:** Rotational Flexibility and Hydrogen Bonding Capacity of Selected Compounds

Pharmacokinetic predictions provided further understanding of the suitability of these compounds as lead candidates. All exhibited high predicted gastrointestinal absorption, a key requirement for effective oral administration. None were predicted to cross the blood–brain barrier, reducing concerns over unintended central nervous system activity. Also, the absence of notable P-glycoprotein substrate

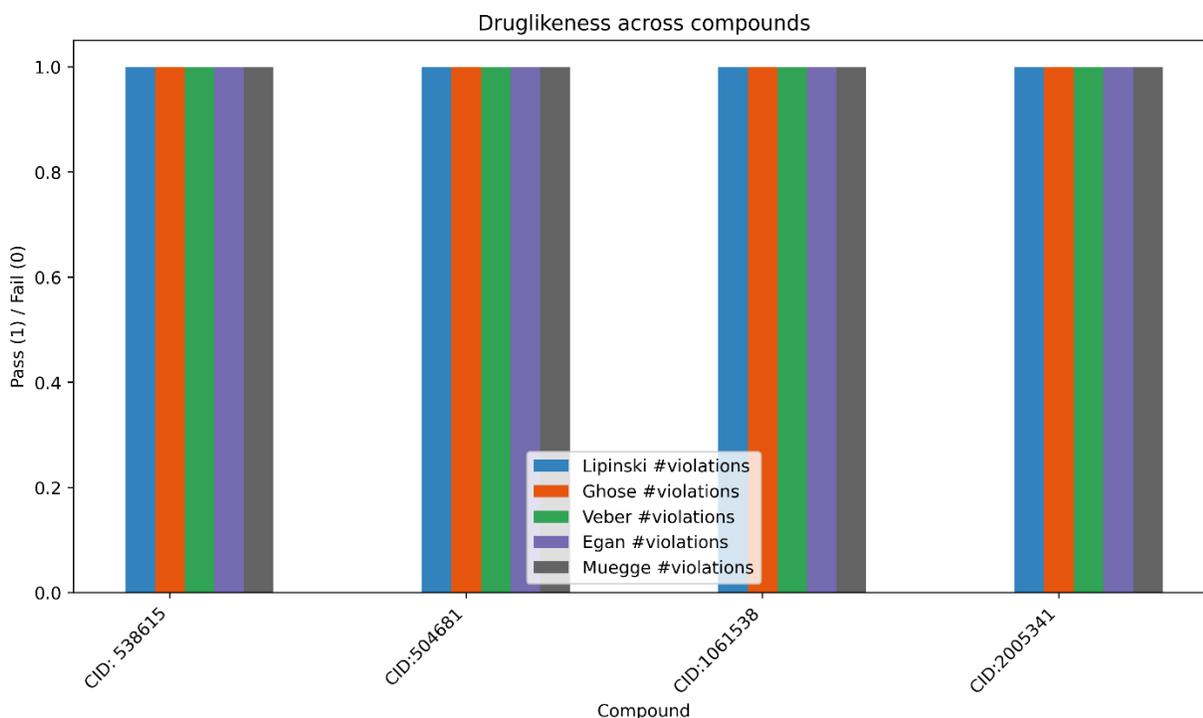
characteristics suggests a lower likelihood of efflux-related reductions in bioavailability, a standard limitation observed with complex natural products (Sharom, 2008). Metabolic predictions indicated no inhibitory effects on primary CYP isoforms, including CYP1A2, CYP2D6, and CYP3A4, suggesting minimal risk for drug–drug interactions or metabolic instability (Zanger & Schwab, 2013) (Figure 3).



**Figure 3:** Predicted Gastrointestinal Absorption, Blood–Brain Barrier Permeation, Efflux Potential, and CYP450 Interaction Profiles

All selected compounds met established drug-likeness guidelines, meeting the Lipinski, Ghose, Veber, Egan, and Muegge criteria without violations. This alignment with empirical property spaces associated with

successful oral therapeutics highlights their potential as promising candidates for further optimisation and biological evaluation (Lipinski *et al.*, 2001; Ghose *et al.*, 1999; Veber *et al.*, 2002) (Figure 4).



**Figure 4:** Evaluation of Drug-Likeness Based on Lipinski, Ghose, Veber, Egan, and Muegge Criteria

The reduction from 132 initial phytochemicals to just four candidates highlights natural products' structural diversity and frequent deviation from conventional drug-like property ranges (Rutz *et al.*, 2022). Nonetheless, these four compounds demonstrate a compelling combination of balanced physicochemical profiles, favourable absorption and metabolic predictions, and compliance with multiple drug-likeness frameworks, supporting their advancement to molecular docking and subsequent studies.

#### Molecular Docking Analysis

Following the physicochemical, pharmacokinetic, and drug-likeness screening, four phytochemicals from *Nigella sativa* advanced to molecular docking studies against the insecticide-resistant AChE1 of *Anopheles gambiae*. This progression highlights the combined necessity of structural compatibility and favourable ADME profiles in prioritizing candidates for receptor engagement (Lipinski *et al.*, 2001; Veber *et al.*, 2002). The docking simulations revealed differentiated binding affinities across the selected compounds, ranging from  $-6.90$  kcal/mol to  $-9.60$  kcal/mol (Table 1). Notably, CID:2005341 demonstrated the strongest predicted interaction energy ( $-9.60$  kcal/mol), indicative of stable complex formation driven by complementary shape and electronic characteristics between the ligand and the active site microenvironment (Morris *et al.*, 2009). This binding

energy is considerably lower (more favourable) than the threshold typically associated with spontaneous ligand accommodation (Ballester *et al.*, 2010), suggesting a strong potential for inhibitory activity.

Analysis of the interaction profiles provides an understanding of the anchoring methods employed by these phytochemicals. CID:538615 formed two hydrogen bonds with Ser283 and Tyr489 at distances of  $3.21$  Å and  $3.23$  Å, respectively. Such proximities are consistent with strong directional H-bonding that can stabilize the ligand orientation within the gorge. At the same time, an extensive network of hydrophobic interactions involving residues Trp245, Gly278, and Phe490 further supports entropic favorability (Figure 5a). These combined contacts imply a dual stabilization mechanism that balances polar and non-polar contributions.

CID:504681 also displayed dual hydrogen bonding with Ser280 and Cys447 at  $2.95$  Å and  $3.35$  Å, respectively. This is significant because the interaction of Cys447 hints at potential modulation of local structural dynamics critical for substrate accommodation, aligning with known sensitivities of AChE peripheral site residues to inhibitor binding (Pang, 2006). The ligand's encapsulation by a cluster of aromatic and aliphatic side chains, such as Tyr282 and Ile446, suggests  $\pi$ -alkyl and van der Waals

contributions that complement its polar contacts (Figure 5b).

CID:1061538 achieved a substantial binding affinity ( $-8.30$  kcal/mol) exclusively through hydrophobic contacts, lacking hydrogen bonds altogether. This highlights the pivotal role of non-polar packing and desolvation in the receptor–ligand association. Interactions with residues Tyr558, Trp686, and Arg404 likely create a hydrophobic shell around the ligand, which compensates for the absence of classical hydrogen bonding and potentially enhances residence time within the active site through reduced solvent accessibility (Figure 5c).

The most favourable binder, CID:2005341, integrated polar and non-polar stabilization techniques. It formed a hydrogen bond with Tyr282 at  $2.94$  Å while embedding within a pocket lined by hydrophobic residues, including Trp245, Phe490, and Trp441 (Figure 5d). This simultaneous exploitation of hydrogen bonding to secure positioning and hydrophobic burial to reduce desolvation penalties is a

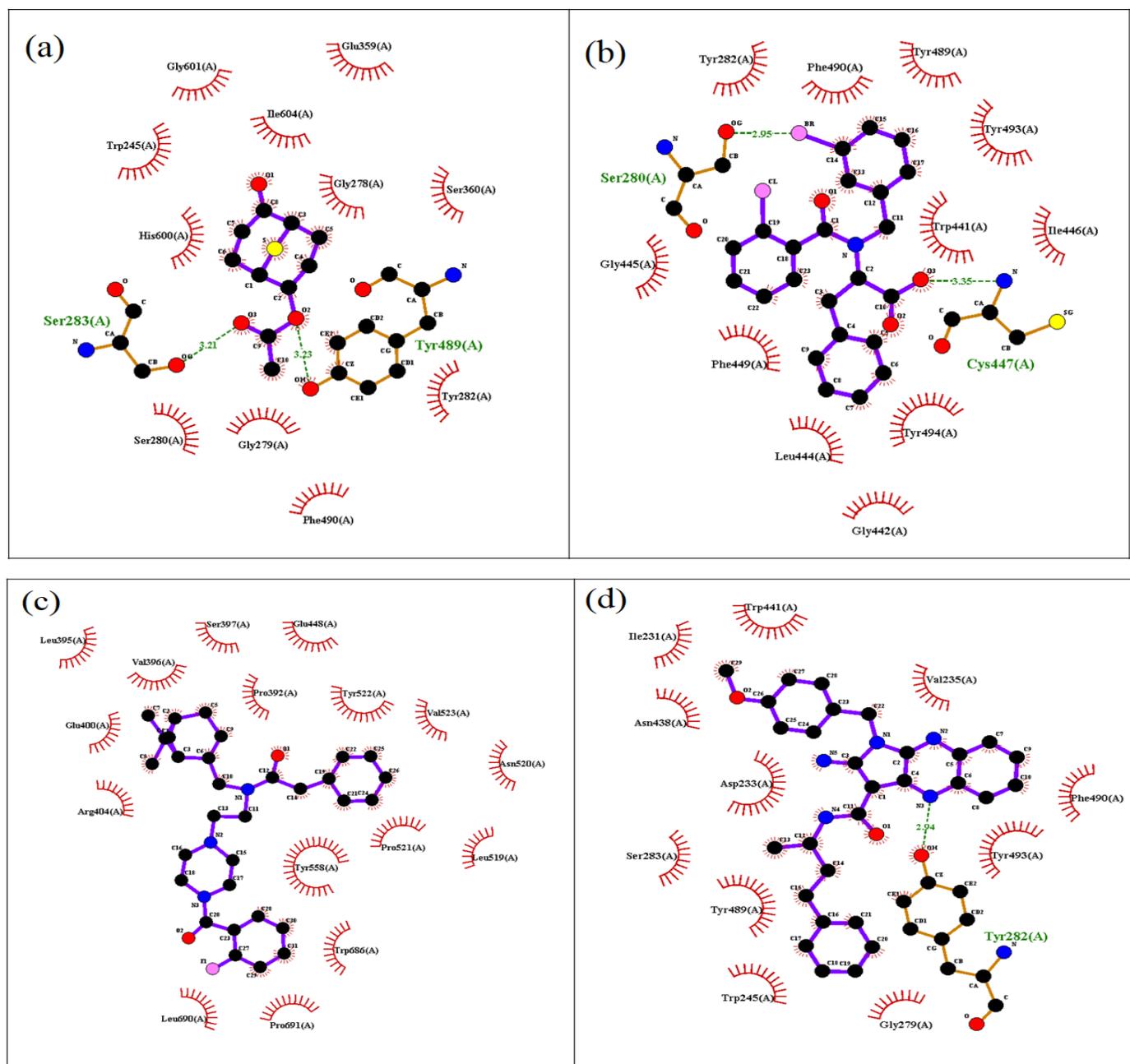
hallmark of high-affinity ligand interactions in enzyme active sites (Laskowski & Swindells, 2011). Moreover, interactions with residues Asp233 and Asn438 could confer additional electrostatic or dipolar stabilization, further rationalizing the superior docking energy.

These docking results reinforce combining ADMET-based preselection with structure-guided modelling and highlight distinct binding paradigms among structurally diverse natural compounds. The interactions observed with residues such as Ser283, Tyr489, and Trp245 align with critical catalytic and structural features reported in AChE homologues, which supports the hypothesis that these ligands may effectively disrupt the enzymatic hydrolysis of acetylcholine, a mechanism central to insecticidal action (Gorecki *et al.*, 2019; Vidal-Albalat *et al.*, 2023).

These findings suggest that select *N. sativa*-derived compounds possess structural attributes conducive to stable binding within the insecticide-resistant AChE1.

**Table 1:** Docking Scores, Hydrogen Bonding, and Hydrophobic Interaction Profiles of Selected *Nigella sativa* Compounds against Insecticide-Resistant AChE1 of *Anopheles gambiae*

S/No.	Compound	Free Binding Energy (kcal/mol)	H-Bond	Distance (Å)	Hydrophobic Interactions
1.	CID: 538615	-6.90	Ser283 Tyr489	3.21 3.23	Trp245, Gly278, Gly279, Ser280, Tyr282, Glu359, Ser360, Phe490, Gly601 and Ile604
2.	CID:504681	-7.70	Ser280 Cys447	2.95 3.35	Tyr282, Gly445, Pro449, Leu444, Gly442, Tyr494, Trp441, Ile446, Tyr493, Tyr489 and Phe490
3.	CID:1061538	-8.30	0	0	Leu395, Val396, Glu400, Arg404, Leu690, Pro691, Trp686, Tyr558, Pro521, Leu519, Asn520, Val523, Tyr522, Pro392, Glu448, and Ser397
4.	CID:2005341	-9.60	Tyr282	2.94	Ile231, Asn438, Asp233, Ser283, Tyr489, Trp245, Gly279, Tyr493, Phe490, Val235 and Trp441



**Figure 5a-d:** 2D Interaction Diagrams of the Top Docked Complexes Illustrating Hydrogen Bonds and Hydrophobic Contacts between Selected *N. sativa* Compounds and the Active Site Residues of Insecticide-Resistant AChE1

## CONCLUSION

The study identified four compounds from *N. sativa* with properties favourable for oral bioavailability, low predicted toxicity, and compatibility with drug-likeness frameworks. Docking analysis against the G119S-mutated acetylcholinesterase of *Anopheles gambiae* revealed meaningful binding energies, supported by key hydrogen bonds and hydrophobic interactions involving catalytic and peripheral site residues. These findings suggest that the selected compounds may interfere with the enzymatic function

in resistant mosquito strains. The work supports further investigation through molecular dynamics and in vitro validation to assess their potential role as components of integrated mosquito control strategies that reduce dependence on synthetic insecticides.

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