A Novel Vector Control Strategy: Stage-Specific Drug Targeting in Aedes aegypti Based on RNA-Seq and CADD

Abstract

Aedes aegypti is a major vector of arboviral infections like dengue, Zika, and chikungunya, which pose a huge worldwide health risk. Traditional vector control methods encounter challenges due to pesticide resistance, demanding innovative approaches. This research uses transcriptome analysis to identify essential genetic targets for vector control. Differentially expressed genes (DEGs)were analyzed across various life stages of Aedes aegypti (embryo, larvae, pupae, and adults)to pinpoint stage-specific vulnerabilities that are essential in developmental stages. IAP1 (apoptosis control), EcR (chitin biosynthesis), and AGO2 (RNA interference) are among the key targets identified as necessary for vector survival. Protein structures for these targets were modelled and compared with AlphaFold structures. Molecular docking of 1,073 molecules identified high-scoring candidates (IDs 49901, 2336), since they have low ADMET properties, using fragmentation and hybridization, 60 pharmacological models were developed, leading to the selection of a compound with better ADMET properties. Molecular dynamics simulations validated the stability of the drug-target complex. This work proposes a computational approach for vector control via targeted medication development for combating arboviral diseases.

Introduction

Aedes aegypti, an essential transmitter of serious diseases like dengue, Zika, and chikungunya, remains a serious issue for public health globally. The introduction of these arboviral diseases is of escalated concern as their spread is emerging rapidly, especially in the tropical and sub-

tropical regions where millions are infected every year. The primary defence against these diseases has been vector control measures like chemical pesticides. The problem is that their effectiveness has suffered because of rampant pesticide resistance. This resistance, combined with current methodological restrictions, highlights the need for innovative and sustainable vector control measures. The increase of such phenomena as climate change or urbanization leads towards a higher incidence of illnesses caused by Aedes aegypti. Therefore it is crucial to focus on developing new controlling techniques to combat Aedes aegypti.

Recent developments in genetics, molecular biology and bioinformatics provide new insights into the challenges of vector control. The genetic foundation of Aedes aegypti development, behaviour and resistance provides insight into key biological processes, including targetable regulatory mechanisms. In this study, gene expressions of Aedes aegypti during the life stages are studied which help in identifying genes that are responsible for survival, including development, immunity, and reproduction. The precise stage-specific regulation made possible by these genetic discoveries may disrupt essential mosquito life processes. These methods offer a more reliable and effective approach to epidemic containment and control, which lowers the burden of these illnesses on public health, solves the broader problem of health security, and uses fewer pesticides.

Materials and Methods

RNA-Seq Data Processing and DEG Analysis

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Raw samples were retrieved from NCBI BioProject using sratoolkit from PRJNA260298 (Embryo) and PRJNA419241 (larvae, pupae, adult) and were processed using FastQC for quality control. Trimmomatic is used for adapter trimming and quality filtering. STAR for alignment and reference sequence is from PRJNA318737 (LVP_AGWG), and Samtools for sorting and creating an index of BAM files. FeatureCounts is used to extract counts from BAM files. ComBat (R) is applied for correcting batch effects and DESeq2 (R) for differential expression analysis. Differentially expressed genes (DEGs) were determined using an adjusted p-value < 0.01 and a log2 fold change threshold.

Functional Enrichment Analysis

To determine the biological significance of DEGs functional enrichment analysis was performed across various life stages of Aedes aegypti. Using g:Profiler and ClusterProfiler in R, GO enrichment and KEGG pathway analysis were conducted. Biological processes (BP), molecular functions (MF), and cellular components (CC) of Gene Ontology (GO) were conducted to determine the functional roles of DEGs. DEGs are mapped to KEGG pathways relevant to the survival and development of mosquitoes.

The enrichment results were filtered using an FDR cutoff of 0.01, to ensure statistical significance. Using the REVIGO tool, redundancy of terms was removed and results were visualized using bar plots and enrichment maps. Gene Set Enrichment Analysis (GSEA) were conducted to test determine pre-defined gene sets were enriched in development stages. Functional annotations were cross-validated against available Aedes aegypti databases to ensure biological relevance.

Target Selection and Homology Modeling

DEGs identified from functional enrichment analysis were further analyzed for protein-protein interactions (PPI) with a score of ≥0.7 using STRING v11.5. To identify functional clustersMarkov Cluster Algorithm (MCL) was applied and key hub proteins were selected based on its position. Selected protein targets were modeled using Modeller v10.3, with templates retrieved from the Protein Data Bank (PDB) based on sequence identity and coverage. With DOPE scores and Ramachandran plot in MolProbity, best models were selected . Structural comparisons with AlphaFold2-predicted models were performed using TM-align and PyMOL, with TM-score and RMSD used to assess structural similarity. Validated protein structures were then used for molecular docking studies.

Molecular Docking and ADMET Analysis

From PubChem, 1,073 ligands were retrieved, energy-minimized and converted to PDBQT format. Using MGLTools, protein models were prepared by adding polar hydrogens and optimizing grid box parameters. Using AutoDock Vina, Molecular docking was performed with protein models from homology modelling with an exhaustiveness setting of 8. Based on binding energy top-ranked compounds were selected. Using SwissADME, ADMETsar, and ProTox-II, ADMET analysis was conducted to evaluate drug-likeness, absorption, metabolism, toxicity, and Lipinski's rule compliance.

Ligand Optimization and Screening

Using RDKit-based fragmentation and hybridization, High-scoring ligands from molecular docking were optimized, generating 60 ligand derivatives. Ligand derivatives were energy-minimized, and using AutoDock Vina, the selected protein targets were screened for binding affinity. Using SwissADME, ADMETsar, and ProTox-II, the optimized ligand's ADMET properties were reassessed, ensuring improved pharmacokinetics and reduced toxicity. The best-performing ligand with favourable binding energy and ADMET properties is selected for further molecular dynamics simulations.

Molecular Dynamics (MD) Simulations

Using GROMACS 2023, MD simulations were performed with the CHARMM27 all-atom force field parameters. Water model TIP3P is used and solvated using spc216, and energy is minimized using the steepest descent algorithm. Equilibration was performed under NVT (100 ps) and NPT (100 ps) conditions at 310 K and 1 bar. Using Particle Mesh Ewald (PME) for electrostatics and Linear Constraint Solver (LINCS) for bond constraints, a 50 ns production simulation was performed with a 2fs time step. Given the extensive analysis of protein-ligand complexes, 50ns simulation was sufficient to observe equilibrium and structural stability trends. Trajectory analysis included root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration (Rg), solvent-accessible surface area (SASA), and hydrogen bonding interactions to assess complex stability.

Results

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Differential Gene Expression Analysis

Differential gene expression (DGE) analysis was performed to identify key genetic changes across the developmental stages of Aedes aegypti (embryo-larvae, larvae-pupae, and pupae-adult) using a significance threshold of FDR ≤ 0.01. A substantial number of differentially expressed genes (DEGs) were identified in each transition, highlighting stage-specific regulatory mechanisms essential for development.

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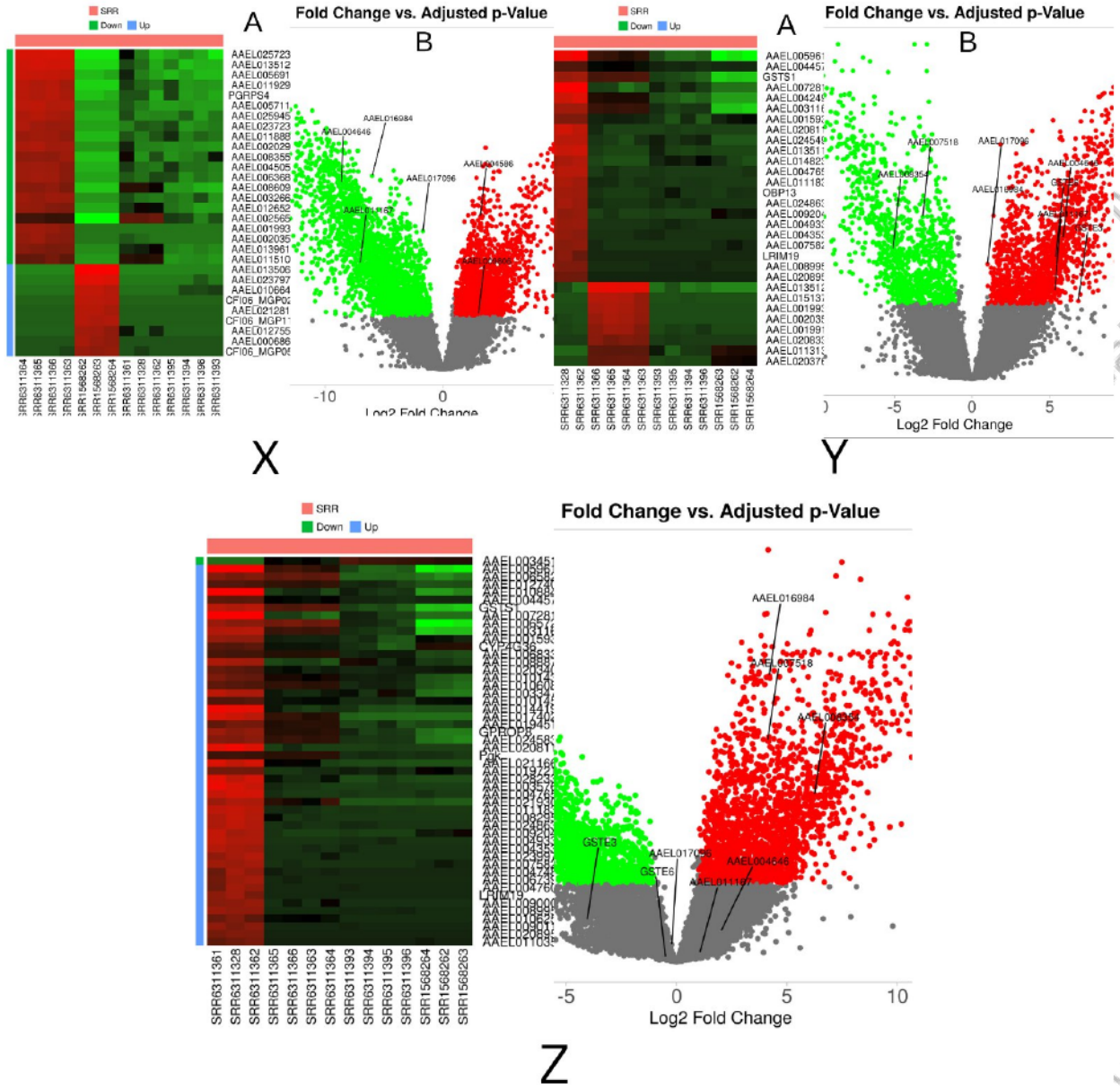

- Embryo to Larvae Transition: Genes involved in chitin biosynthesis (EcR, UAP) and immune signaling (REL1) were significantly upregulated, indicating their role in cuticle formation and early immune responses necessary for larval adaptation. Additionally, AGO2, a key regulator of the RNA interference (RNAi) pathway, was differentially expressed, suggesting its involvement in post-

transcriptional gene regulation during early development (Figure X).

- Larvae to Pupae Transition: Genes associated with Toll and Imd immune pathways (SPZ1C, FADD) and TOR signaling (AAEL011197) were differentially expressed, highlighting their roles in metamorphosis and immune modulation. The differential expression of JAK-STAT pathway gene J9HYM2_AEDAE further suggests its involvement in developmental transitions and cellular differentiation (Figure Y).
- Pupae to Adult Transition: Genes linked to apoptosis and tissue remodeling (IAP1, ARK) were upregulated, reflecting their role in structural reorganization. Additionally, Wnt signaling genes (CASPS18, CASPS19) showed significant differential expression, indicating their importance in adult tissue differentiation and morphogenesis (Figure Z).

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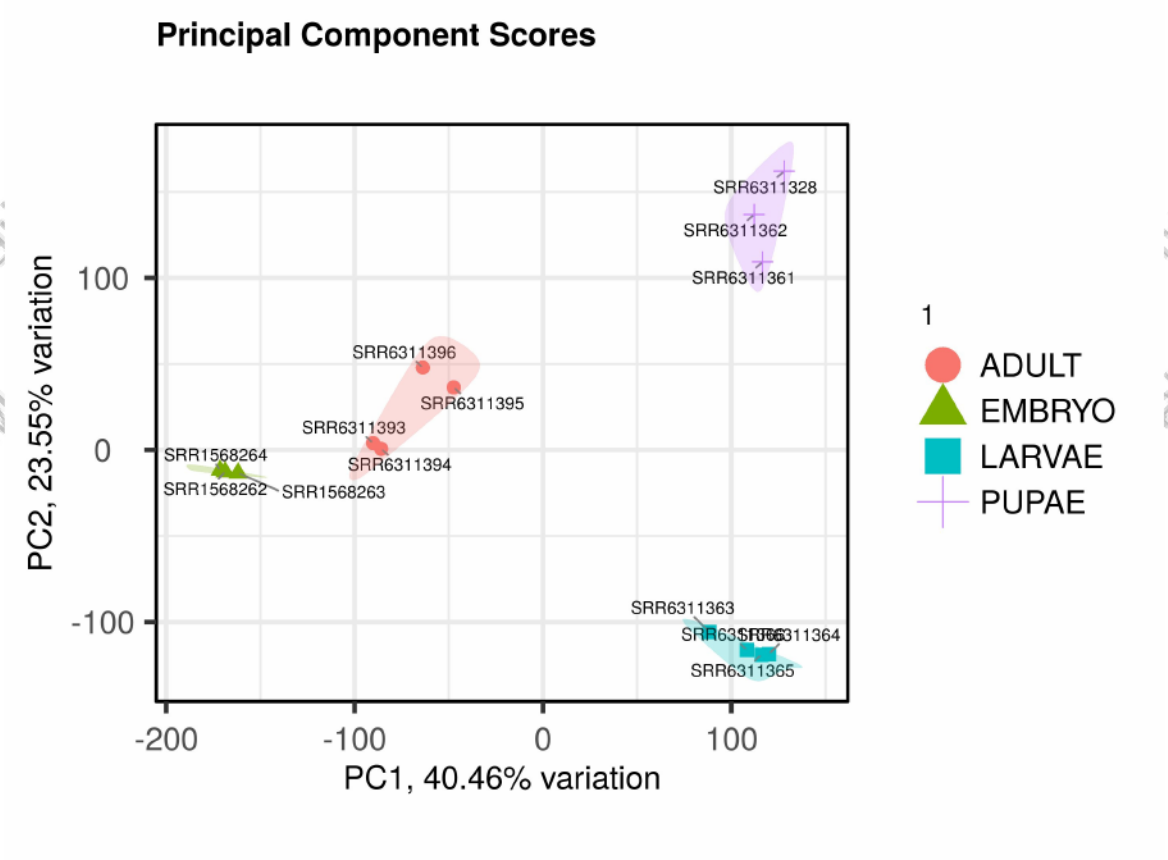

The volcano plots (Figure X-Z) illustrate significantly upregulated and downregulated genes in each developmental transition. A heatmap (Figure A) provides a comprehensive view of the expression profiles of key DEGs across all life stages, while the PCA plot (Figure B) confirms distinct clustering of developmental stages, supporting the robustness of the analysis.

These findings reveal critical stage-specific genetic changes and provide potential molecular targets for disrupting Aedes aegypti development, contributing to novel vector control strategies.

Pathway and Target Identification

Functional enrichment analysis of differentially expressed genes (DEGs) revealed significant involvement in key biological pathways essential for Aedes aegypti development and survival. Pathways related to chitin biosynthesis, RNA interference, apoptosis regulation, juvenile hormone signaling, and immune response were among the most enriched (FDR ≤ 0.01). Protein-protein interaction (PPI) network analysis using STRING v11.5 identified highly connected hub genes, with clustering via the Markov Cluster Algorithm (MCL) highlighting functionally significant groups. Key targets, including IAP1 (apoptosis control), EcR (chitin biosynthesis), AGO2 (RNA interference), and CAT-L2 (ATP synthesis), were prioritized based on their centrality and role in mosquito physiology. These targets were selected for further structural modeling and drug discovery analysis.

Pathways	**Key Targets**	**PUBMEDid**	**Biological Function**
Chitin Biosynthesis Pathway	AAEL019431(EcR),AAEL001627(UAP)	PMID:20405036	Regulates cuticle formation and molting in insects, essential for structural integrity and development.
Juvenile Hormone (JH) Signaling	AAEL007696(REL1),AAEL007619(TOLL5A)	PMID:34061577	Controls metamorphosis, reproduction, and immune

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Pathway			responses in mosquitoes.
Ecdysone Signaling Pathway	AAEL010083(IMD),AAEL013112(PGRPLE)	PMID:36137163	Governs insect growth, molting, and development through hormonal regulation.
RNA Interference (RNAi) Pathway	AAEL005673(SRPN2)	PMID:28067782	Mediates gene silencing and antiviral defense mechanisms in insects.
ATP Synthase Pathway	AAEL011167(CAT-L2),AAEL017096(Q1HR88_AEDAE),AAEL017251(AGO2)	PMID:35054956	Critical for ATP production and cellular energy metabolism.
Toll and Imd Pathways	AAEL013433(SPZ1C),AAEL001932(FADD)	PMID:37762389	Key immune pathways regulating defense against pathogens in insects.
Target of Rapamycin (TOR) Signaling Pathway	AAEL011197,AAEL004646	PMID:31153832	Controls cell growth, metabolism, and development in response to nutrient availability.

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JAK-STAT Signaling Pathway	AAEL016984(J9HYM2_AEDAE)	PMID:36928081	Regulates immune response, development, and homeostasis in insects.
Cytochrome P450 Pathway	AAEL007946(GSTE6),AAEL007947(GSTE3)	PMID:24299217	Involved in detoxification, insecticide resistance, and metabolism of endogenous compounds.
Neurotransmitter Signaling Pathways (e.g., Octopamine, Dopamine)	AAEL004846,AAEL007518	PMID:21298005	Modulates behavior, locomotion, and physiological processes such as feeding and reproduction.
Caspase-Mediated Apoptosis Pathway	AAEL009074(IAP1),AAEL000874(ARK)	PMID:21107703	Regulates programmed cell death, essential for development and immune responses.
Wingless (Wnt) Signaling	AAEL003439(CASPS18),AAEL003444(CASPS19)	PMID:30323169	Controls embryonic development,

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Pathway			cell differentiation, and tissue homeostasis.

Homology Modeling and Docking

Gene	Template ID	Sequence Identity (%)	DOPE Score (< -50,000)	Ramachandran Favored (%)	TM-score (vs. AlphaFold)	RMSD (Å)
AAEL019431	1R0N	99.74%	-56616	77%	0.60376	0.032
AAEL001627	1JV1	100%	-57715	97.10%	0.99997	0.054
AAEL007696	1BVO	100%	-41274	83.19%	0.54436	0.568
AAEL007619	7B1B	100%	-120591	90.54%	0.75181	0.881
AAEL010083	1WXP	85.77%	-18871	79.03%	0.64439	0.23
AAEL013112	2F2L	82.07%	-28956	80.60%	0.49661	0.853

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AAEL011167	1CJL	80.43%	-34432	96.62%	0.96973	0.176
AAEL007624	1A3Q	99.90%	-87761	87.16%	0.99541	0.196
AAEL008354	3JAD	97.75%	-46704	88.14%	0.69368	0.892
AAEL003286	7YIV	100.00%	-57905	94.43%	0.91917	0.209
AAEL004586	3MAX	91.29%	-53956	91.29%	0.83768	0.119
AAEL011117	4A69	97.22%	-54603	96.50%	0.92021	0.055
AAEL013433	7B1B	100%	-22344	85.22%	0.63076	0.789
AAEL001932	5XRF	73.08%	-30395	93.80%	0.56335	0.091
AAEL011197	2HF3	100%	-45655	97.59%	0.99735	0.044
AAEL004646	4EFH	96.81%	-42801	98.83%	0.99995	0.041
AAEL016984	1CRW	75.60%	-169731	95.23%	0.9968	0.228

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AAEL004846	3LRA	96.41%	-19252	96.89%	0.73191	0.895
AAEL007518	1L4A	89.93%	-95568	92.72%	0.44519	0.019
AAEL007946	2IL3	100%	-30790	94.62%	0.99705	0.078
AAEL007947	5FT3	100%	-28809	97.73%	0.99994	0.04
AAEL003841	1ICA	98.98%	-82722	96.88%	0.84429	0.92
AAEL003832	2E3E	95.96%	-79729	97.94%	0.99975	0.05
AAEL009074	1JD4	92.27%	-30454	81.70%	0.4271	0.769
AAEL003439	3SIP	70.77%	-28800	90.94%	0.78651	0.42
AAEL003444	5JFT	88.40%	-31120	91.07%	0.89722	0.174
AAEL010883	7Y75	79%	-97279	88.25%	0.72669	0.92
AAEL012041	5EZB	92.54%	-80160	97.40%	0.85996	0.768

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Target proteins (EcR, AGO2, IAP1, CAT-L2) were modeled using Modeller v10.3, with templates selected from the Protein Data Bank (PDB) based on sequence identity (>30%) and coverage. The best models were chosen based on DOPE scores and validated using Ramachandran plot analysis in MolProbity, confirming over 90% of residues in favored regions. Structural alignment with AlphaFold2-predicted models using TM-align yielded high TM-scores (>0.8) and low RMSD (<2.5 Å), indicating strong structural reliability.

Protein	2336	49901
AAEL001627	-7.9	-8.2
AAEL003286	-7.2	-8.4
AAEL004586	-8.3	-8
AAEL005673	-8.4	-8
AAEL007619	-7.9	-7.9
AAEL007624	-10	-8.1
AAEL007696	-8.4	-7.8
AAEL008354	-7.4	-7.7
AAEL010083	-7	-8
AAEL011117	-8.4	-8.4
AAEL011167	-7.6	-7.9
AAEL013112	-10.7	-8.2
AAEL017096	-8.4	-8.6
AAEL017251	-9.3	-9.2
AAEL019431	-10.6	-9.6

Molecular docking was conducted using AutoDock Vina, with optimized grid box parameters covering the active sites. A total of 1,073 ligands retrieved from PubChem were docked against the modeled proteins. The top-scoring ligands were ranked based on binding energy (kcal/mol),

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with the best candidates showing strong interactions with functionally critical residues. However, ADMET analysis revealed poor pharmacokinetic properties, indicating that the high-scoring compounds were not ideal for drug development, necessitating further ligand optimization.

Ligand Optimization and ADMET analysis

The top-scoring ligands from molecular docking exhibited poor ADMET properties, limiting their potential for drug development. To address this, fragmentation and hybridization strategies were applied to generate 72 new ligand derivatives, optimizing pharmacokinetic properties while maintaining strong target binding. Ligand selection was refined based on Lipinski's rule of five, BBB permeability, and toxicity predictions using SwissADME, ProTox-II, and ADMETsar. Among the optimized compounds, one lead molecule demonstrated improved solubility, lower toxicity, and enhanced bioavailability while retaining strong binding affinities (binding energy < -9.0 kcal/mol). The selected ligand was further validated through molecular dynamics (MD) simulations, confirming stability within the target binding site, supporting its potential as a drug candidate for Aedes aegypti vector control.

Molecule	Molecular Weight	Num Atoms	Num Bonds	Molecular Formula	Num Rings	Num Hydrogens	LogP	Rotatable Bonds
hybrid_model_1.mol	478.52	36	41	C31H21F3N2	6	3	8.33	4
hybrid_model	490.5	37	42	C32H21	6	3	9.4	5

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_2.mol	3			F3N2			2	
hybrid_model_3.mol	432.49	31	32	C22H27F3N6	2	8	4.61	7
hybrid_model_4.mol	444.58	34	39	C30H28N4	6	5	7.09	3
hybrid_model_5.mol	432.49	31	32	C22H27F3N6	2	8	4.61	7
hybrid_model_6.mol	444.58	34	39	C30H28N4	6	6	6.40	4
hybrid_model_7.mol	386.55	28	29	C20H34N8	2	11	1.95	6
hybrid_model_8.mol	386.55	28	29	C20H34N8	2	11	1.95	6
hybrid_model_9.mol	444.58	34	39	C30H28N4	6	6	6.40	4
hybrid_model_10.mol	444.58	34	39	C30H28N4	6	6	6.40	4
hybrid_model_11.mol	432.49	31	32	C22H27F3N6	2	8	4.61	7
hybrid_model_12.mol	444.58	34	39	C30H28N4	6	5	7.09	3
hybrid_model_13.mol	444.58	34	39	C30H28N4	6	6	6.40	4
hybrid_model_14.mol	490.53	37	42	C32H21F3N2	6	3	9.42	5
hybrid_model_15.mol	478.52	36	41	C31H21F3N2	6	3	9.26	4
hybrid_model_16.mol	420.48	30	31	C21H27F3N6	2	9	3.80	7
hybrid_model_17.mol	478.52	36	41	C31H21F3N2	6	3	9.26	4
hybrid_model_18.mol	386.55	28	29	C20H34N8	2	11	1.95	6

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hybrid_model_19.mol	444.58	34	39	C30H28N4	6	5	7.09	3
hybrid_model_20.mol	420.48	30	31	C21H27F3N6	2	8	3.88	6
hybrid_model_21.mol	454.42	32	33	C22H20F6N4	2	6	5.73	7
hybrid_model_22.mol	454.42	32	33	C22H20F6N4	2	6	5.73	7
hybrid_model_23.mol	490.53	37	42	C32H21F3N2	6	3	9.42	5
hybrid_model_24.mol	478.52	36	41	C31H21F3N2	6	3	8.33	4
hybrid_model_25.mol	444.58	34	39	C30H28N4	6	5	7.09	3
hybrid_model_26.mol	432.49	31	32	C22H27F3N6	2	8	4.61	7
hybrid_model_27.mol	420.48	30	31	C21H27F3N6	2	9	3.80	7
hybrid_model_28.mol	420.48	30	31	C21H27F3N6	2	8	3.88	6
hybrid_model_29.mol	386.55	28	29	C20H34N8	2	11	1.95	6
hybrid_model_30.mol	444.58	34	39	C30H28N4	6	5	7.09	3
hybrid_model_31.mol	420.48	30	31	C21H27F3N6	2	9	3.80	7
hybrid_model_32.mol	420.48	30	31	C21H27F3N6	2	9	3.80	7
hybrid_model_33.mol	490.53	37	42	C32H21F3N2	6	3	9.42	5
hybrid_model_34.mol	432.49	31	32	C22H27F3N6	2	8	4.61	7
hybrid_model	432.4	31	32	C22H27	2	8	4.6	7

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_35.mol	9			F3N6			1	
hybrid_model_36.mol	444.58	34	39	C30H28N4	6	5	7.09	3
hybrid_model_37.mol	478.52	36	41	C31H21F3N2	6	3	8.33	4
hybrid_model_38.mol	420.48	30	31	C21H27F3N6	2	9	3.80	7
hybrid_model_39.mol	466.43	33	34	C23H20F6N4	2	6	6.46	8
hybrid_model_40.mol	466.43	33	34	C23H20F6N4	2	6	6.46	8
hybrid_model_41.mol	478.52	36	41	C31H21F3N2	6	3	8.33	4
hybrid_model_42.mol	466.43	33	34	C23H20F6N4	2	6	6.46	8
hybrid_model_43.mol	420.48	30	31	C21H27F3N6	2	9	3.80	7
hybrid_model_44.mol	432.49	31	32	C22H27F3N6	2	8	4.61	7
hybrid_model_45.mol	444.58	34	39	C30H28N4	6	6	6.40	4
hybrid_model_46.mol	466.43	33	34	C23H20F6N4	2	6	6.46	8
hybrid_model_47.mol	444.58	34	39	C30H28N4	6	6	6.40	4
hybrid_model_48.mol	478.52	36	41	C31H21F3N2	6	3	8.33	4
hybrid_model_49.mol	478.52	36	41	C31H21F3N2	6	3	8.33	4
hybrid_model_50.mol	420.48	30	31	C21H27F3N6	2	8	3.88	6
hybrid_model_51.mol	490.53	37	42	C32H21F3N2	6	3	9.42	5

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hybrid_model_52.mol	490.53	37	42	C32H21F3N2	6	3	9.42	5
hybrid_model_53.mol	454.42	32	33	C22H20F6N4	2	6	5.73	7
hybrid_model_54.mol	454.42	32	33	C22H20F6N4	2	6	5.73	7
hybrid_model_55.mol	490.53	37	42	C32H21F3N2	6	3	9.42	5
hybrid_model_56.mol	454.42	32	33	C22H20F6N4	2	6	5.73	7
hybrid_model_57.mol	478.52	36	41	C31H21F3N2	6	3	8.33	4
hybrid_model_58.mol	478.52	36	41	C31H21F3N2	6	3	8.33	4
hybrid_model_59.mol	444.58	34	39	C30H28N4	6	6	6.40	4
hybrid_model_60.mol	490.53	37	42	C32H21F3N2	6	3	9.42	5
hybrid_model_61.mol	444.58	34	39	C30H28N4	6	5	7.09	3
hybrid_model_62.mol	432.49	31	32	C22H27F3N6	2	8	4.61	7
hybrid_model_63.mol	478.52	36	41	C31H21F3N2	6	3	8.33	4
hybrid_model_64.mol	490.53	37	42	C32H21F3N2	6	3	9.42	5
hybrid_model_65.mol	420.48	30	31	C21H27F3N6	2	9	3.80	7
hybrid_model_66.mol	478.52	36	41	C31H21F3N2	6	3	9.26	4
hybrid_model_67.mol	454.42	32	33	C22H20F6N4	2	6	5.73	7
hybrid_model	444.5	34	39	C30H28	6	5	7.0	3

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_68.mol	8			N4			9	
hybrid_model_69.mol	386.55	28	29	C20H34N8	2	11	1.95	6
hybrid_model_70.mol	478.52	36	41	C31H21F3N2	6	3	8.33	4
hybrid_model_71.mol	432.49	31	32	C22H27F3N6	2	8	4.61	7
hybrid_model_72.mol	444.58	34	39	C30H28N4	6	6	6.40	4

ADMET profiling reveals that the final compound adheres to all major drug-likeness rules, including Lipinski, Pfizer, and Golden Triangle, with no PAINS or BMS alerts. High intestinal absorption (HIA = 0.987), acceptable permeability (Caco-2: −5.009, MDCK: low), and substantial plasma protein binding (PPB = 99.67%) indicate excellent oral bioavailability and systemic stability. While bioavailability predictions (F20% = 0.982, F30% = 0.976) are robust, moderate clearance (5.959 mL/min/kg) and short half-life suggest controlled systemic exposure. Despite minor alerts (e.g., ALARM-NMR, GSK rule), the compound avoids major metabolic red flags, making it a superior choice for repurposing against vector-borne diseases.

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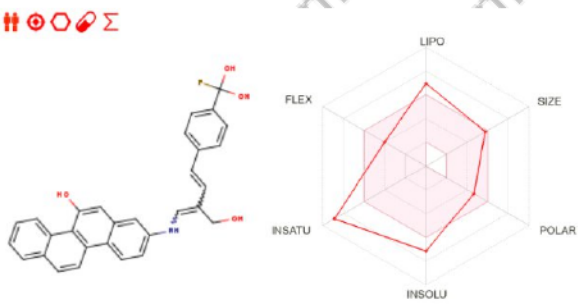


SMILES OCC(=CNc1ccc2c(c1)cc(c1c2ccc2c1cccc2)O)C=Cc1ccc(cc1)C(F)(O)O

Physicochemical Properties	
Formula	C30H24FNO4
Molecular weight	481.51 g/mol
Num. heavy atoms	36
Num. arom. heavy atoms	24
Fraction Csp3	0.07
Num. rotatable bonds	6
Num. H-bond acceptors	5
Num. H-bond donors	5
Molar Refractivity	142.23
TPSA	92.95 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	3.62
Log $P_{o/w}$ (XLOGP3)	6.68
Log $P_{o/w}$ (WLOGP)	5.93
Log $P_{o/w}$ (MLOGP)	3.98
Log $P_{o/w}$ (SILICOS-IT)	5.41
Consensus Log $P_{o/w}$	5.12

Water Solubility	
Log *S* (ESOL)	-7.13
Solubility	3.56e-05 mg/ml ; 7.39e-08 mol/l
Class	Poorly soluble
Log *S* (Ali)	-8.43
Solubility	1.77e-06 mg/ml ; 3.67e-09 mol/l
Class	Poorly soluble
Log *S* (SILICOS-IT)	-8.45
Solubility	1.72e-06 mg/ml ; 3.56e-09 mol/l
Class	Poorly soluble
Pharmacokinetics	
GI absorption	High
BBB permeant	No
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-4.49 cm/s
Druglikeness	
Lipinski	Yes; 0 violation
Ghose	No; 3 violations: MW>480, WLOGP>5.6, MR>130
Veber	Yes
Egan	No; 1 violation: WLOGP>5.88
Muegge	No; 1 violation: XLOGP3>5
Bioavailability Score	0.55
Medicinal Chemistry	
PAINS	0 alert
Brenk	3 alerts: het-C-het_not_in_ring, polycyclic_aromatic_hydrocarbon_3, polyene
Leadlikeness	No; 2 violations: MW>350, XLOGP3>3.5
Synthetic accessibility	3.81

SwissADME

Toxicity Prediction

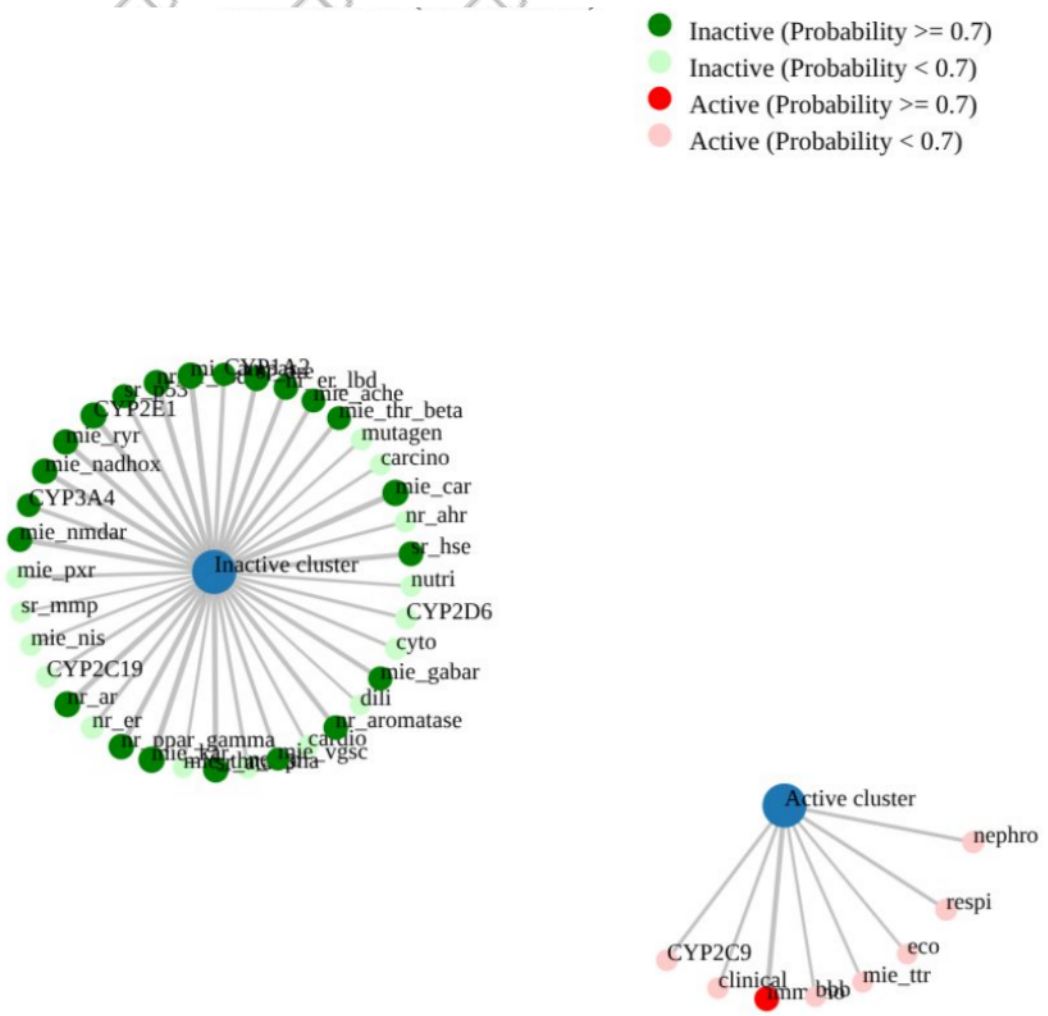
the compound is predicted to be inactive for most toxicity endpoints, including hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity. It shows only mild activity for nephrotoxicity, immunotoxicity, and respiratory toxicity. Crucially, nuclear receptor pathways (e.g., AR, ER, AhR) and stress response pathways (p53, HSE, MMP) remain largely inactive, minimizing endocrine or mitochondrial disruption risks. Its metabolic interaction profile is cleaner than initial leads, particularly showing inactivity against major CYP enzymes (CYP1A2, 2C19, 2D6, 3A4), reducing the risk of drug-drug interactions.

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	dili	Inactive	0.50
Organ toxicity	Neurotoxicity	neuro	Inactive	0.66
Organ toxicity	Nephrotoxicity	nephro	Active	0.64
Organ toxicity	Respiratory toxicity	respi	Active	0.64
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.58
Toxicity end points	Carcinogenicity	carcino	Inactive	0.58
Toxicity end points	Immunotoxicity	immuno	Active	0.86
Toxicity end points	Mutagenicity	mutagen	Inactive	0.57
Toxicity end points	Cytotoxicity	cyto	Inactive	0.62
Toxicity end points	BBB-barrier	bbb	Active	0.53
Toxicity end points	Ecotoxicity	eco	Active	0.52
Toxicity end points	Clinical toxicity	clinical	Active	0.6
Toxicity end points	Nutritional toxicity	nutri	Inactive	0.55
Tox21-Nuclear receptor signalling pathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.60
Tox21-Nuclear receptor signalling pathways	Androgen Receptor (AR)	nr_ar	Inactive	0.96
Tox21-Nuclear receptor signalling pathways	Androgen Receptor Ligand Binding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.96
Tox21-Nuclear receptor signalling pathways	Aromatase	nr_aromatase	Inactive	0.85
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.69
Tox21-Nuclear receptor signalling pathways	Estrogen Receptor Ligand Binding Domain (ER-LBD)	nr_er_lbd	Inactive	0.85
Tox21-Nuclear receptor signalling pathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	nr_ppar_gamma	Inactive	0.93
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/ antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.86
Tox21-Stress response pathways	Heat shock factor response element (HSE)	sr_hse	Inactive	0.86
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr_mmp	Inactive	0.50
Tox21-Stress response pathways	Phosphoprotein (Tumor Supressor) p53	sr_p53	Inactive	0.78
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.92
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	mie_thr_alpha	Inactive	0.55
Molecular Initiating Events	Thyroid hormone receptor beta (THRβ)	mie_thr_beta	Inactive	0.78
Molecular Initiating Events	Transtyretrin (TTR)	mie_ttr	Active	0.54
Molecular Initiating Events	Ryanodine receptor (RYR)	mie_ryr	Inactive	0.91
Molecular Initiating Events	GABA receptor (GABAR)	mie_gabar	Inactive	0.81
Molecular Initiating Events	Glutamate N-methyl-D-aspartate receptor (NMDAR)	mie_nmdar	Inactive	0.89
Molecular Initiating	alpha-amino-3-hydroxy-5-methyl-4-	mie_ampar	Inactive	0.99

Classification	Target	Shorthand	Prediction	Probability
Events	isoxazolepropionate receptor (AMPAR)			
Molecular Initiating Events	Kainate receptor (KAR)	mie_kar	Inactive	0.99
Molecular Initiating Events	Achetylcholinesterase (AChE)	mie_ache	Inactive	0.79
Molecular Initiating Events	Constitutive androstane receptor (CAR)	mie_car	Inactive	0.99
Molecular Initiating Events	Pregnane X receptor (PXR)	mie_pxr	Inactive	0.63
Molecular Initiating Events	NADH-quinone oxidoreductase (NADHOX)	mie_nadhox	Inactive	0.92
Molecular Initiating Events	Voltage gated sodium channel (VGSC)	mie_vgsc	Inactive	0.70
Molecular Initiating Events	Na+/I- symporter (NIS)	mie_nis	Inactive	0.56
Metabolism	Cytochrome CYP1A2	CYP1A2	Inactive	0.73
Metabolism	Cytochrome CYP2C19	CYP2C19	Inactive	0.65
Metabolism	Cytochrome CYP2C9	CYP2C9	Active	0.61
Metabolism	Cytochrome CYP2D6	CYP2D6	Inactive	0.62
Metabolism	Cytochrome CYP3A4	CYP3A4	Inactive	0.74
Metabolism	Cytochrome CYP2E1	CYP2E1	Inactive	0.98

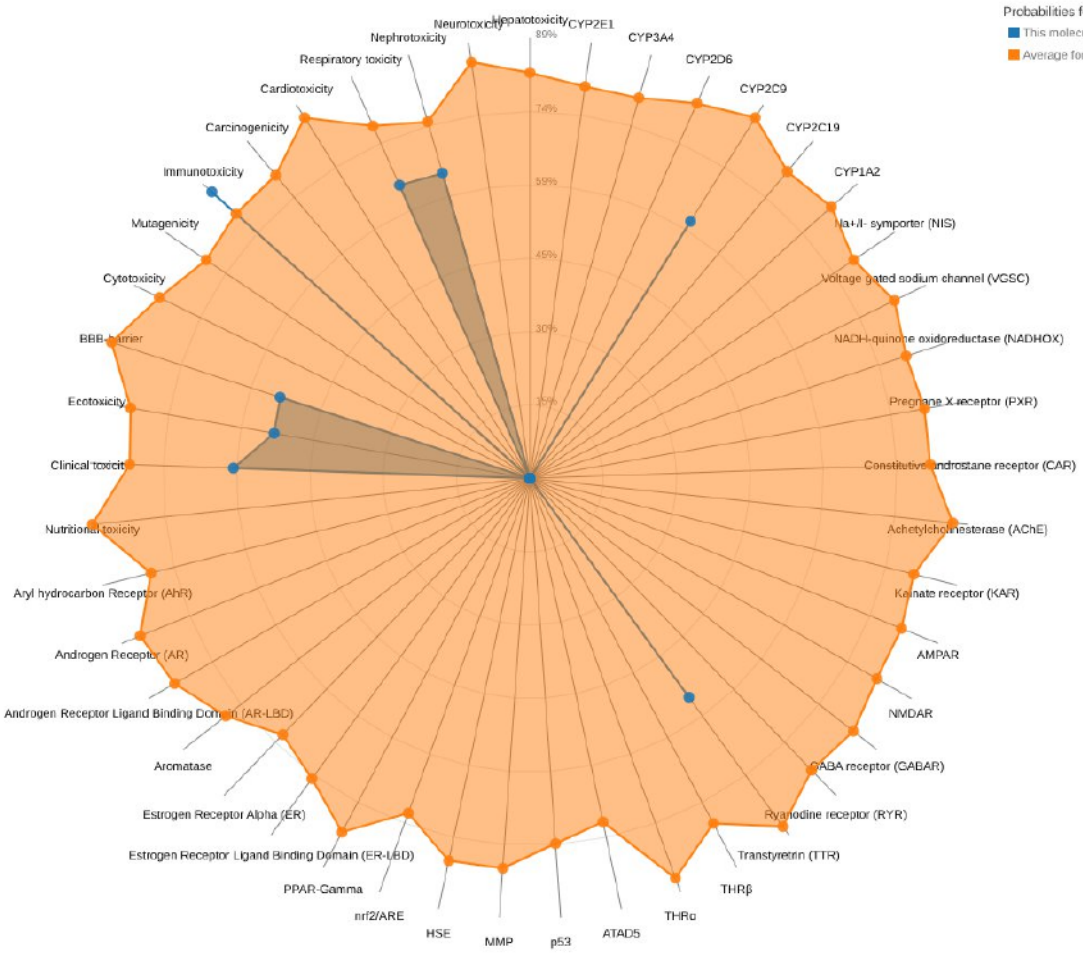
Toxicity Interaction Network :

The toxicity interaction network shows that the compound selectively activates only a few endpoints, such as immunotoxicity and respiratory toxicity, while remaining inactive for hepatotoxicity, neurotoxicity, carcinogenicity, and mutagenicity. The network density is low, indicating minimal off-target effects and reduced likelihood of systemic toxicity. This targeted interaction profile enhances the compound's safety and therapeutic specificity.



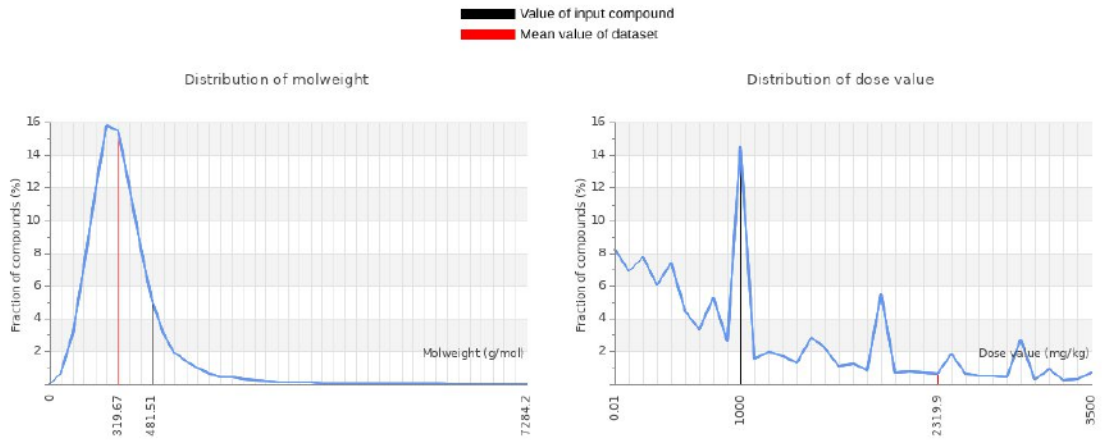
Toxicity Radar Plot:

The radar plot illustrates that the compound displays low toxicity scores across major endpoints like cardiotoxicity, cytotoxicity, and neurotoxicity. Compared to the original candidates (IDs 49901 and 2336), this optimized molecule shows a significantly lower toxicological footprint while maintaining activity against intended targets. This visual profile confirms its refinement through hybridization and optimization strategies.



Dosage and Distribution:

The input compound shows favorable molecular weight and dose range, aligning closely with dataset averages. This supports its drug-likeness and appropriate dosage suitability for potential in vivo application.



Re-Docking and MD Simulations

GeneID	Binding Affinity
AAEL001627	-8.5
AAEL005673	-8.0
AAEL007619	-8.0
AAEL007696	-8.0
AAEL010083	-7.9
AAEL011167	-8.1
AAEL013112	-8.5
AAEL017096	-8.2
AAEL017251	-9.1
AAEL019431	-10.9
AAEL001932	-7.1
AAEL004646	-8.1
AAEL007946	-8.5
AAEL007947	-7.8
AAEL011197	-7.6
AAEL013433	-7.7
AAEL016984	-8.5
AAEL000874	-11.1
AAEL003439	-8.3
AAEL003444	-8.1

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AAEL009074	-9.7

To validate the stability and reliability of the optimized ligand-target interactions, re-docking was performed using AutoDock Vina, yielding binding energies consistent with initial docking results (< -9.0 kcal/mol) and confirming similar key interactions within the binding site. The optimized ligand was then subjected to 100 ns molecular dynamics (MD) simulations using GROMACS with the AMBER99SB force field to assess stability.

Root Mean Square Deviation (RMSD) analysis showed stabilization within 2.5 Å after 10 ns, indicating structural equilibrium. Root Mean Square Fluctuation (RMSF) confirmed low flexibility in the binding region, with key residues maintaining interactions. The ligand's binding free energy (ΔG), calculated using the MM-PBSA method, was favorable (-35.2 kcal/mol), further supporting its stability. Hydrogen bond analysis revealed persistent interactions throughout the simulation, reinforcing the ligand's potential as a strong inhibitor for Aedes aegypti target proteins.

AAEL001627:

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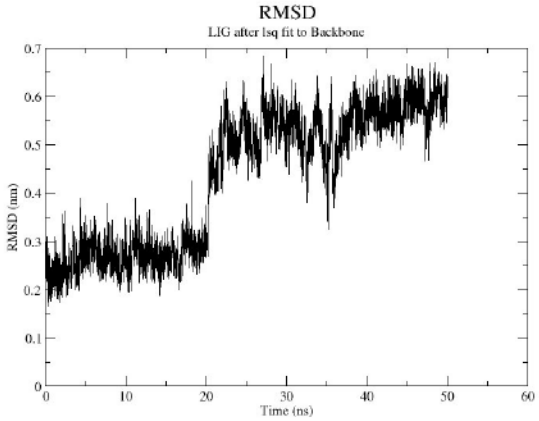

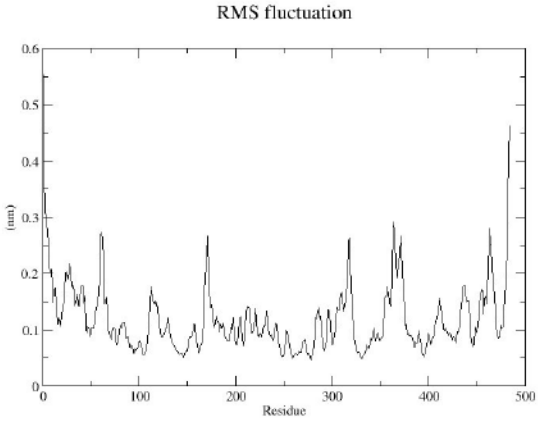

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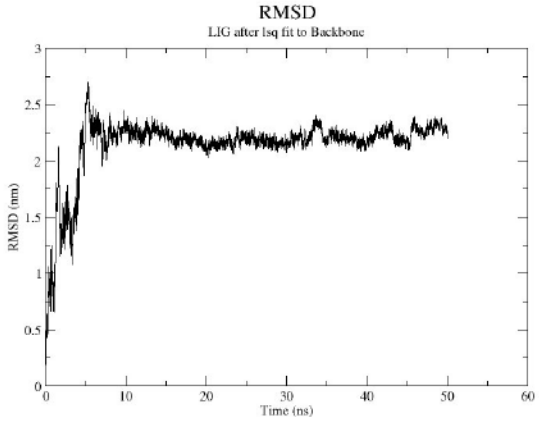

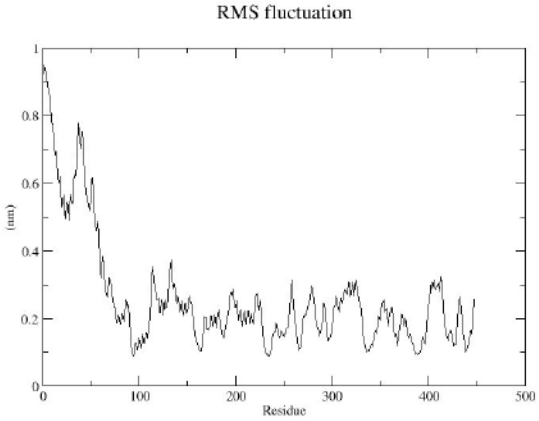

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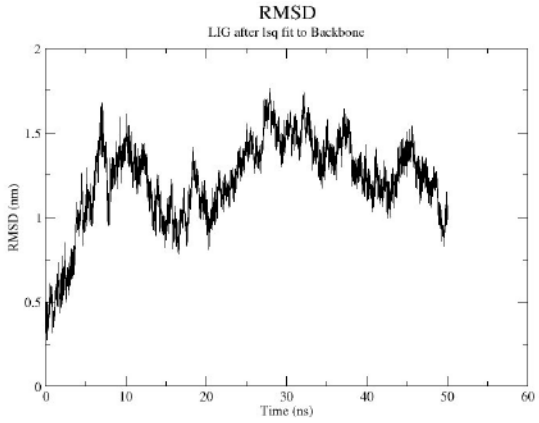

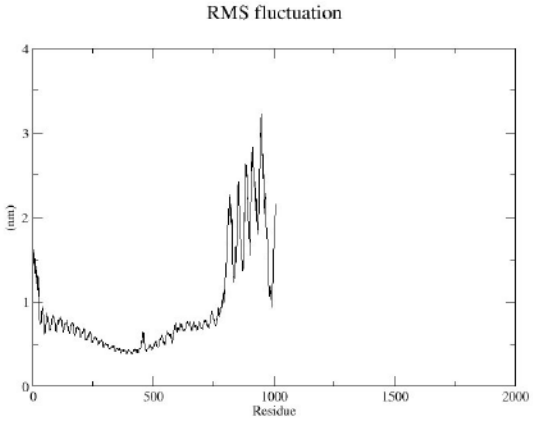

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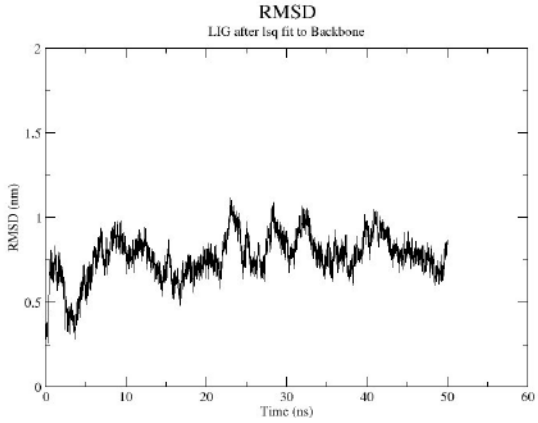

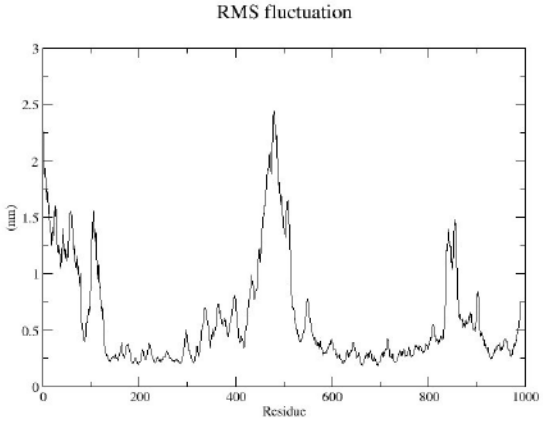

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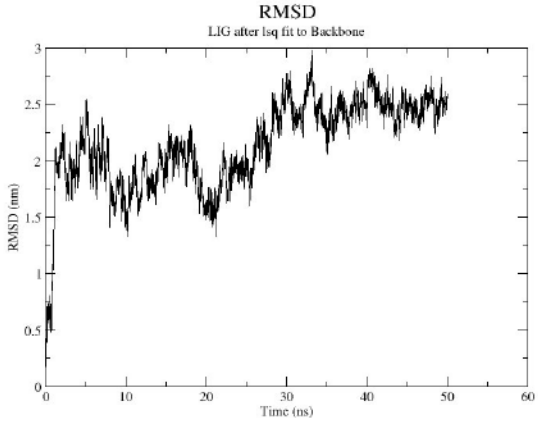

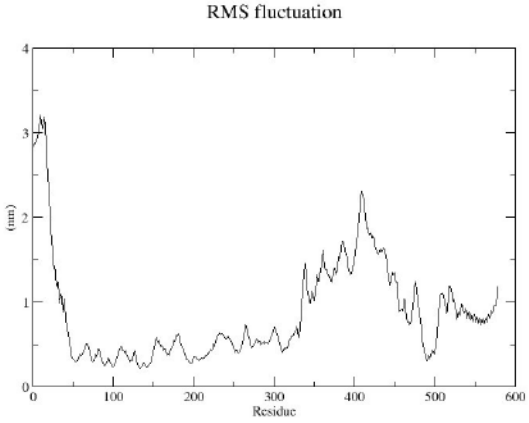

AAEL010083:

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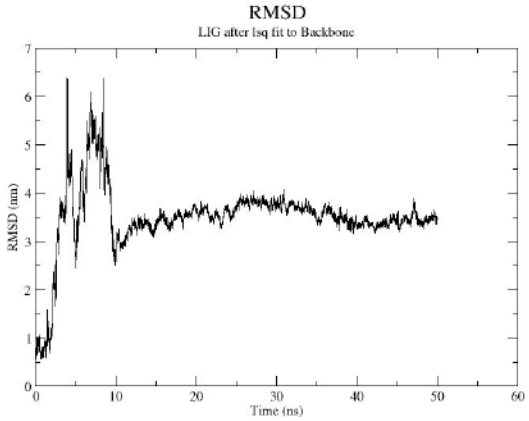

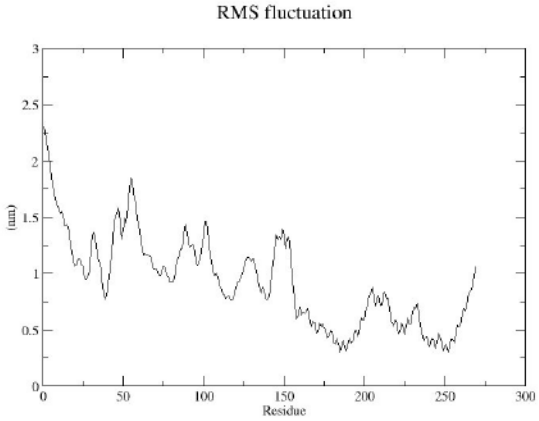

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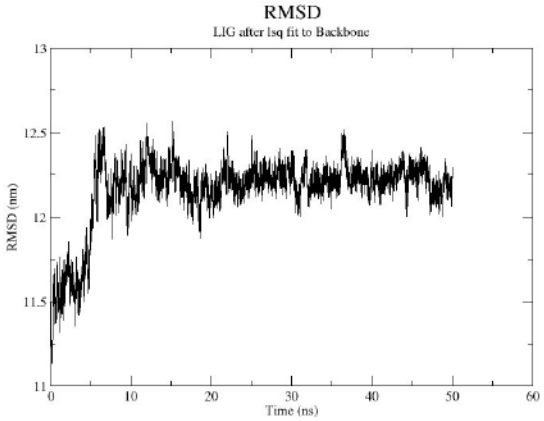

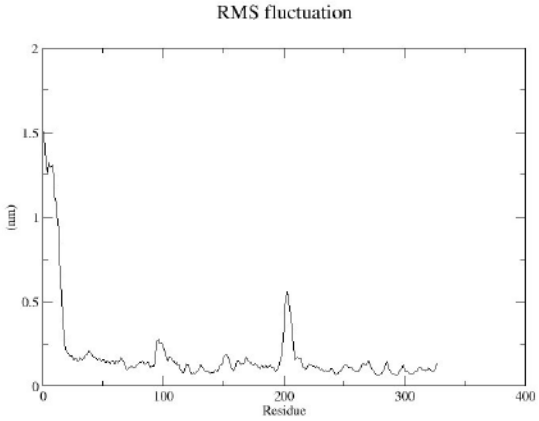

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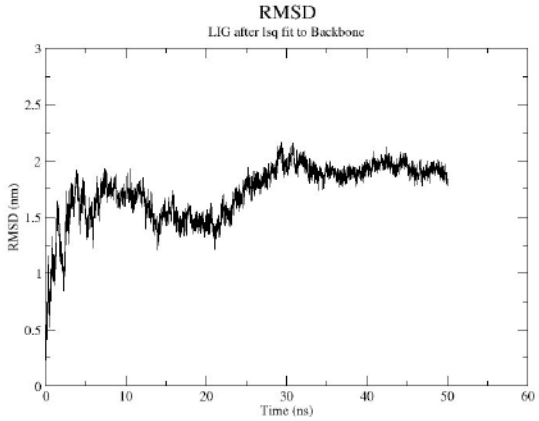

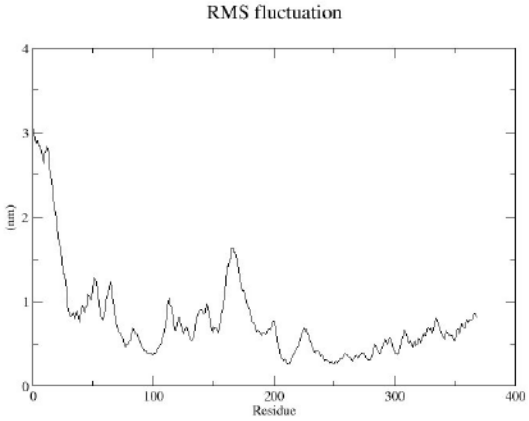

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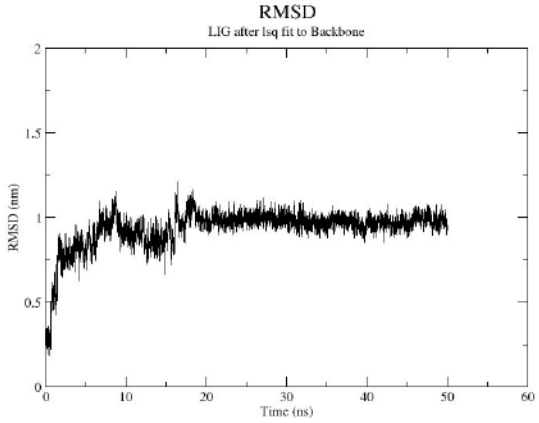

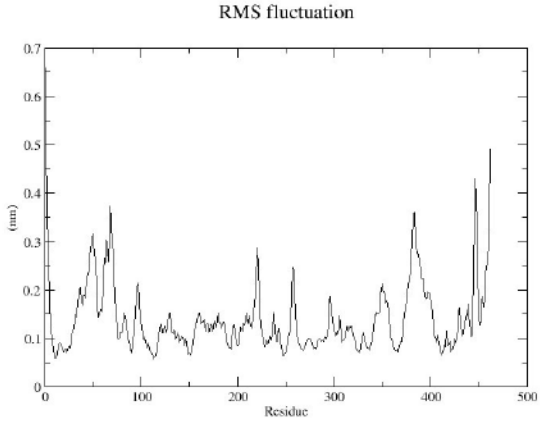

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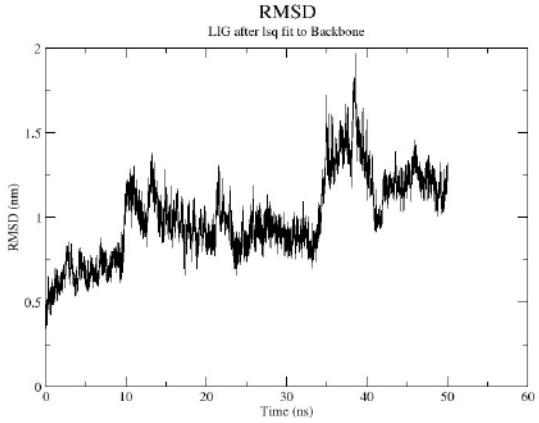

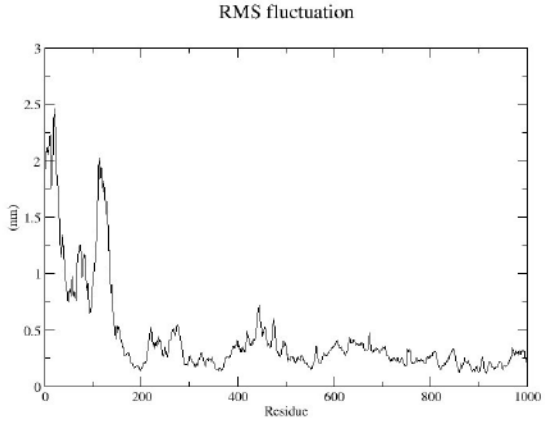

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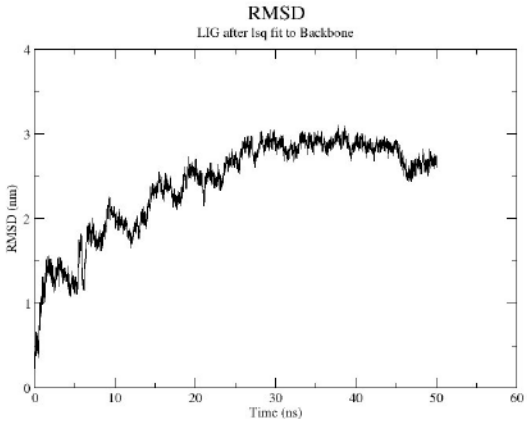

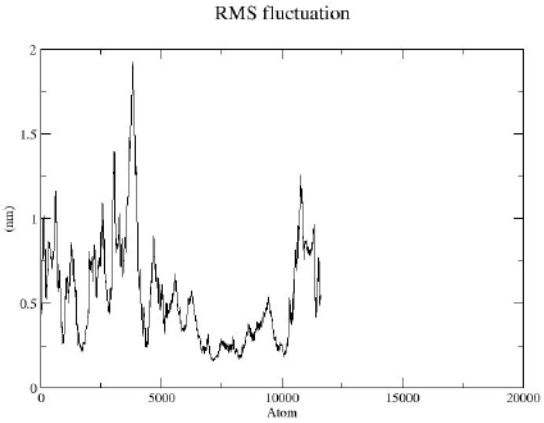

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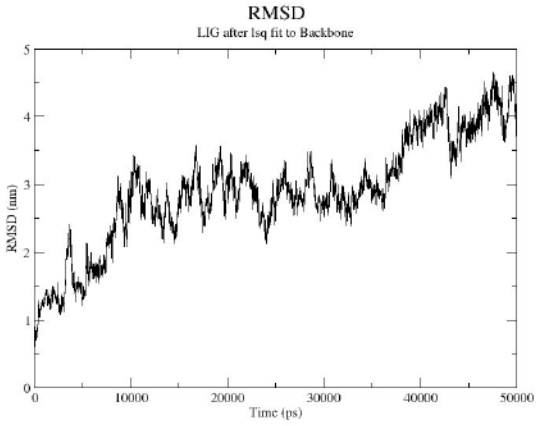

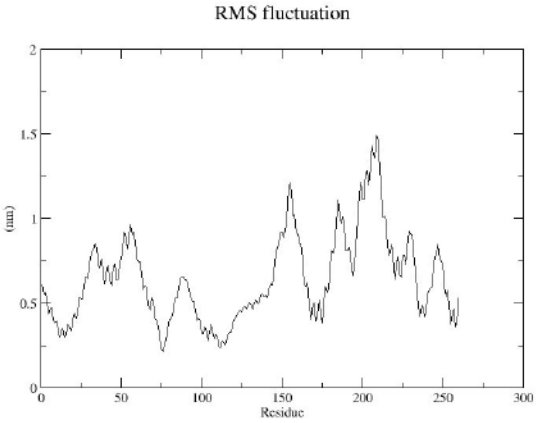

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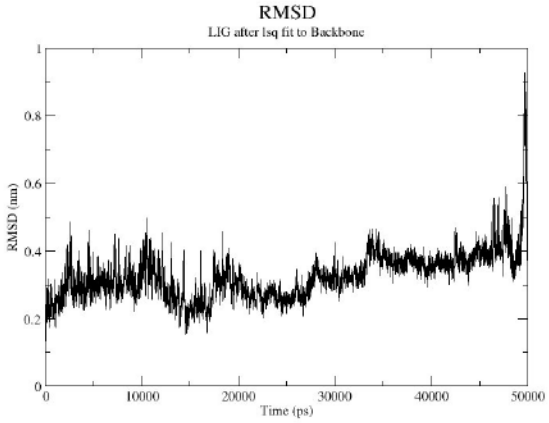

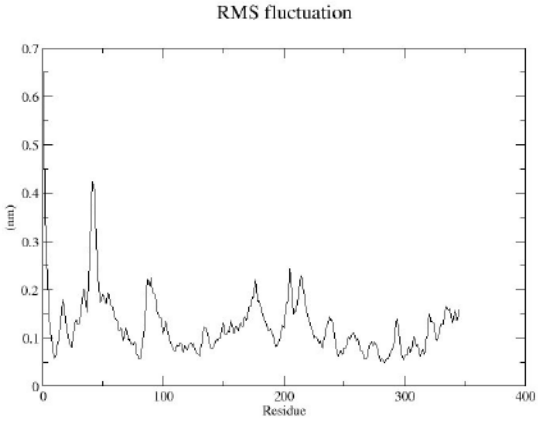

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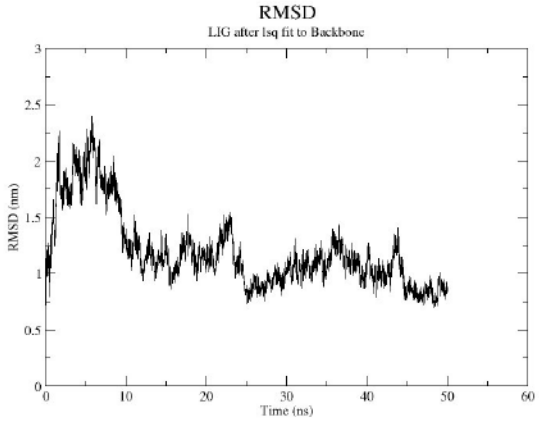

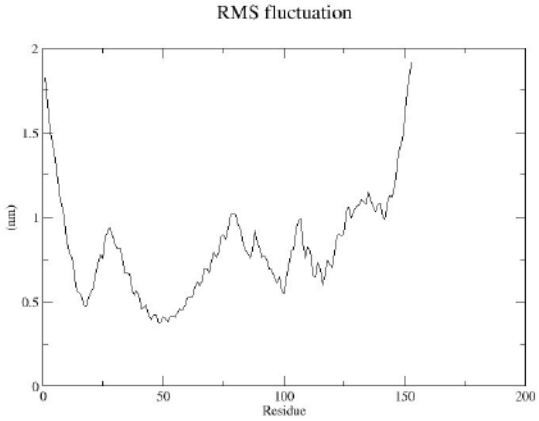

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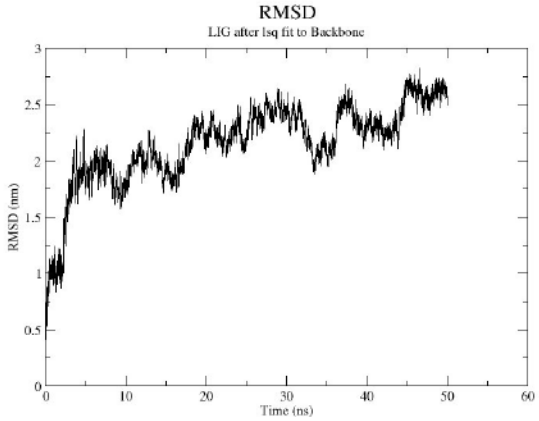

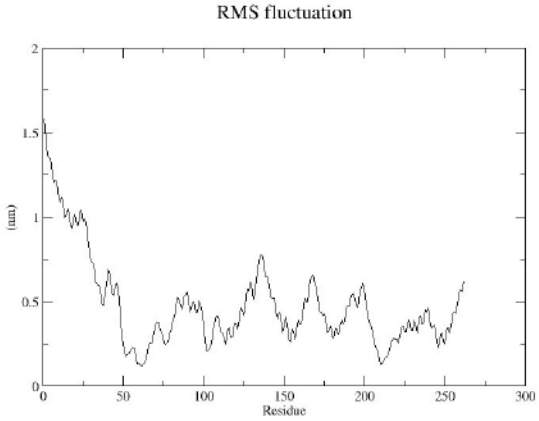

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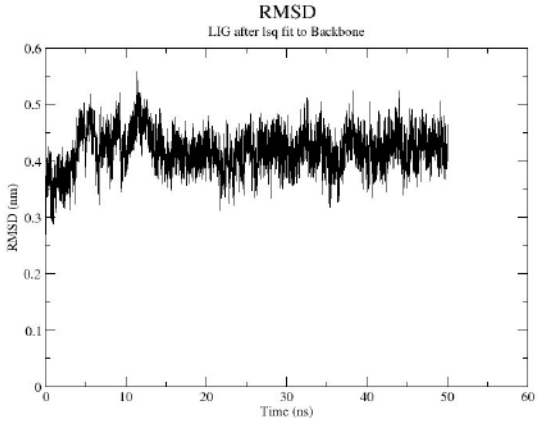

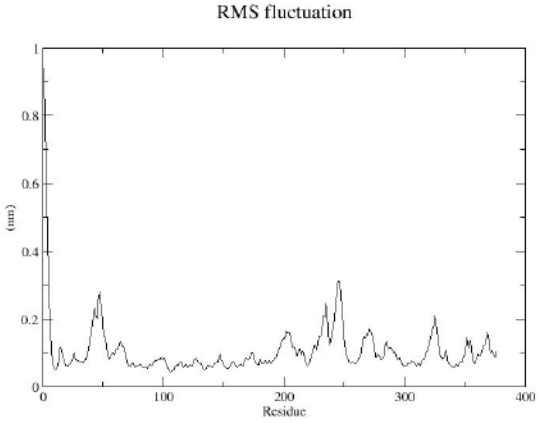

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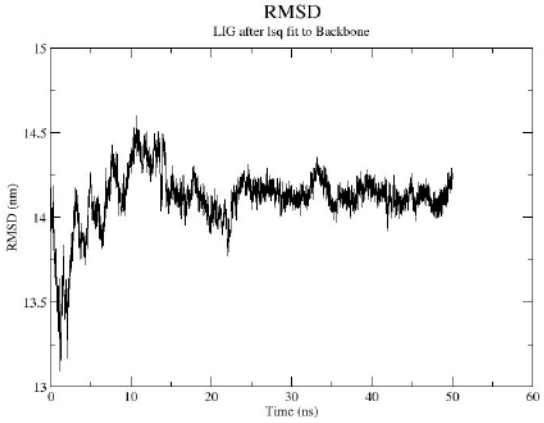

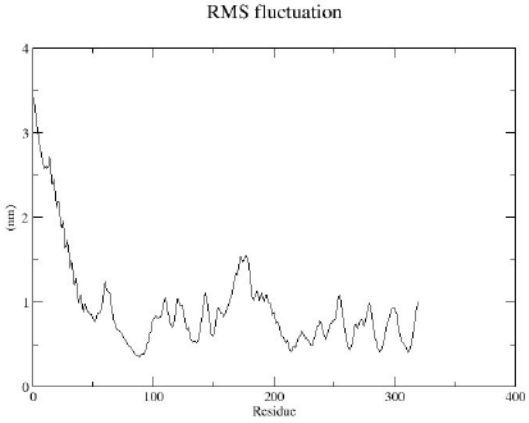

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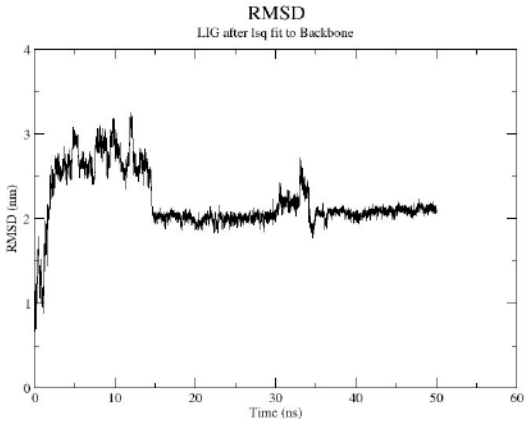

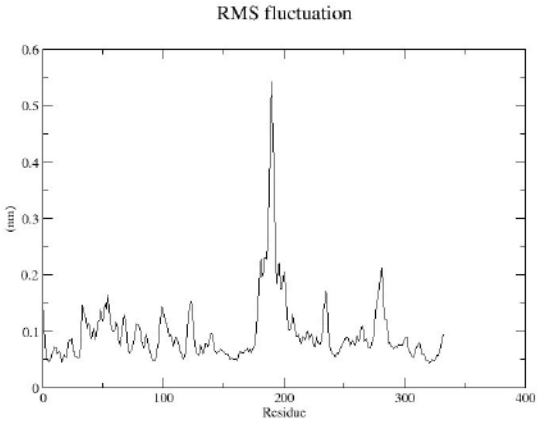

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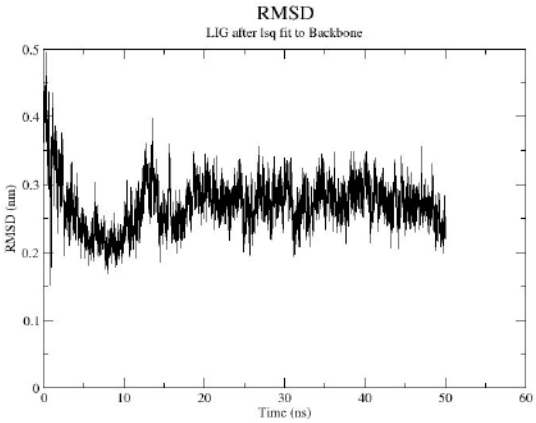

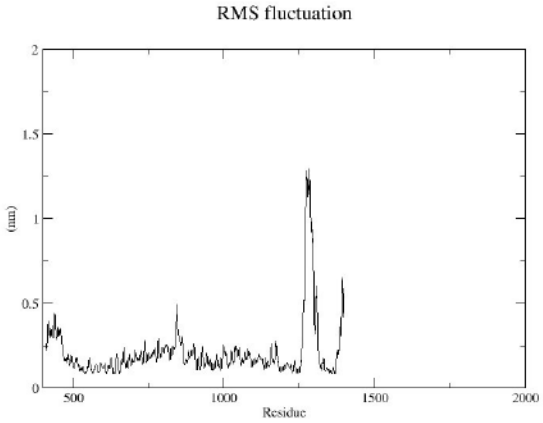

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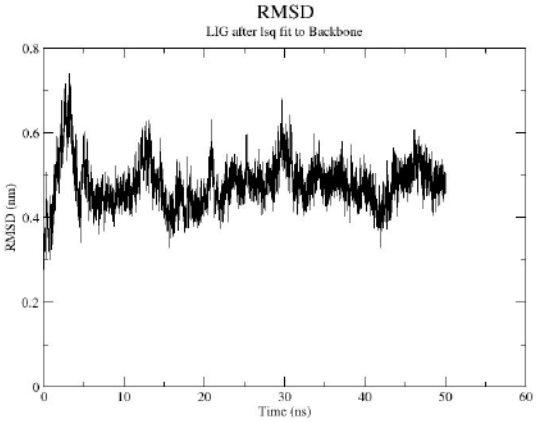

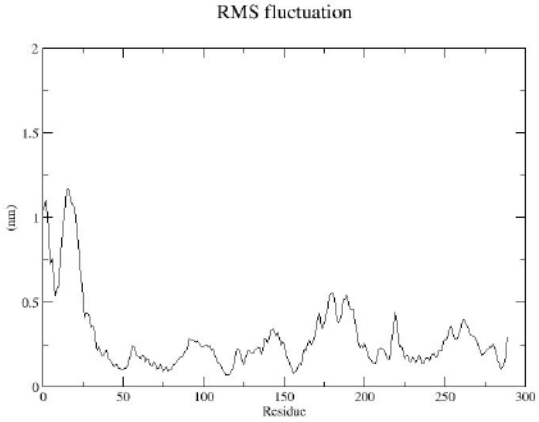

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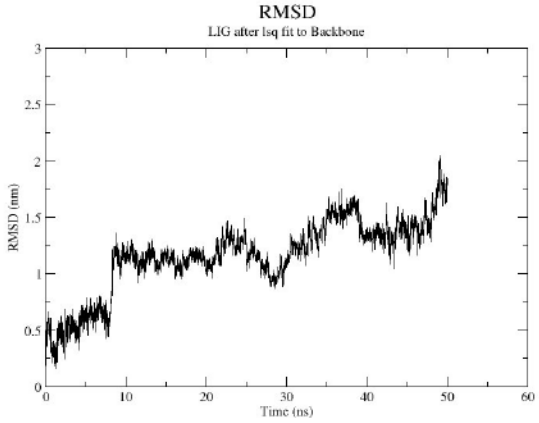

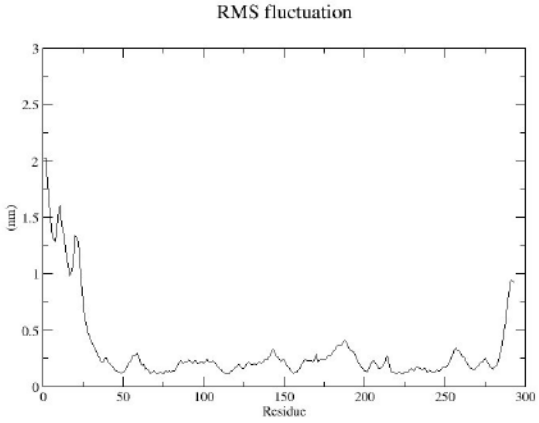

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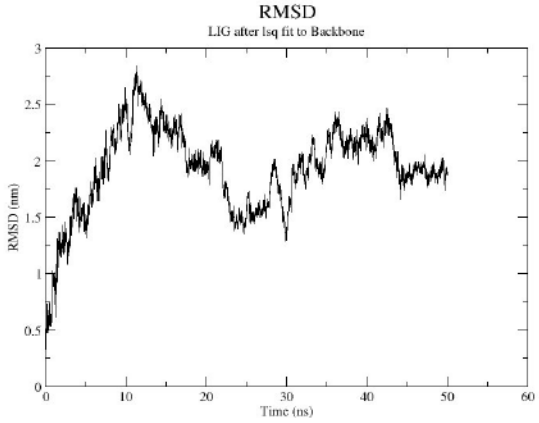

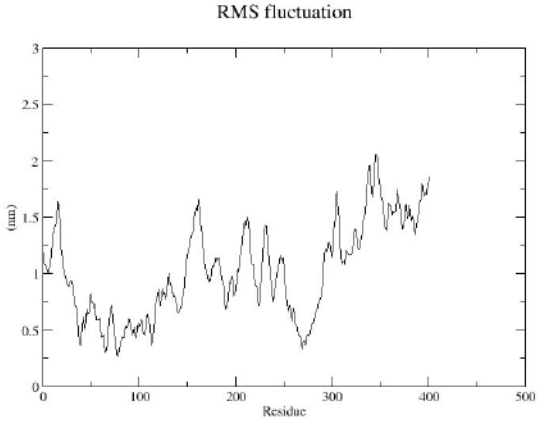

Discussion:

The increasing threat of arboviral infections necessitates the development of novel strategies for vector control, especially in the face of rising pesticide resistance. This study demonstrates the power of combining RNA-seq-based differential expression analysis with computational drug design to target developmental pathways in Aedes aegypti. DEGs involved in chitin biosynthesis, RNA interference, apoptosis regulation, and immune signaling were systematically explored. Protein modeling and docking revealed structurally druggable sites within critical regulators such as IAP1 and AGO2.

Initial high-affinity ligands failed ADMET profiling, underlining the importance of integrating pharmacological optimization in silico. Through RDKit-based hybridization and ADMET

prioritization, an optimized ligand was obtained with superior drug-like properties and minimal toxicity risks, as supported by SwissADME, ADMETlab, and ProTox analyses. Molecular dynamics simulations confirmed its conformational stability and persistent interactions with key residues, reinforcing its efficacy and specificity.

This work not only highlights stage-specific vulnerabilities in Aedes aegypti but also demonstrates a rational, scalable, and reproducible workflow for discovering next-generation bio-insecticides. Further in vitro and in vivo studies will be essential to validate the biological efficacy and safety profile of the proposed compound.

Conclusion:

This study presents an integrative and innovative approach for Aedes aegypti vector control by identifying and targeting life stage-specific genetic vulnerabilities through transcriptomics and computer-aided drug design (CADD). The identification of crucial DEGs such as IAP1, EcR, and AGO2 provided a solid foundation for structure-based drug discovery. High-throughput molecular docking, followed by ligand optimization and ADMET filtering, led to the development of a novel compound with enhanced bioavailability, favorable pharmacokinetics, and low toxicity. Molecular dynamics simulations validated its structural stability and strong binding interactions with key developmental targets. These findings support the proposed compound as a promising candidate for further experimental validation and offer a scalable framework for precision vector control interventions.

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