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INSECTICIDE RESISTANCE MANAGEMENT STRATEGY FOR *Aedes aegypti* L. AND *Anopheles gambiae* G. THROUGH PREDICTION OF POTENTIAL CHORION PEROXIDASE INHIBITORS USING COMPUTER AIDED DRUG DESIGNING APPROACH (CAAD)

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Mosquitoes, *Aedes aegypti* L. and *Anopheles gambiae* G. are very important insect vectors for transmission of infectious diseases like dengue and malaria. Extensive use of insecticides provided rapid control of these insect vectors but gradually they have developed insecticide resistance. Target site insensitivity is one the main reasons for insecticide resistance development. Development of new insecticides targeting different target sites can be a useful approach for management of insecticide resistance in insect pests. Chorion peroxidase inhibitors have been predicted by using computer aided drug designing (CAAD) approach. 3D structure of chorion peroxidase of *A. aegypti* and *A. gambiae* was predicted by using I-TASSER and refined by 3Drefine and GalaxyWEB servers. Ligand binding sites were predicted by using 3DLigandSite, RaptorX and COACH servers. Ligands/Inhibitor compounds were obtained from literature and drug bank. Protein-ligand docking was performed by AutoDock Vina. The compounds with low binding energy and good interactions were selected for both species. Pharmacophore models were generated by using LigandScout. Screening of zinc library was performed against the features of designed pharmacophores. Top ten compounds with best pharmacophore fit score for each species were selected for docking with chorion peroxidase. Protein ligand interactions were determined through LIGPLOT. One lead compound showing lowest binding energy and best interactions was selected for each species, i.e., ZINC04581496 (*A. aegypti*) and ZINC15675298 (*A. gambiae*). Protein-Protein interaction was studied by STRING database to find the co-expressing proteins in network of chorion peroxidase. Ten interaction partners were found for *A. aegypti* while five for *A. gambiae*. These pharmacophore models may provide theoretical basis for designing effective insecticides for *A. aegypti* and *A. gambiae* control. The efficacy of computationally predicted lead molecules can be confirmed by testing *in vitro* and *in vivo.*

Keywords: Mosquito, chorion peroxidase, insecticide resistance management, computer aided drug designing.

INTRODUCTION

An integral part of the global strategy of mosquito associated diseases management is the control of vectors (mosquitoes). *Aedes aegypti* Linnaeus and *Anopheles gambiae* Giles are considered very important insect vectors for dissemination of important life threatening diseases, *i.e*., dengue fever (Ponlawat *et al*., 2005) and malaria (Lindsay *et al.*, 1998), respectively. Insecticides are commonly used for insect vector management. A few decades back, extensive use of insecticides resulted in good control of mosquitoes and eradication of mosquito borne diseases (Hemingway *et al*., 2002). However, after some year's mosquito borne diseases were starting rising with more lethal effects because of increasing mosquito resistance to insecticides (Kelvin, 2011). This resulted in a number of outbreaks of mosquito related diseases in different areas of the world. The widespread development of resistance in mosquitoes to the most commonly used insecticides has become a serious problem in mosquito management strategies (Zaim and Guillet, 2002). Research on insecticide resistance in mosquitoes had started since 1950s, when the first report of mosquito resistance to chlorinated-hydrocarbon insecticides was published (Gjullin and Peters, 1952). Many studies have indicated that multiple resistance mechanisms are involved in development of insecticide resistance even in a single mosquito species (Hemingway *et al*., 2002; Vontas *et al*., 2005; Liu *et al*., 2007; Ranson *et al*., 2011; Li and Liu, 2014) Two mechanisms, *i.e*., increased metabolic detoxification of insecticides and target-site insensitivity, have been extensively studied and their importance in insecticide resistance is now widely accepted (Ranson *et al*., 2011; Li and Liu, 2014; Yang and Liu, 2014; Ali *et al*., 2017). Most of the insecticides used for mosquito control use nervous system related target sites *i.e*., Sodium

channels (Pyrethroids), Acetylcholinesterase AchE (OP and carbamates), γ-aminobutyric acid GABA (Cyclodiene and Fipronil). Target site insensitivity is an important aspect of insecticide resistance mechanism to be addressed in developing insecticide resistance management program.

Pyrethroids, due to their safety and efficacy, are widely used as indoor insecticides for mosquito control. These insecticides alter the function of the voltage-gated sodium channels in the nerve membranes of insects and prevents repolarization phase of the action potentials (Narahashi, 1996; Sattelle and Yamamoto, 1998). Mosquitoes have developed resistance to pyrethroids and DDT (Dichlorodiphenyltrichloroethane) through structural modifications of their target proteins, voltage-gated sodium channel proteins, which resulted in insensitivity to insecticides (Casida and Durkin, 2013). The proteins of voltage-gated sodium channels, acetylcholinesterase and GABA receptor, have been reported to be involved in increased target site insensitivity to applied insecticides (Feyereisen, 2013).

The principle of the 3R's (Replacement, Reduction and Refinement) has become an integral part of insecticide development legislation (Saini and Kumar, 2014). *In-silico* tools have been proved a very good alternative of animal experimentation and time taking screening processes. These computational methods are gaining popularity worldwide in drug designing because it economizes resources and time. Protein Data Bank (PDB) contains over 92,505 three-dimensional (3D) protein structures. In case of unavailability of experimentally identified 3D protein structure several free online servers and tools are available for 3D structure prediction. These tools/ servers use different approaches for 3D molecular modeling of proteins *i.e.*, homology modeling, iterative threading, modeling protein folding etc. We can easily study the proteins computationally and identify different mutations and potential target site for drug. We can have a "from genome to drug" drug design protocol by integrating genomics, proteomics and molecular modeling (Reiss, 2001; Dean and Zanders, 2002).

The pharmacodynamics and tools used in computer aided pesticide designing (CAPD) are the same as used for computer aided drug designing (CADD), except pharmacokinetic considerations (Tice, 2001). The number of known experimental structures of targets sites in the field of pesticide chemistry is significantly smaller than those in medicinal chemistry (Bordas *et al*., 2003). Hence, pesticide chemists have to rely more upon indirect ligand based pesticide design methodologies. The major *in-silico* tools used in pesticide design are molecular modeling, protein-ligand docking, pharmacophore generation, virtual screening and QSAR (Saini and Kumar, 2014). Virtual screening is used for screening of very large libraries of compounds to find the related compound of desired features in pharmacophore models (Walters *et al*., 1998). The aim of virtual screening is to identify molecules of novel chemical structure that bind to the macromolecular target of interest. Thus, success of virtual screening is measured in terms of finding interesting new scaffolds rather than number of hits. Virtual screening is already being successfully used in the field of medicinal chemistry (Rester, 2008; Rollinger *et al*., 2008) but its use in pesticide design and development is still inadequate.

The chorion or eggshell is a protein structure of insect egg. It undergoes a hardening process during the last stage of egg development, leading to the formation of an insoluble chorion (Margaritis, 1985a). Peroxidase-catalyzed chorion protein crosslinking through dityrosine formation has been considered a major mechanism contributing to the formation of a hardened chorion in insect egg (Petri *et al*., 1976; Mindrinos *et al*., 1980; Margaritis, 1985b). This process is catalyzed by peroxidase in the presence of H_2O_2 and provides physical and biological protection to the developing embryo (Dias *et al*., 2013).

Researchers in mosquito resistance management programs are trying to understand the mechanisms for development of insecticide resistance in mosquitoes to develop more effective and targeted insecticides. Development of new insecticides based on different target sites can be a useful approach to be adopted for management of insecticide resistance in insect pests.

In the current study we have predicted potential inhibitors of chorion peroxidase enzyme in *A. aegypti* and *A. gambiae.* 3D structure prediction, protein ligand docking and virtual screening was done to identify the lead compounds for inhibition of chorion peroxidase in both the species.

MATERIALS AND METHODS

Amino acid sequences of chorion peroxidase for *Aedes aegypti* and *Anopheles gambiae* were retrieved from UniProt and saved in FASTA format for further use in analysis.

***3D protein model prediction, refinement and evaluation*:** 3D structure and related templates for homology modeling were not present in PDB (Protein Data Bank). The amino acid sequences were submitted to I-TASSER (Yang and Zhang, 2015) for 3D model prediction. Predicted models were refined by using online available servers, i.e., 3Drefine (Bhattacharya *et al*., 2016), GalaxyWEB (Ko *et al*., 2012) and ModRefiner. The refined structures were evaluated through ERRAT, VERIFY3D and PDBsum.

***Active site prediction*:** Active site prediction was done by using three online available tools, i.e., 3DLigandSite (Wass *et al*., 2010), COACH (Yang *et al*., 2014) and RaptorX.

Molecular docking: Inhibitors/ ligands for chorion peroxidase in *A. aegypti* and *A. gambiae* were retrieved from literature survey and DrugBank. The selected ligand molecules were docked with target protein through targeted docking performed by AutoDock Vina.

***Pharmacophore modeling*:** The ligand molecules showing low binding energy after docking were selected for

pharmacophore generation (Trott and Olson, 2010). LigandScout (Wolber and Langer, 2005) was used for pharmacophore generation.

Virtual screening and molecular docking: ZINC library of synthetic compounds was screened out to get the compound having more closely related features to selected pharmacophore model. The compounds with best pharmacophore fit score were selected. The binding pose and binding energy of selected compounds were predicted by protein-ligand docking using AutoDock Vina. Protein-Ligand interaction was analyzed by LIGPLOT (Wallace *et al*., 1995). One lead compound for each species was selected.

***Protein-Protein interactions*:** Protein-Protein interaction was explored, from STRING database (Mering *et al*., 2003), to find out the interactions of target protein to other proteins which might be selected for alternate target site in future.

RESULTS AND DISCUSSION

Insecticide resistance is a major issue now days. Insects have become resistant to most of the insecticides used. There is a need to introduce new and effective insecticides. One of the promising methods to effectively manage insect pests might be by using enzyme inhibitors (Laskowski and Jr Kato, 1980). In the current study we tried to find the inhibitors for chorion peroxidase enzyme using computer aided drug designing (CADD) and suggested a new target site and effective chemicals for control of *A. aegypti* and *A. gambiae*. 3D protein model prediction, refinement and evaluation.

The first step in a proteomic study is to have the 3D structure of the protein. 3D structures of the selected protein in both species were not found in Protein Data Bank (PDB). First step of our study was prediction of 3D model of selected proteins. Homology modeling was not possible because it needs closely related templates from PDB for structure prediction (Eswar *et al*., 2008). Therefore, 3D models of chorion peroxidase for *A. aegypti* and *A. gambiae* were predicted through I-TASSER (Iterative Threading Assembly Refinement). The best models with highest confidence scores (C-score) were selected. The selected models were further refined and evaluated. The best models on basis of evaluation score were selected for further analysis (Fig. 1-2). I-TASSER is a protein modeling tool which uses a hierarchical approach based on secondary structure enhanced profile threading alignment (Wu and Zhang, 2007). It generates three-dimensional (3D) atomic models from multiple threading alignments and iterative structural assembly simulations (Zhang, 2008). For each submitted sequence, I-TASSER gives up to five predicted models ranked based on C-score. High C score is related to good quality of predicted structure (Zhang and Skolnick, 2004).

The predicted models were refined and evaluated by ERRAT, VERIFY3D and PDBsum. The models with highest refinement score were selected for both species (Table 1).

Table 1. Structure refinement score of selected 3D models of chorion peroxidase of *Aedes aegypti* L. and *Anopheles gambiae* G.

Organism	ERRAT	VERIFY3D	PDBsum*
Aedes aegypti	87.084	79.49	93.1
Anopheles gambiae	84.321	79.92	92.6

***The score is sum of amino acids in favored and allowed region.

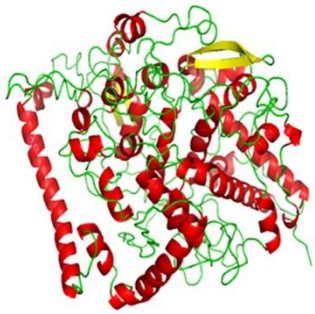


Figure 1. Predicted 3D structure of chorion peroxidase of *Aedes aegypti* L.

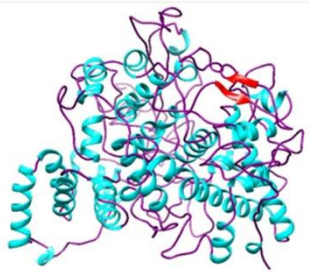


Figure 2. Predicted 3D structure of chorion peroxidase of *Anopheles gambiae* G.

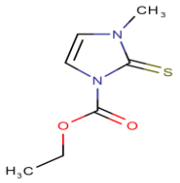
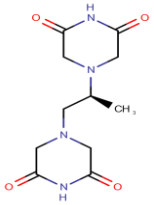
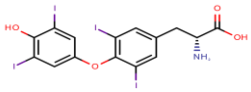
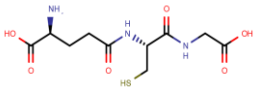
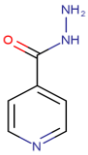
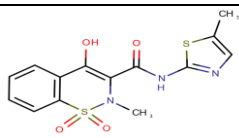
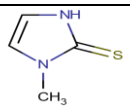
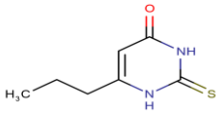
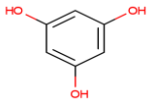
***Active site prediction*:** Active sites mainly contain polar amino acids, e.g. Asp, Ser, Cys, His, these may be involved in binding activities (Ouzounis *et al*., 1998; Villar and Kauvar, 1994). The predicted active sites contain 24 and 20 residues in *Aedes aegypti* (P82600) and *Anopheles gambiae (*Q7QH73), respectively. Two Ser rsidues (Ser 304 and Ser 310) and one His residue (His 547) were found in predicted binding sites of chorion peroxidase of *A. aegypti*. Same was the case with *A. gambiae i.e*., Ser283, Ser289 and His529 (Table 2)

Table 2. Predicted chorion peroxidase binding sites in *Aedes aegypti* L. and *Anopheles gambiae* G.

Organism	Active site amino acids
Aedes aegypti	Met297, Gly300, Gln301, Ser304, Thr308, Leu309, Ser310, Arg447, Gln450, Leu451, Ala544, His547, Arg548, Tyr549, Gly550, His551, Val554, Ile572, Phe576, Leu611, Leu615, Phe616, Leu626, Arg633
Anopheles gambiae	Met276, Gly279, Glu280, Ser283, Thr287, Arg288, Ser289, Arg425, Gln428, Ile429, Tyr482, Gly522, Phe525, Arg526, Gly528, His529, Thr531, Val532, Ile550, Phe554

***Molecular docking*:** Ligands for chorion peroxidase of both species were retrieved from extensive literature survey and DrugBank (Table 3). Docking of ligands with chorion peroxidase of *A. aegypti* and *A. gambiae* was performed by AutoDock Vina. The ligands showing low binding energies and good interactions with target protein were selected for further analysis.

Table 3. List of the ligands selected as chorion peroxidase inhibitors in *Aedes aegypti* L. and *Anopheles gambiae* G.

Sr	Ligand	Molecular weight (g/mol)	2D structure
1.	Carbimazole	186.229	
2.	Dexrazoxane	268.273	
3.	D-Thyroxine	776.874	
4.	Glutathione	307.321	
5.	Isoniazid	137.142	
6.	Meloxicam	351.395	
7.	Methimazole	114.166	
8.	Propylthiouracil	170.230	
9.	Phloroglucinol	126.111	

Meloxicam (Zinc 13129998) showed best interaction with chorion peroxidase in *A. aegypti* and showed lowest (-7.9 Kcal/mol) binding energy (Table 4). It is a Nonsteroidal Anti-inflammatory drug. It acts as a cyclooxygenase inhibitor.

Table 4. Ligands and chorion peroxidase docking results for *Aedes aegypti* L.

Ligands	Binding interactions	Bond distance (Å)	Binding energy (Kcal/mol)
Carbimazole	No	No	-5.1
Dexrazoxane	No	No	-7.6
D-Thyroxine	03-Arg619:NE	2.98	-6.6
	O3-Arg619:NH2	3.08	
	N-Thr314:OG1	3.34	
	N-Phe313:O	3.04	
	O2-Arg633:NE	3.16	
Isoniazid	N3-Thr718:O	2.90	-5.6
	N1-Thr718:O	2.81	
	N1-Asn528:O	2.75	
Meloxicam	O4-Trp 648:O	3.19	-7.9
Methimazole	N2-Met147:O	2.99	-3.7
Propylthiouracil	No		-5.3
Phloroglucinol	O2-Gln602:NE2	3.13	-6.5
	O2-Tyr23:O	3.00	
	O2-Tyr23:N	2.92	
	O3-Asp604:OD2	3.04	
	O3-Thr17:O	2.92	
	O1-Phe21:N	3.12	

Table 5. Ligands and chorion peroxidase docking results for *Anopheles gambiae* G.

Ligands	Binding interactions	Bond distance (Å)	Binding energy (Kcal/mol)
Carbimazole	No		-4.8
Dexrazoxane	O4-Asn26:ND2	2.90	-7.8
	O3-Ala182:N	3.23	
D-Thyroxine	O3-Asn340:ND2	3.19	-6.3
	O3-Asn340:OD1	2.86	
	N-Asn340:OD1	3.19	
	N-Asn340:O	3.18	
	N-Ser602:OG	3.10	
Isoniazid	N1-Ser283:OG	2.87	-5.4
Meloxicam	O3-Asn427:N	2.88	-7.6
	O3-Val426:N	3.06	
Methimazole	N2-Tyr695:OH	3.07	-4.0
Propylthiouracil	N2-Tyr200:O	3.15	-5.0
Phloroglucinol	O3-Ser40:OG	3.04	-5.3
	O3-His42:ND1	2.91	
	O1-Leu189:N	2.95	
	O2-Glu52:OE1	2.97	
	O2-Asn376:ND2	3.10	

It interacts with target protein through hydrogen bonding between Trp 648 and O-4 of ligand with a bond distance of 3.19 Å. The best inhibitor selected in case of *A. gambiae* was Dexrazoxane (Zinc 87515509) (Table 5). The binding energy was -7.8kcal/mol, which interacted with target protein through two hydrogen bonds *i.e*., Asn 26 and Ala 82 with bond distance of 2.9 Å and 3.23Å, respectively. It is derivative of Ethylene diamine tetraacetic acid (EDTA). As a derivative of EDTA, dexrazoxane chelates iron and thus reduces the number of metal ions complexed with anthracycline and decrease the formation of superoxide radicals (Jones, 2008).

Pharmacophore modeling: Pharmacophore modeling involves merging of different chemical compounds to find the new compound with desired features. LigandScout is a freely available tool used for rapid and accurate generation of three dimensional structures of pharmacophores from provided structural data, of ligand- protein complexes, in an automated and expedient manner (Steindl *et al*., 2006). The LigandScout can be executed on all commonly used operating systems and so many noticeable examples of its successful application are available in literature (Schuster and Langer, 2005; Schuster *et al*., 2006) . In ligand-based molecule structure design the pharmacophore designing is a popular approach to find out common chemical features among a large number of diversified structures. Pharmacophore model can be used as a query to search chemical databases for finding some unique chemical structures to be used as ligands (Ci *et al*., 2007). The generated 3D pharmacophore model gives some important information, which can be used to develop new and more effective insecticides (Li *et al*., 2008). From the docking results, the ligand with low binding energy and strong interaction were selected for pharmacophore generation. The ligands selected in case *A. aegypti* were D- Thyroxine, Meloxicam and Phloroglucinol (Table 6). Lowest binding energy (-7.9 Kcal/mol) was observed for Meloxicam but exhibited only one hydrogen bond interaction with target protein. Phloroglucinol had binding energy of -6.5 Kcal/mol but its six sides were involved in hydrogen bonding with target protein with hydrogen bond distance ranging from 2.92 to 3.13Å. Three best interacting ligands, Dexrazoxane, D-Thyroxine and Meloxicam were selected for pharmacophore generation in case of *A. gambiae* (Table 7). Three-dimensional pharmacophores were constructed which were used for virtual screening of related compounds (Wolber and Langer, 2005). Pharmacophores were generated by merging the properties of selected ligands and their pharmacophore features were studied (Table 8). 3D images of the pharmacophores were generated. Green circles represent Hydrogen bond donor (HBD) and red color showed Hydrogen bond acceptor (HBA). The pharmacophore generated for *A. aegypti* had three HBA and two HBD (Fig. 3) that is for *A. gambiae* contained two HBA and four HBD (Fig. 4)

Table 6. Features of selected compound for pharmacophore generation for chorion peroxidase of *Aedes aegypti* L.

Selected ligands	Molecular weight (g/mol)	HBD	HBA	Aromatic rings	Rotatable bonds
D-Thyroxine	776.876	3	5	2	5
Meloxicam	351.935	2	7	3	2
Phloroglucinol	126.111	3	3	1	3

* HBD= Hydrogen bond donor, HBA= Hydrogen bond acceptor

Table 7. Features of selected compound for pharmacophore generation for chorion peroxidase of *Anopheles gambiae* G.

Selected ligands	Molecular weight (g/mol)	HBD	HBA	Aromatic rings	Rotatable bonds
D-Thyroxine	776.876	3	5	2	5
Meloxicam	351.935	2	7	3	2
Dexrazoxane	265.273	2	6	2	3

HBD= Hydrogen bond donor, HBA= Hydrogen bond acceptor

Table 8. Features of pharmacophores generated by merging the properties of selected chorion peroxidase inhibitors in *Aedes aegypti* L. and *Anopheles gambiae* G.

Organism	Hydrogen bond acceptors	Hydrogen bond donor
Aedes aegypti	3	2
Anopheles gambiae	2	4

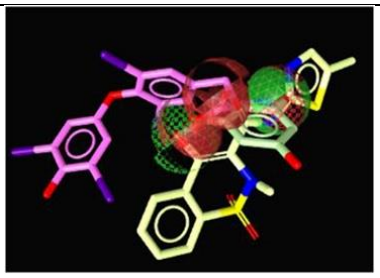


Figure 3. Pharmacophore model for chorion peroxidase inhibitor in *Aedes aegypti* L.

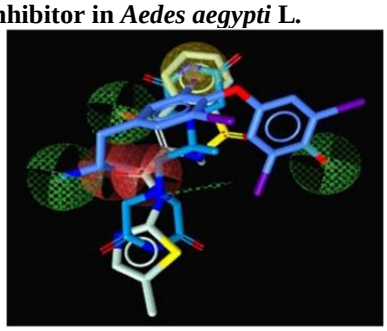


Figure 4. Pharmacophore model for chorion peroxidase inhibitor in *Anopheles gambiae* G.

Table 9. Top 10 compounds selected after virtual screening for chorion peroxidase inhibition in *Aedes aegypti* L.

ZINC ID	Molecular weight (g/mol)	Binding energy (Kcal/mol)	Fit score	Binding interactions	Bond distance (Å)
ZINC03897411	268.24	-7.3	51.37	$O3^{+}$-Gly650: O	2.98
				$O5^{+}$-Gly650: O	2.90
				$O5^{+}$-Thr190: OGI	2.99
ZINC04533921	377.35	-8.7	51.31	N2-Gln193:O	3.19
				O2-Lys158:NZ	3.35
ZINC49947047	314.4	-7.2	51.26	O5-Gly650:O	2.99
				O5-Thr190:OGI	2.96
ZINC05223665	304.15	-6.8	51.44	$O5^{+}$-Gly650:O	2.87
				$O3^{+}$-Gly650:O	3.00
				$O5^{+}$-Thr190:OGI	2.98
ZINC03870267	268.24	-7.9	51.35	$O5^{+}$-Gly196:N	3.02
				$O5^{+}$-Gln193:O	2.89
ZINC03830679	180.16	-6.9	51.26	O2-Thr17:O	2.87
				O2-Asp604:OD2	2.85
				O3-Tyr23:O	2.70
				O4-Phe21:O	3.17
				O5-Tyr23:N	2.80
				O5-Tyr23:O	2.90
				O5-Gln602:NE2	3.18
				O5-Gln602:OE1	2.97
				O6-Phe21:N	2.83
				O6-Gly20:N	3.10
ZINC05439384	324.28	-8.9	51.26	O5-Met147:O	2.75
				O7-Gly650:O	3.14
				O8-Gly650:O	2.95
				O8-Thr190:OGI	2.93
ZINC05439386	324.28	-8.5	51.27	O5-Met147:O	2.85
				O7-Gly650:O	3.13
				O8-Gly650:O	2.92
				O8-Thr190:OGI	2.94
ZINC05733294	354.31	-8.5	51.39	O7-Gly650:O	3.20
				O8-Thr190:OG1	3.08
				O8-Gly650:O	3.10
				O9-Gln168:OE2	2.80
ZINC04581496	354.31	-9.1	51.39	N2-Gln193:O	3.2

In silico screening and molecular docking: Virtual screening of ZINC library was performed through LigandScout and top 10 hits with best pharmacophore fit score were retrieved for both species separately. Molecular docking of the selected compounds with the target proteins was performed by using AutoDock Vina.

Three compounds with molecular weight ranging from 324.28 g/mol to 354.31 g/mol were found to have strong binding and good interactions with *A. aegypti* chorion peroxidase, i.e., ZINC04581496 (-9.1 kcal/mol), ZINC05439384 (-8.9 kcal/mol), and ZINC05733294 (-8.5 kcal/mol) (Table 9). One lead compound, ZINC04581496 (*N-[1-[3,4-dihydroxy-5-(hydroxymethyl) tetrahydrofuran-2-yl]-2-oxo-pyrimidin-4-yl]-4-methyl-benzamide)* was selected (Table 5). The amino acid residues interacting with the top scoring compound ZINC04581496 is presented in Figure 5. One residue, Glu193 showed hydrogen binding to N2 of ZINC04581496 with 3.2 Å bond distance. In case of *A. gambiae* compound having strong interaction with chorion peroxidase were ZINC15675298 (-9.9 kcal/mol), ZINC15675295(-9.6 kcal/mol) and ZINC12603668(-9 kcal/mol) (Table 10).

ZINC15675298 *(N-[[(2R,3S,4R,5S)-3,4-dihydroxy-5-[2-oxo-2-[4-(2-pyridyl)piperazin-1-yl]ethyl]tetrahydrofuran-2-yl]m*) was selected as lead compound for chorion peroxidase inhibition. Ser35, Ser41, Ser44 and Glu52 of chorion peroxidase were involved in hydrogen bonding to O4, N4, O5 and N3 of ZINC15675298 with bond distances 3.19 Å, 3.18Å, 3.15Å and 3.11Å, respectively (Fig. 6). Dashed lines represent the hydrogen bonding but residues shown as an arc with spokes represent hydrophobic interactions (Liu *et al*., 2008). The interaction between these hydrophobic regions of the binding site with the ligand is responsible for providing driving force for binding (Kelly and Mancera, 2005). Thirteen residues from chorion peroxidase in *A. aegypti* were involved in hydrophobic interactions with the ligand and those of *A. gambiae* those were fourteen.

Table 10. Top 10 compounds selected after virtual screening for chorion peroxidase inhibition in *Anopheles gambiae* G.

ZINC ID	Binding energy (Kcal/mol)	Fit score	Mol. Weight (g/mol)	Binding interactions	Bond distance (Å)
ZINC03900055	-7.6	51.73	314.29	O2-Tyr356:N	3.05
				O2-Tyr356:O	3.14
				O5-Arg171:O	3.11
				O5-Leu177:O	3.20
				O6-Asn26:ND2	2.80
				O7-Lys186:NZ	3.22
ZINC12153092	-8.3	51.20	478.41	O2-Arg187:NH1	3.15
				O7-Asn376:ND2	2.80
				O8-Ser35:OG	2.89
				O10-Arg288:NH2	3.25
				011-Arg288:NH2	3.13
				O12-Ser291:O	2.78
ZINC08624294	-8.4	51.33	353.41	O3-Arg348:NH2	3.07
				O3-Tyr356:O	3.03
				O4-Tyr356:N	3.05
				F-Ala182:N	3.18
ZINC15675295	-9.6	51.55	512.00	O5-Ser35:OG	3.07
				O6-Ser44:OG	3.24
				N4-Ser41:OG	3.17
ZINC15675298	-9.9	51.40	455.53	O4-Ser35:OG	3.19
				O5-Ser44:OG	3.15
				N3-Glu52:OE1	3.11
				N4-Ser41:OG	3.18
ZINC08643389	-8.5	51.25	348.40	O5-Tyr356:N	2.94
				N2-Leu183:O	3.31
ZINC08643392	-8.3	51.23	366.39	O4-Gln280:NE2	3.12
				O4-His529:NE2	3.23
				O4-Gln428:NE2	3.15
				O4-Gln428:OE1	3.07
				O5-Gln428:OE1	3.06
ZINC77262433	-7.2	51.21	354.47	N2-His284:NE2	3.34
ZINC12603668	-9.0	51.69	405.47	O4-Ser35:OG	3.15
				N3-Ser41:OG	3.24
ZINC12603952	-8.6	51.27	421.49	O5-HiS529:NE2	3.07
				O5-Gln428:NE2	3.02
				O6-Gln428:NE2	2.96
				N3-Ser283:OG	2.85

The selected lead compounds exhibited good interaction and strong binding with target protein (Table 11). The Hydrogen bond distance for both compounds was laid between 3.1 and 3.2Å. The preferred interaction region for a hydrogen bond between carbonyl oxygen and amide nitrogen is 2.5-3.5 Å (Hubbard and Haider, 2010). So the hydrogen binding was more stable and strong between lead molecules and target protein. Both selected compounds have low binding energy (ranging between -9.9 and -9.1), which reflects the strong and stable interaction with target protein (Ajay and Murcko, 1995). The selected lead molecules have HBD 4 and HBA 9 each. HBA less than 10 and HBD less than 5 reflect good membrane permeability and bioavailability of drug molecule (Lipinski *et al*., 1997). The number of rotatable bonds in a molecule is very important for its stability and bioavailability. If numbers of rotatable bonds in a molecule are more than 10 then it has very poor bioavailability (Veber *et al*., 2004). The selected lead molecules have less than 10 rotatable bond i.e., 4 in ZINC04581496 and 6 in ZINC15675298, which renders good permeability and bioavailability to the lead molecule.

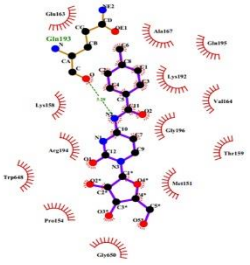
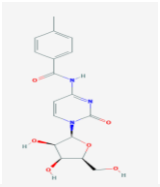
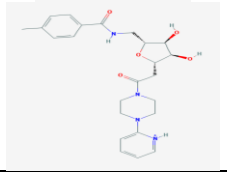


Figure 5. Interactions of ZINC04581496 with chorion peroxidase in *Aedes aegypti* L.

Table 11. Properties of lead compounds selected as chorion peroxidase inhibitor in *Aedes aegypti* L. and *Anopheles gambiae* G.

Organism	Selected ligands	Binding energy (Kcal/mol)	Hydrogen bond distance (Å)	HBD	HBA	Molecular weight (g/mol)	Rotatable bonds	Aromatic rings	2D Structure
Aedes aegypti	ZINC04581496	-9.1	3.20	4	9	361.354	4	2	
Anopheles gambiae	ZINC15675298	-9.9	3.19 3.15 3.11 3.18	4	9	455.535	6	2	

HBD= Hydrogen bond donor, HBA= Hydrogen bond acceptor

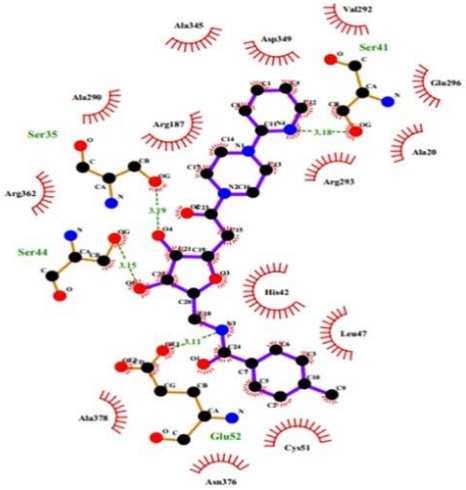


Figure 6. Interactions of ZINC15675298 with chorion peroxidase in *Anopheles gambiae* G.

***Protein-Protein interactions*:** Protein-Protein interaction networks (PPIN) is used to understand biological processes in organisms. Protein network analysis has been used to study proteins involved in different diseases (Feldman *et al*., 2008; Glaab *et al*., 2010). Recent studies (Hooda and Kim, 2012; Wang *et al*., 2012; Nishi *et al*., 2013) on integration of protein structural information and PPIN proved successful implementation protein network studies to find association between cellular mechanism and protein complexes on large scales. In 2008, it was found that more than six hundred thousand PPIs should be present in humans (Stumpf *et al*., 2008) but only very low numbers of these estimated interactions have been proved experimentally (Lehne and Schlitt, 2009). STRING database (Franceschini *et al*., 2013) was used to find out the interaction partner of chorion peroxidase in *A. aegypti* and *A. gambiae*. Most of the interacting proteins showed co-expression with the chorion peroxidase of both the species (Figs 7-8.). Eleven nodes and thirty six edges with average clustering coefficient of 0.829 were observed in case of *A. aegypti*. The interaction with ten proteins in the network was based on co-expression and text mining. AAEL000442 showed highest homology score (0.798) through co-expression followed by that of AAEL00923 (0.689) through text mining and co-expression and AAEL007657 (0.682) through co-expression. AAEL000442 is an uncharacterized hypothetical protein with unknown function. AAEL007657 is a calcium ion binding protein and an integral protein of cell membrane (Nene *et al*., 2007). In case of *A. gambiae* five nodes and five edges with average local clustering coefficient of 0.833 were shown in interaction network. AGAP003545 was the strongest interaction partner through co-expression and homology score 0.753. AGAP 008072 showed interaction through co-expression, text mining and experimental evidences with homology score of 0.43. AGAP006098 (*nos*) had homology score of 0.405. AGAP008072 was experimentally proved to be involved in interaction with chorion peroxidase of *A. gambiae*. All the proteins are unreviewed but some functions and cellular localizations are reported. AGAP006098 is RNA and zinc binding protein also involved in regulation of translation (Sharakhova *et al*., 2007). AGAP 011828 is involved in cystein type endopeptidase and proteolytic activities of catabolism. AGAP008072 is an integral part of cell membrane. It is involved in Calcium ion binding and also acts as a super oxidase, performing NADPH oxidase activity (Holt *et al*., 2002). These results can help the researchers to select other target proteins for more effective insecticides development and to avoid frequent insecticide resistance.

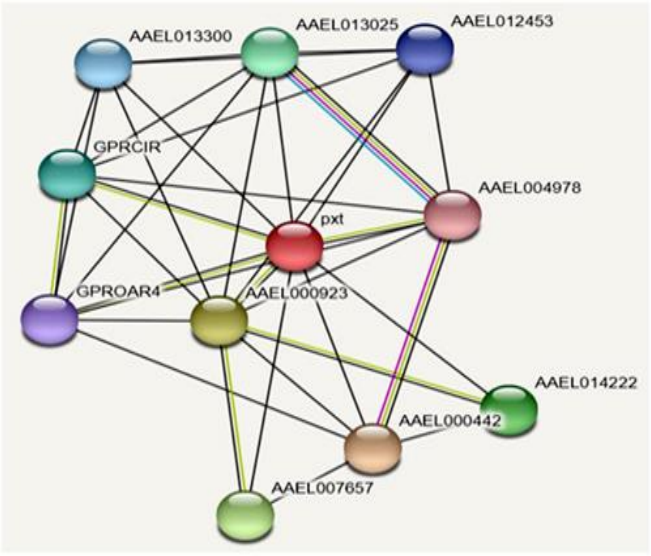


Figure 7. Protein-Protein interaction of chorion peroxidase (P82600) in *Aedes aegypti* L.

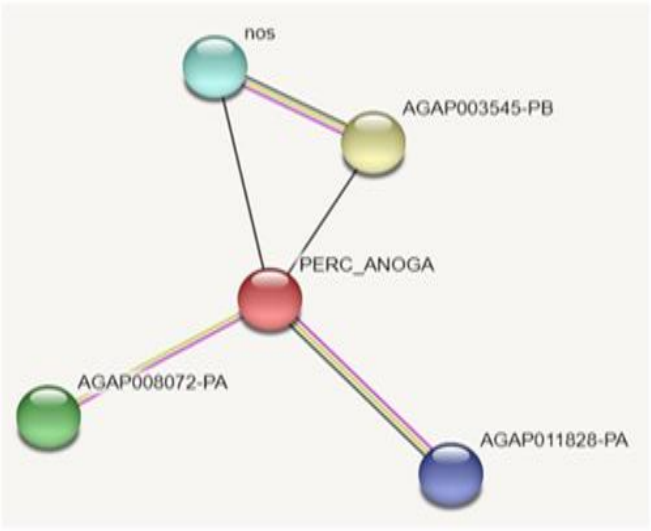


Figure 8. Protein-Protein interaction of chorion peroxidase (Q7QH73) in *Anopheles gambiae* G.

***Conclusion*:** Target site insensitivity is one of the common causes of insecticide resistance in insects. New insecticide compounds can be developed for better efficacy by changing their target sites, which will be helpful in managing the insecticide resistance. This study was designed to find novel compounds that can act as inhibitors against the chorion peroxidase of mosquitoes, *A. aegypti* and *A. gambiae*. Chorion peroxidase are involved in the formation of a rigid and insoluble egg chorion by catalyzing chorion protein cross-linking through dityrosine formation and responsible for hardening of eggshell. Pharmacophore modeling, virtual screening and molecular docking were used to filter the compounds having high binding energy with target proteins. It is concluded that ZINC04581496 and ZINC15675298 were effective lead compounds in case of *A. aegypti* and *A. gambiae*, respectively. These compounds are harmless to human because they target only the chorion peroxidase in insects. These pharmacophore models may provide theoretical basis for designing effective insecticides for *A. aegypti* and *A. gambiae*. The efficacy of computationally predicted lead molecules can be confirmed by testing *in vitro* and *in vivo*. In the current study we have screened the compounds from library and saved time and resources by providing baseline information for further wet lab experimentations

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