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Neonicotinoids in vector control: *In silico* approach

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Abstract

Insecticide based intervention remains the primary control tool for vector borne diseases. Many mosquito vectors have developed resistance to the insecticides which are in use in public health programmes. There is a need to search for alternative insecticides to manage the insecticide resistance. Neonicotinoids are one of the emerging new promising insecticides with novel mode of action against agricultural pests. To understand the neonicotinoids efficacy in vector control, *In silico* docking study has been explored between the neonicotinoids and nicotinic acetylcholine receptor of *Anopheles gambiae* Giles (Diptera: Culicidae). The format of nicotinic acetylcholine receptor (nAChR) protein subunits sequence of the *An. gambiae* was retrieved from NCBI database. Three dimensional structure of nAChR protein subunits has been predicted using Modeller 9.10. The predicted protein structure was energy minimized by Swiss Pdb viewer 4.1. Stereo chemical analysis of a structure was performed using PROCHECK Ramachandran plots from SAVES server. Validated protein structure was visualized by UCSF Chimera 1.5.3 and models were submitted in Protein Model Database (PMDB). Molegro virtual docker 5.0 was used for nAChR protein subunits and neonicotinoid compounds docking study. Nicotinic acetylcholine receptor subunit's three dimensional structure predicted models were submitted in Protein Model Database having PMID PM0079637, PM0079638, PM0079639, PM0079640, PM0079641, PM0079642, PM0079643, PM0079644, PM0079645 and PM0079646. Each neonicotinoid compound selectively bound to the receptor subunits individually. The best conformation with the least binding energy was selected. Out of seven compounds, dinotefuran has the least binding energy value of –110 Mol Dock score and Re rank score of –94.63 with more number of H-bond interactions showing the highest activity in nAChR α3 subunit. The docking scores of neonicotinoid compounds against nicotinic acetylcholine receptor subunit of *An. gambiae* are in the order of dinotefuran >thiamethoxam >clothianidin >imidacloprid >nitenpyram >acetamiprid> thiacloprid. Among the seven neonicotinoids, dinotefuran has shown the least docking score with highest activity and possibly this molecule can be used in control of mosquito vectors.

Keywords: Neonicoinoids, *Anopheles gambiae*, insecticidal activity, in silico, docking.

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INTRODUCTION

Mosquitoes (Diptera: Culicidae) transmit diseases such as malaria, dengue, chikungunya, japanese encephalitis, yellow fever, lymphatic filariasis, etc. These vector borne diseases pose much threat to the economy of developing countries and cause enormous morbidity and mortality in more than 100 countries world over. Insecticide based intervention remains the primary control tool for vector borne diseases. One of the important challenges in vector control is the development of resistance to insecticides. To ensure long-term effectiveness of insecticides and to prevent or delay the onset of resistance to chemical insecticides, there is an immediate need to introduce new insecticide molecules with novel mode of action and their optimization for use [1]. Several new promising insecticides are effective against agricultural pests and neonicotinoid group is one among them. The neonicotinoids are systemic toxins that target nAChRs in the insect nervous system. Neonicotinoid group contains seven compounds, namely acetamiprid, dinotefuran, clothianidin, nitenpyram, imidacloprid, thiacloprid and thiamethoxam and comes under Group 4A nicotinic acetylcholine receptor agonists according to IRAC mode of classification [2]. The neonicotinoids have attracted great attention for their high efficacy, no-cross resistance and unique mode of action [3]. In general, these compounds possess low mammalian toxicity in comparison to other toxins and also relatively non-toxic to non-target species. Further, neonicotinoids are selective to insects because of the differential sensitivity of insect and vertebrate nAChRs subtypes [4].

Neonicotinoid group of compounds can be classified based on structure as follows: imidacloprid, thiacloprid and thiamethoxam as cyclic forms whereas acetamiprid, dinotefuran, clothianidin and nitenpyram as non cyclic forms. Based on common pharmacophore, these are classified into three groups as follows: imidacloprid, clothianidin, and nitenpyram as nitromethylenes; thiamethoxam and dinotefuran as n-nitroguanidines; thiacloprid, and acetamiprid as n-cyanoamidines [5].

Neonicotinoids have unique properties, due to these they act as novel candidate molecules in agriculture pest control. nAChRs that mediate fast cholinergic synaptic transmission in the insect nervous system. nAChR gene family was characterized in the genome of major malaria vector *Anopheles gambiae* Giles (Diptera: Culicidae). nAChR gene family in *An. gambiae* possess 10 subunits, nine α subunits and one β subunit [6].

Hence, the *in silico* molecular docking study has been attempted to explore the activities of neonicotinoids against the nAChRs gene family of *An. gambiae*.

METHODS

Data collection:

The format of nAChR protein subunit's sequence of the *An. gambiae* α1, α2, α3, α4, α5, α6, α7, α8, α9 and β1 were retrieved from NCBI database which have the accession numbers AAU12503, AAU12504, AAU12505, AAU12506, AAU12507, AAU12508, AAU12509, AAU12511, AAU12512 and AAU12513, respectively. The sdf format of the seven neonicotinoid compounds, acetamiprid, dinotefuran, imidacloprid, nitenpyram, clothianidin, thiacloprid and thiamethoxam were downloaded from the Pubchem database which have the IDs CID 213021, CID 197701, CID 86418, CID 3034287, CID 213027, CID 115224, and CID 5821911 respectively.

Protein structure prediction and validation:

In this study, three dimensional structure of nAChR protein subunits was predicted by using Homology modeling. Homology modeling is the reliable method for protein structure prediction with the suitable template. The potential template for nAChR protein subunits of *An. gambiae* was searched by BLASTP against Pdb database [7]. Three dimensional structure of nAChR protein subunits has been predicted by Modeller 9.10 using the selected templates [8]. Once the 3D model was generated, energy minimization was performed by GROMOS 96 force field in a Swiss-Pdb viewer V4.1 [9]. Stereo chemical analysis of a structure was performed using PROCHECK Ramachandran plot from SAVES server [10]. Visualization of generated models was performed using UCSF chimera 1.5.3 [11]. The generated protein models were submitted in Protein Model Database (PMDB) [12]. Protein Model Database was repository for submitting the protein models predict by theoretical methods.

Ligand preparation:

Neonicotinoid compound sdf format was converted to Pdb format by Open babel 2.2.1 software [13] and was used for further docking studies.

Molecular docking simulation:

Molegro virtual docker 5.0 (trial version) [14] is an automated docking software used for nAChR protein and neonicotinoid compounds docking study. Protein and ligand structures were prepared and binding site for protein was predicted using detect cavities option. Finally, protein and ligand docking was performed by using the docking wizard. The default parameters were used for docking at a grid resolution of 0.30 A0 for a grid generation and a 15 Aº radius for the template as the binding site. We used the Moldock optimizer as a search algorithm and the number of runs was set to 10. A population size of 50, maximum iteration of 2000, scaling factor of 0.50, cross over rate of 0.90 and variation based termination scheme for parameter

settings were used. The maximum number of poses was set to a default value of 5.

RESULTS AND DISCUSSION

More than one template used in modeling the protein structure. The BLASTP for nAChR protein subunits resulted in various templates and those with best identity with query sequence was used for modeling are given in (Table 1). The template percent identity was ranged from minimum 30% to maximum 65%. The template structure and query sequence were aligned in Modeller 9.10 and three dimensional protein structure was generated [15-16]. Three dimensional structure provides information on the function and also help us to analyze its interactions with the suitable inhibitors. Predicted protein structure was energy minimized by Swiss-Pdb Viewer. The refined structure was then submitted in SAVES server PROCHECK module for evaluation. Ramachandran plot was used to visualize dihedral angles phi against psi of amino acid residues in protein structure. Amino acid distribution in core, allowed, generously allowed and disallowed regions have been shown in Table 2. Most of the amino acids were present in the favoured region in the plot which confirms that the protein structure is good with stable conformation and can be used for further studies [17]. Validated protein structure has been visualized through Chimera software and used for further docking studies (Fig.1) [18]. The generated models were submitted in Protein Model Database having PMID PM0079637, PM0079638, PM0079639, PM0079640, PM0079641, PM0079642, PM0079643, PM0079644, PM0079645 and PM0079646. The nAChR protein target predicted structure was used for further studies. The binding compatibility of neonicotinoid compounds (Kcal/mol) with the nAChR protein subunits of *An. gambiae* is given in Table 3 and docking conformation in Fig. 2. Docking results were evaluated based on Mol Dock and Re-ranking scores [19-20]. The least binding energy values with best fit posses in the protein cavity [21-23] are a stable docking conformation [24-25]. Each neonicotinoid compound bound selectively to the receptor subunits individually with the best docking energy (Table 3). Out of seven compounds, dinotefuran compound has the least binding energy value of –110 Mol Dock score and Re rank score of –94.63 with more number of H-bond interactions showing the highest activity in nAChR α3 subunit. The docking scores show the order of neonicotinoid compound activity against nAChR protein subunit of *An. gambiae* as follows: dinotefuran >thiamethoxam >clothianidin >imidacloprid >nitenpyram >acetamiprid >thiacloprid. It was observed that the binding affinity and docking score were approximately similar to the pharmacophore mode of action. Both the dinotefuran and thiamethoxam compounds from n-nitroguanidines have the similar docking score. Similarly, imidacloprid, nitenpyram and clothianidin from nitromethylenes have similar docking score. Thiacloprid and acetamiprid from n-cyano amidine group have similar docking score. The docking scores of neonicotinoid pharmacophore are as follows: n-nitroguanidines >nitromethylenes >n-cyano amidines. One more interesting was open chain compounds dinotefuran, acetamiprid and clothianidin have higher docking scores than ring or closed structure compounds. Probably this is a first attempt to show each neonicotinoid compound's activity with the member of *An. gambiae* nAChR gene family. Each neonicotinoid compound has specific interaction with the receptor subunit with specific site.

The intrinsic toxicity of dinotefuran was by larval bioassay and topical application against different mosquito strains of *An. gambiae*, *Culex quinquefasciatus*, and *Aedes aegypti* [26]. Results showed that susceptible mosquitoes has relatively low toxicity of dinotefuran than resistant mosquitoes and the absence of cross resistance with common insecticides used in public health (pyrethroids, carbamates, and organophosphates) makes dinotefuran potential candidates for disease vector control, especially in area where mosquitoes are resistant to insecticides [26]. Results of the docking study also supported above observations and seven insecticide molecules indicated that dinotefuran has shown least docking score with highest activity and possibly can be used in vector control.

nAChR protein subunit	Template	Identity (%)
α1	2QC1 B	48
	2KSR A	65
α 2	2BG9 B	44
	1OED A	55
α 3	1OED C	51
	3SQ6 A	35
α 4	2BG9 E	42
	2LM2 A	57
α 5	2BYP A	31
	2BR7 A	32
α 6	2QC1 B	44
	1OED B	33
α 7	2QC1B	41
	3PMZ A	30
α 8	4AFG A	30
	1OED E	33
α 9	2BG9 A	30
	1KH1 A	47
β1	2BG9 C	46
	2LLY A	61

Table 1: Summary of the templates resulted from BLAST for *Anopheles gambiae* nAChR protein subunit and used for protein structure prediction

nAChR protein subunit	Core region (%)	Allowed region (%)	Generously allowed region (%)	Disallowed region (%)
α 1	77.0	18.2	2.7	2.0
α 2	84.9	12.0	2.0	1.2
α 3	80.0	16.8	1.9	1.3
α 4	83.7	11.3	2.4	2.6
α 5	88.4	9.9	0.6	0.6
α 6	81.4	16.8	1.1	0.7
α 7	74.2	20.4	2.9	2.4
α 8	79.7	16.1	1.7	2.6
α 9	93.1	5.5	1.1	0.3
β1	81.9	14.7	2.4	1.1

Table 2: *Anopheles gambiae* nAChR protein model validation by using SAVES Procheck

Compound	MVD		Receptor	No of H-Bonds formed	Residues involved
	Mol dock score	Re rank score			
Acetamiprid	–88.7137	–73.608	α 5	4	thr 433, ser 322, ser 326
Clothianidin	–104.192	–88.5853	α 4	4	thr 261, ser 320, ser 258
Dinotefuran	–110	–94.6378	α 3	10	asp 450, ser 407, ser 406, ser 408, met 452
Imidacloprid	–102.724	–83.4848	α 2	6	lys 177, tyr 552, tyr 232, tyr 125
Nitenpyram	–93.7762	–78.4386	α 6	4	gly160, lys206
Thiacloprid	–86.417	–70.5594	α 8	3	leu 510, asn 507
Thiamethoxam	–106.616	–90.6216	α 7	6	thr 409, asn 367

Table 3: Docking score of seven neonicotinoid compounds and nACHR subunits of *Anopheles gambiae* using Molegro virtual docker 5.0

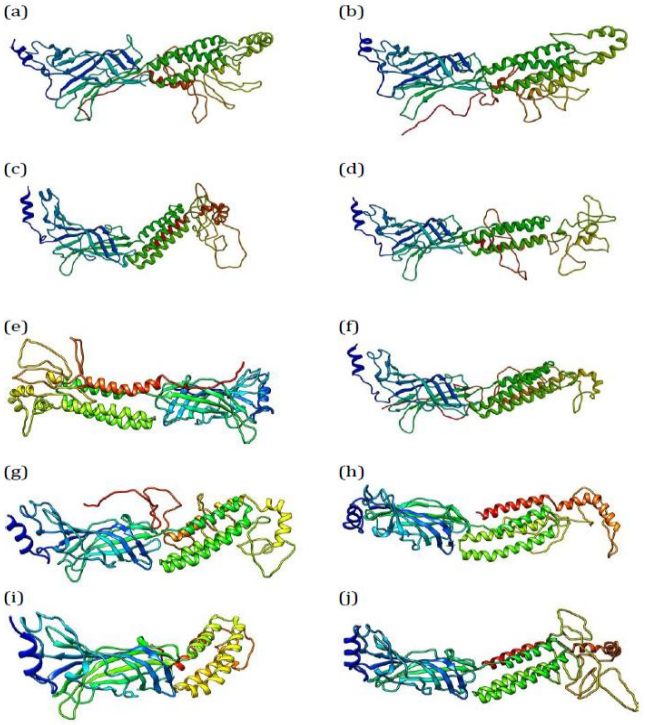

Fig.1 : *Anopheles gambiae* predicted structure of nAChR protein subunit (a) α 1 (b) α 2 (c) α 3 (d) α 4 (e) α 5 (f) α 6 (g) α 7 (h) α 8 (i) α 9 (j) β1

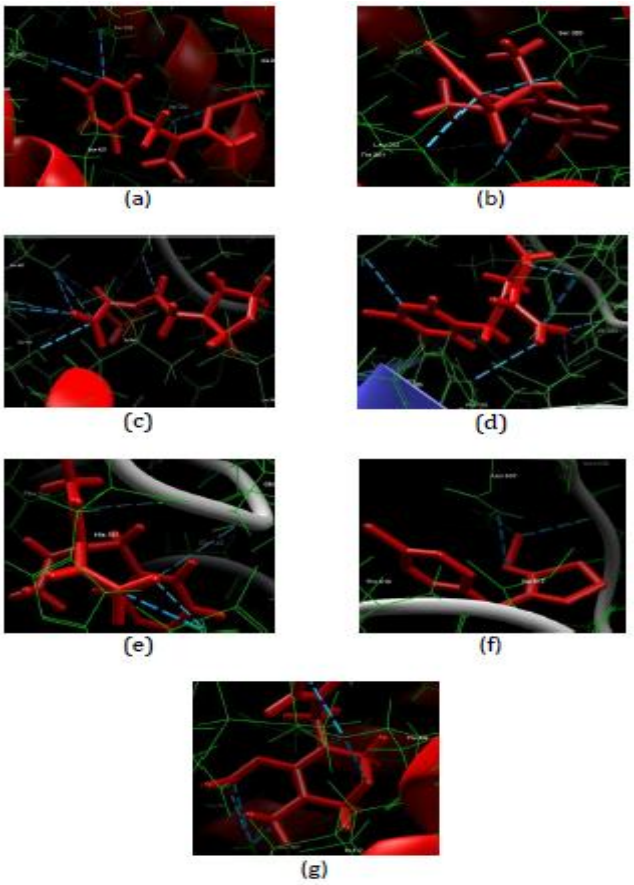

Fig. 2: Docking conformation of (a) acetamiprid (b) clothianidin (c) dinotefuran (d) imidacloprid (e) nitenpyram (f) thiacloprid (g) thiamethoxam with nAChR protein subunits of *Anopheles gambiae* by using Molegro virtual docker 5.0

CONCLUSION

This study clearly shows that each neonicotinoid compound acts selectively with the nAChR protein subunit of the *An. gambiae*. Out of seven insecticide molecules, dinotefuran has shown least docking score with highest activity indicating its possible use in vector control. More studies are needed for assessing the feasibility of using neonicotinoids for their efficacy in controlling adult and larval stages of mosquitoes.

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