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In Silico Studies on Acetylcholine Receptor Subunit Alpha-L1 for Proposal of Novel Insecticides against *Aphis craccivora* †

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Abstract: *Aphis craccivora* is an aphid which damages many species of plants and is also a vector for numerous plant viruses. Since neonicotinoids are a well-known class of effective insecticides with less toxicity against mammals and other vertebrates, a small dataset of compounds retrieved from literature based on similarity with highly active neonicotinoids was used. Thus, homology modelling was involved in the building of the 3D structure of the acetylcholine receptor subunit alpha-L1 of *Aphis craccivora*. The homology model was involved in virtual screening experiments using the Fred docking tool of the OpenEye software. The aforementioned dataset was used to explore the intermolecular ligand-target interaction patterns for a rational design of desired and relatively safe insecticides.

Keywords: homology model; docking; neonicotinoids; aphid

1. Introduction

Aphis craccivora (*A. craccivora*) is known as one of the most common aphids in the tropics [1] nowadays and has an extended distribution worldwide. This aphid is an important pest especially in groundnuts, cowpeas, and numerous other leguminous crops. Being considered the most harmful insect of groundnut throughout Africa [2], *A. craccivora* makes direct feeding and indirect damages, being a vector for numerous plant viruses [3]. It was observed resistance to organophosphate, nicotine, pyrethroids and organochlorine insecticides in *A. craccivora* populations on various plants in India [4]. Therefore, finding new potential pesticides to prevent the resistance issue is a legitimate goal. After pyrethroid chemicals, neonicotinoids represent a major advance in terms of insecticides [5]. They are a well-known class of effective pesticides with less toxicity against mammals and other vertebrates [6].

In this study, for a better understanding of the mechanism of action of neonicotinoid and neonicotinoid-like compounds we conducted an in silico experiment consisting of homology modelling and molecular docking procedures. The 3D structure of the neonicotinoid target is experimentally unavailable, therefore the homology model of the acetylcholine receptor subunit alpha-L1 of *A. craccivora* was first built. Further, a small dataset of 648 compounds retrieved from literature based on the similarity with highly potent neoticotinoids was docked in the binding site of the homology model. Knowing that neonicotinoids are pesticides involved in the decline of bees [7], the dataset was preliminarily investigated using the BeeTox tool [8] to predict the bee toxicity of these compounds and they were found to be safe. The aforementioned dataset was used to explore the intermolecular ligand-target interaction patterns for a rational design of desired insecticides. The

docking results were analyzed, by inspecting the key ligands – target interactions and the ranking of the docking scores.

2. Methods

The homology model for the acetylcholine receptor subunit alpha-L1 of *A. craccivora* (GenBank ID: KAF0770983.1) was realized using the Swiss Model server [9,10]. In the first instance, the KAF0770983.1 sequence was used to find the most appropriate template. As the complex of human alpha3beta4 nicotinic acetylcholine receptor with nicotine (6PV7 PDB ID) was identified by the Swiss Model software, taking into account the best coverage score, it was further used as template in the homology modelling process. It was observed that the binding site of the 6PV7 protein is located between the chains of two monomer units. Thus, the sequence alignment of the A and B chains of the 6PV7 receptor and the KAF0770983.1 sequence of the target was achieved using the Blast server [11]. The alignment obtained for each chain was then submitted to "alignment of the target sequence and template structure(s)" module of the Swiss Model server. Finally, the raw homology model consisting of two chains (A and B) was obtained and a process of refining and optimizing was undergone using the Maestro software from the Schrodinger suite (https://www.schrodinger.com/) and the Deep View Swiss-Pdb Viewer version 4.1.0 program [12]. The loops that have been observed as having problems were remodelled and the steric clashes between the side chains of the amino acid residues have been removed by choosing the right rotamers for them. To attest the stereochemical quality of the homology model the PROCHECK server was involved [13].

A dataset of 648 compounds retrieved from literature [14–34] was used in the virtual screening experiments based on their similarity with high active neonicotinoids. In advance, the compounds were prepared for docking studies using the OMEGA software [35,36] using the 94s MMFF (Merck Molecular force field) option for the minimization of the generated conformers.

The homology structure was prepared with the Make Receptor 3.5.0.4 and then the Fred 3.5.0.4 tool (Open Eye Scientific Software) [37–39] was utilized for molecular docking. Every conformer was docked into the protein binding site with the aid of the FRED program, which uses an exhaustive rotation–translation search algorithm.

3. Results and Discussion

The sequence alignment between the target and template resulted from the Blast server and further used for the building of the quaternary homology model is presented in Figure 1. The visualization of the alignment was done using the BIOVIA software [40].

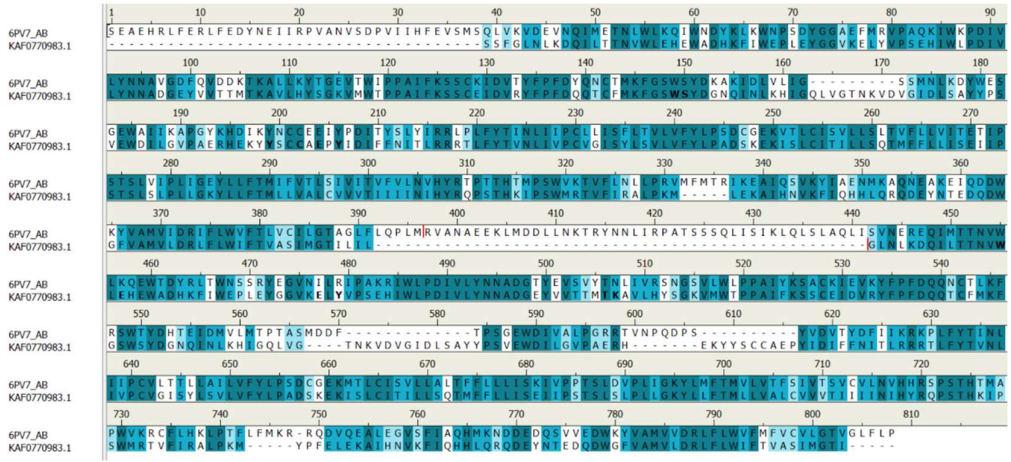


Figure 1. The sequence alignment was achieved between the query (KAF0770983.1—GenBank ID, acetylcholine receptor subunit alpha-L1 [Aphis craccivora]) and the template (6PV7|Chains A, B|—PDB ID, Fusion protein of Neuronal acetylcholine receptor subunit alpha-3 and Soluble cytochrome b562|Homo sapiens). The similarity character of the alignment is highlighted by increasing the color

intensity (white—dissimilar amino acids, dark blue—identical amino acids). The identified binding site amino acid residues are bold depicted.

The raw homology model was evaluated using the PROCHECK server [13] for checking its stereochemical quality, through a series of plots for the analysis of its overall and residue-by-residue geometry. Especially Ramachandran map is an important tool for the validation of a good protein`s geometry. A good quality protein structure would be expected to have over 90% residues in the most favoured regions. The refined homology model has 564 residues (90.4%) in the most favoured regions [A,B,L]; 59 residues (9.5%) in the additional allowed regions [a,b,l,p], 1 (0.2%) residue in the generously allowed regions [~a,~b,~l,~p], and 0 residues in disallowed regions (Figure 2). The main and side chain parameters were also analyzed and were observed to be in the allowed mean or even better. After iterative steps of refining and evaluation, the final model was validated as it results from the plots shown in Figures 2 and 3.

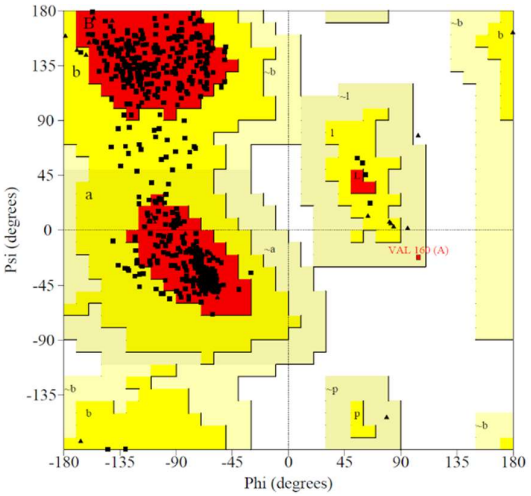
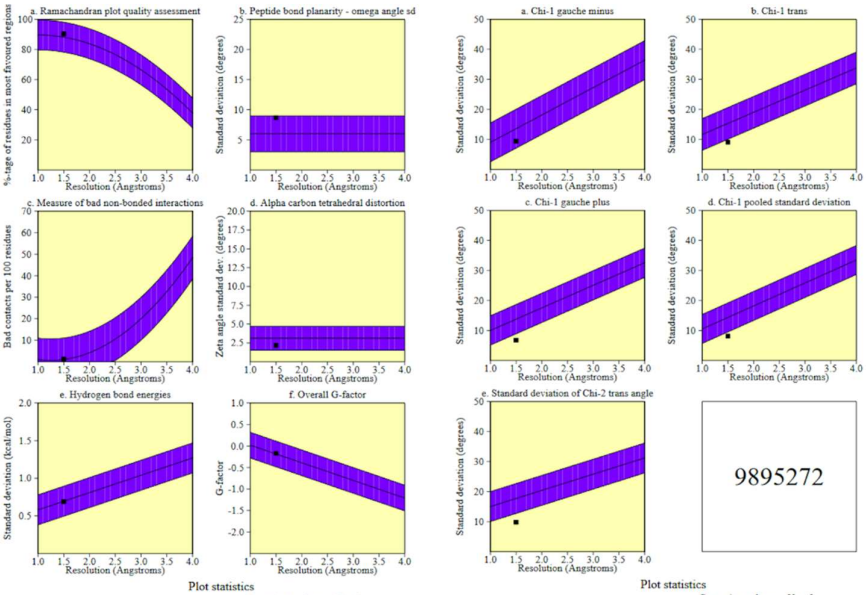


Figure 2. The Ramachandran plot of the refined homology model with 90.4% residues in most favoured regions [A,B,L].



(**a**) (**b**)

Figure 3. Main-chain (**a**) and side-chain parameters (**b**).

The dimeric structure of the homology model and the identified binding site located between its two monomer units is presented in Figure 4.

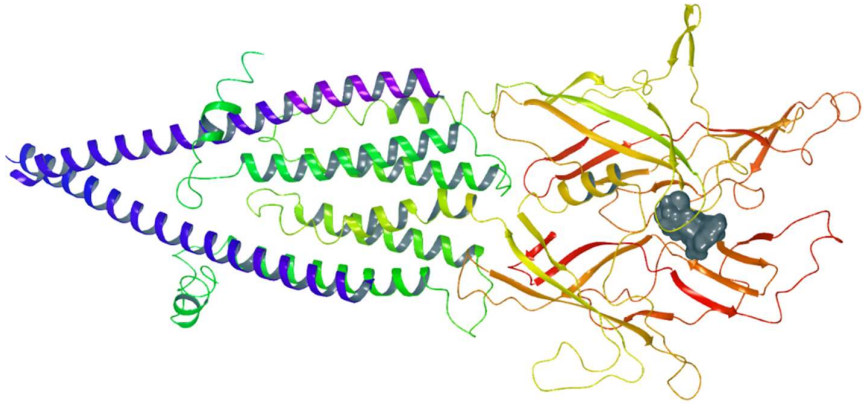
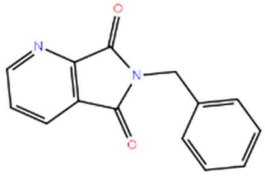
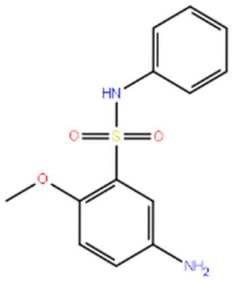
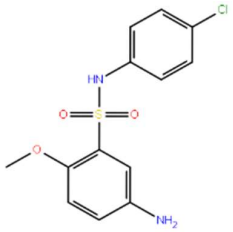
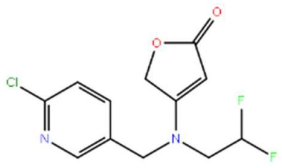


Figure 4. The homology model of the acetylcholine receptor of *A. craccivora*. The binding site is represented by the grey surface.

The docking of the selected dataset of 648 compounds led to their ranking. Thus, considering the best FRED Chemgauss4 score values the top four compounds were chosen for further analyses (Table 1).

Table 1. Top four compounds as resulted from the FRED docking ranking.

2D Structure	FRED Chemgauss4 Score	Reference
	−10.759	15liu2015
	−10.748	14bavadi2017
	−10.493	15bavadi2017
	−10.353	2jeanmart2016

The visual inspection of the docking outcome for these four top ranked compounds is presented in Figure 5.

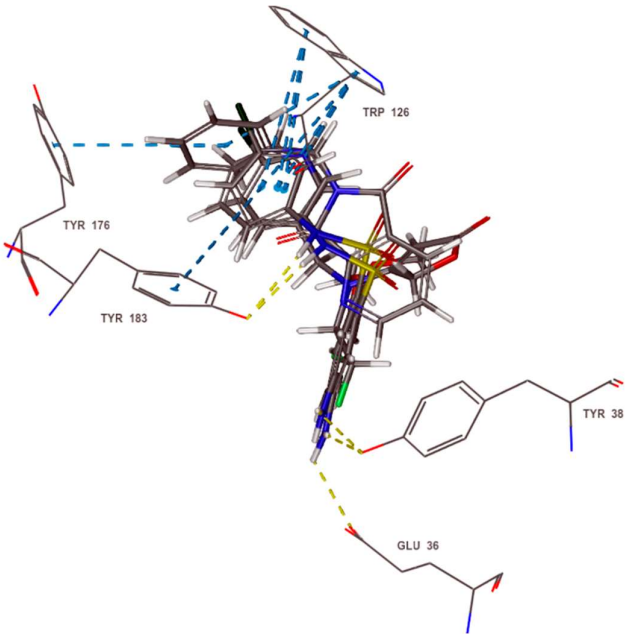


Figure 5. The binding site of acetylcholine receptor homology model along with the four top ranked compounds. The compounds are represented by sticks and the binding site amino acids by lines. The hydrophobic interactions are highlighted by blue dashed lines, while the yellow dashed lines represent the hydrogen bonds.

From the superposition of the compounds in the binding site (Figure 5), it can be observed that the most common is a hydrophobic interaction with Trp126(A) residue. Our finding is also supported by another study [41], in which the equivalent residue Trp147(A) of the crystal structure of acetylcholine-binding proteins (3C79-PDB ID) makes important hydrophobic interaction with the co-crystallized pesticide: imidacloprid. Further, the hydrophobic interactions of the ligands through aromatic rings with the other two amino acid residues (Tyr183(A) and Tyr176(A)) lead to their stabilization into the binding site.

It has been found that Tyr residues play an important role in the acetylcholine-binding protein site [41,42], both by forming hydrophobic interactions and, also, hydrogen bonds (Figure 5). As one can see, an amino acid from chain B, namely Glu36, participates additionally as an acceptor in the formation of a hydrogen bond.

4. Conclusions

In order to realize a structure-based virtual screening experiment, a qualitative homology model for the acetylcholine receptor subunit alpha-L1 of *Aphis craccivora* was built. A dataset of structures retrieved from literature was docked in the binding site of the receptor. Four top ranked compounds were further analyzed. Their poses showed important hydrophobic and hydrophilic interactions with the residues, which were, also, confirmed by other studies as being key amino acids in the acetylcholine receptor – neonicotinoid insecticide interactions. Additionally, the toxic character on bee of these compounds was evaluated with the aid of the BeeTox server. They were predicted to be safe, thus, we can conclude that the compounds proposed by us could be further tested to confirm their potential insecticidal activity against *A. craccivora*.

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Conflicts of Interest: The authors declare no conflict of interest.

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