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Molecular docking study of triterpenoid azadirachtin A on acetylcholinesterase of Drosophila melanogaster (Diptera: Drosophilidae)

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Abstract: Organic molecules of botanical origin can offer a source of compounds of pest management that are more environmentally acceptable and an efficient alternative to replace persistent synthetic insecticides. The molecular docking study using Molegro Virtual Docker software identified that the triterpenoid azadirachtin A showed stable conformations, with lower energy in the ligand-receptor complex of the compounds analyzed in this study, thus having a high affinity for the active site of the enzyme acetylcholinesterase, from a variety of interactions, which can determine its insecticidal potential against the species Drosophila melanogaster.

Keywords: Drosophila, Docking, Triterpenoid

1. Introduction

Pest control has mainly depended on insecticides. Organophosphates, carbamates, pyrethroids and neonicotinoids show the development of insects resistant to various insecticides. To evaluate insecticide toxicities, Drosophila melanogaster is an interesting model (ARAIN et al., 2017).

Organic molecules of botanical origin can offer a source of pest control compounds that are more environmentally acceptable and an efficient alternative to replace persistent synthetic insecticides. The limonoids, which is present mainly in the seeds of the neem tree (*Azadirachta* increasing interest in the potential of secondary metabolites in pest control favors the search for new sources of biologically active natural products with low mammalian toxicity, low persistence in the environment, and biodegradability (CESPEDES et al., 2013). Bio-insecticides are safer than synthetic pesticides due to rapid degradation in the environment and low toxicity to vertebrates (DERE et al., 2015).

Therefore, one of the alternatives is the use of botanical insecticides, Azadirachtin A, is a triterpenoid belonging to the class *indica*) (MORGAN 2009) and is one of the most biologically natural insecticides

active (BOULAHBEL et al., 2015). Recently this compound was evaluated as a significant biopesticide and used for increasingly in pest control programs (BAJWA and AHMAD, 2012).

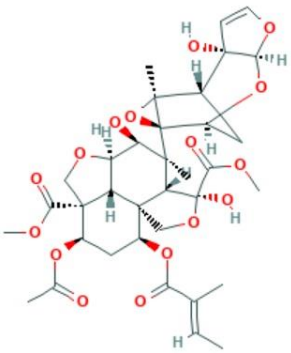
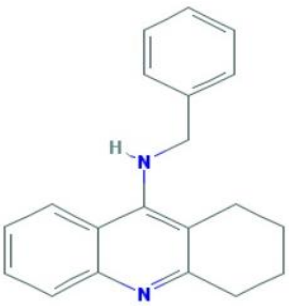
Therefore, the objective of this work was to verify the interactions between triterpenoid molecules azadirachtin A, the active principle of a synthetic insecticide carbofuran and the PDB ligand in the active site of the enzyme acetylcholinesterase of the species Drosophila melanogaster, helping to understand the determining characteristics of the interaction ligand-receptor.

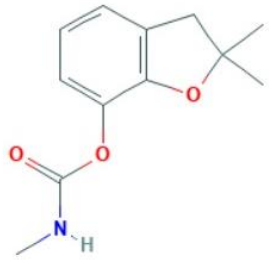
2. Results and Discussion

In the molecular docking study it was possible to verify that the activity of the selected triterpenoid Azadirachtin A, shows a greater affinity with the acetylcholinesterase enzyme than the commercial insecticide carbofuran and 9-n-phenylmethylamino-tacrine (PDB ligand).

In the Table 1, we can observe that the triterpenoid Azadirachtin A presented the lowest binding energy value, in relation to 9-n-phenylmethylamino-tacrine (PDB ligand) and to the active principle, carbofuran. This demonstrates that Azadirachtin A presented more stable conformations, thus, as the greater number of interactions with the amino acid residues in the enzyme acetylcholinesterase. Analyzes of the interactions identified by the amino acid residue of the enzyme with the ligands under study were also performed.

Table 1: Results of the best energy poses of the Docking Molecular study of the ligands tested and the crystallographic ligand in the active site of the enzyme acetylcholinesterase.

Name	Ligand	Moldock Score	HBond
Azadirachtin ($C_{35}H_{44}O_{16}$)		-206.492	-11.3839
9-n-phenylmethylamino-tacrine (PDB ligand)		-129.853	0

Carbofuran (2,2-dimethyl-3H-1-benzofuran-7-yl) N-methylcarbamate.		-100.335	-5

For the triterpenoid molecule azadirachtin A, the lower energy pose identified that the compound had several interactions with the amino acid residues of the active site of the enzyme acetylcholinesterase. Amino acids Tyr 162, Thr 154, Gly 155, Met 153, Gly 151, Gly 150, Phe 330, Tyr 370, Try 71, Phe 371, His 480, Asp 482 and Trp 83 perform steric interactions with the oxygen atoms of the groups esters and ether in the molecule. Interactions of hydrogen bonds with Trp 83 residues with the ether group were also identified as well as interactions with residues Asp 482, His 480, Leu 479, Tyr 71, Tyr 370, Thr 154 also with the oxygen atoms of the esters . And interactions of Van der waals with amino acid residues Phe 152, Tyr 324, Trp 321, Glu 80, Gly 79, Ans 84, Gly 79, lle 82, Gly 149, Leu 159, Tyr 148 and Glu 485. These interactions may be observed in Figure 1. No electrostatic interactions were identified.

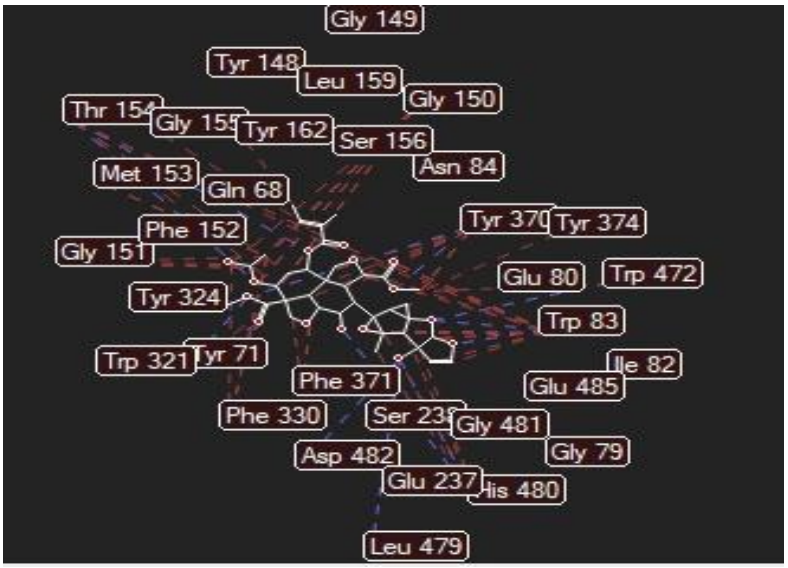


Figure 1: Representation of triterpenoid (azadirachtin A) at the active site of the acetylcholinesterase enzyme in molecular docking. The interactions of the hydrogen bonds are observed in the dotted lines in blue and the red ones represent the steric interactions, as well as interactions of van der waals were identified.

In the molecule of the active principle, carbofuran (**Figure 2**), hydrogen bonding interactions were identified with amino acid residues His 480 with carbonyl and Glu 237 with the nitrogen atom. And the steric interaction between the ether oxygen and the Trp residue 83. As well as Wan der Waals binding interactions were observed with residues Gly 79, Trp 472, Leu 479, Try 370, Gly 481, Tyr 162, Gly 149, Gly 150, Ser 238, lle 484. In this molecule no electrostatic interactions were identified.

In the 9-n-phenylmethylamino-tacrine compound (PDB ligand) (Figure 3) steric interactions with the benzene ring were identified with residues Tyr 71 and Phe 330, as well as interactions of the ether group with Trp 83 and Gly 150 on carbon 3 of cyclohexane. And interactions of Van der

waals with amino acid residues Trp 321, Gly 151, 371, Ser 238, His 480, Try 162, Gly 149, Glu 237, lle 484, Gly 79, Gly 481, Leu 479, Trp 472, Glu 80 and Thr 154. In this molecule no electrostatic interactions were identified, nor were hydrogen bonds observed.

In the analysis of the results it was possible to identify that the steric interaction with the amino Phe

acid residue Tryptophan 83 was observed in the three compounds. Steric interactions with the amino acid residues Glycine 150, Tyrosine 71, Phenylalanine 330 were observed only in the triterpenoid (azadirachtin A) and in the PDB ligand.

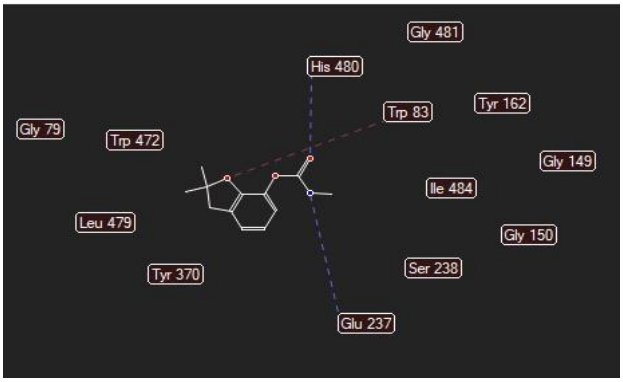


Figure 2: Representation of the active principle carbofuran in the active site of the enzyme acetylcholinesterase in molecular docking. The hydrogen bond interactions are observed on the dotted lines in blue, while the steric interactions are identified on the dotted lines in red.

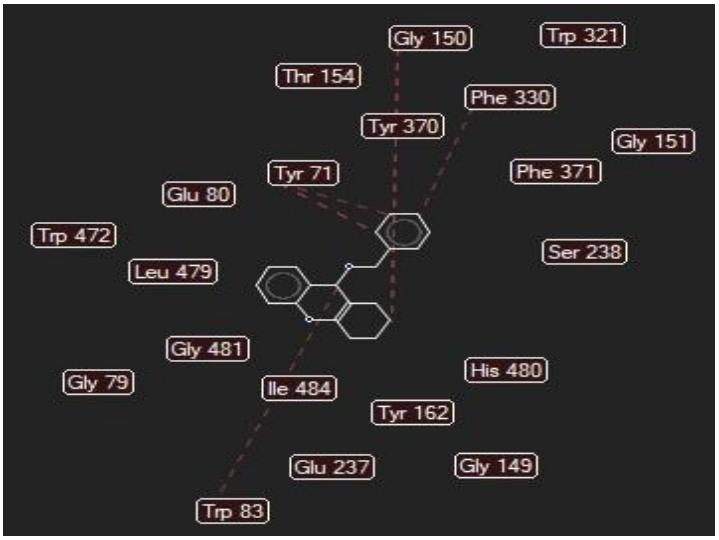


Figure 3: Representation of 9-n-phenylmethylamino-tacrine (PDB ligand) at the active site of the acetylcholinesterase enzyme in molecular docking. The steric interactions are identified on the dotted lines in red.

3. Method

The compounds selected for this work were: a triterpenoid (Azadirachtin A) that was isolated from the species *Azadirachta indica* (Meliaceae) published by (Morgan 2009), an active principle of a synthetic insecticide (carbofuran) and the ligand PDB (9-n -phenylmethylamino-tacrine). These molecules were designed using Hyperchem v. 8.03. Then, they were subjected to geometry optimization, conformational analysis and energy minimization, initially using the MM molecular mechanics method (HOCQUET & LANGGARD, 1998) and then the semi-empirical quantum method AM1 (Austin Model 1) (DEWAR et al., 1985).

Protein Data Bank (PDB)

The acetylcholinesterase enzyme was selected because of its importance in the nervous system of insects, because in order for nerve impulses to be transmitted through synapses, it is necessary for a neurotransmitter, acetylcholine (ACh), to transmit these impulses from one neuron to another, until it reaches the cell to be excited. After this excitation is performed the acetylcholine needs to return to the inside of the neuron where the nerve cell returns to the resting state and can be excited again. This return is accomplished by the enzyme acetylcholinesterase that breaks Acetylcholine into choline + acetate, which within the neuron rejoins acetylcholine for a new transmission.

The active principles of insecticides, such as Carbofuran, which belong to the chemical group of organophosphates and carbamates and act by binding to the enzyme acetylcholinesterase inhibiting its action, resulting in an accumulation of acetylcholine in the synapse causing hyperexcitability due to continuous transmission and uncontrolled nervous impulses including tremors, seizures, collapse of the central nervous system and death (MATIAS, 2016).

According to Cespedes et al. (2013), global agricultural systems consistently use pesticides of synthetic origin, such as carbamates and organophosphates. These active pesticide targets target acetylcholinesterase and have resulted in a generation of new insect strains resistant to the original pesticides. The development of resistance is related to the modification of receptors involved in the mechanisms and targets of action of a given molecule.

Docking

Initially, the crystallized structure of the acetylcholinesterase protein was obtained in the PDB (Protein Data Bank) under the code 1DX4, being this protein of origin of the species Drosophila melanogaster. The resolution of the crystallographic structure deposited in the PDB is 2.7Å. Anchoring of the molecules was performed using a 15Å GRID in the radius and 0.30Å resolution at the enzyme binding site with the structures.

Molecular docking calculations were performed in the Molegro Virtual Docker v.6.0.1 software and the Algorithm Molde Score algorithm. The water molecule and cofactors were removed from the protein to aid in understanding the ligand-receptor interaction. A template was created on the enzyme using the 9-n-phenylmethylamino-tacrine linker, which was obtained along with the pdb file. The Moldock score algorithm was used as a score function to predict the best interaction between ligand and receptor. Next, a docking wizard was created, in which the enzyme molecules and the ligands were inserted, to analyze the stability of the system through the interactions identified with the active site of the enzyme, taking as reference the value of MolDock Score energy.

4. Conclusion

In conclusion, the molecular coupling study using the Molegro Virtual Docker software identified that the triterpenoid azadirachtin A showed more stable conformations, with a lower energy in the ligand-receptor complex of the compounds analyzed in this study, thus having a high affinity for the active site of the enzyme acetylcholinesterase, from a variety of interactions, which may determine its insecticidal potential against the species Drosophila melanogaster. In relation to the active principle of carbofuran, this showed a lower binding affinity with the amino acid residues of the enzyme, although it is widely used as a commercial insecticide (Furadan). Therefore, docking studies have proved to be a useful tool capable of identifying electronic affinity and helping to understand the ligand-receptor interaction.

5. References

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