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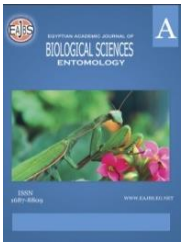

Molecular Modelling of Insecticide Binding Sites of the Voltage-Gated Sodium Channels of Fall Armyworm, (*Spodoptera frugiperda)*

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ABSTRACT

Fall armyworm is a polyphagous migratory pest. Insecticides are used as major components of integrated pest management to control the pest, however, full dependence on insecticides has made the pest evolve resistance to most insecticide classes. The involvement of voltage-gated sodium channels in the excitation of cells makes them a primary target site of a large number of synthetic and naturally occurring neurotoxins. Consequently, it is imperative to delineate the molecular determinants that mediate interactions between insecticides with voltage-gated sodium channels. The present study sought to identify residues involved in the binding of these insecticides and to demarcate the most efficacious insecticides depending on their binding affinity to the voltage-gated sodium channels. The study took an *in-silico* approach to identify docking sites on voltage-gated sodium channels along with the interactions between known insecticides and specific amino acids on the voltage-gated sodium channels. The homology of the *Spodoptera frugiperda* voltage-gated sodium channels was developed to predict the binding sites of different known insecticides that target the insect. The current study identified amino acid residues that insecticides could target to enhance their effectiveness against the fall armyworm. Insecticides that do not target voltage-gated sodium channels also showed interactions with this channel, indicating the possibility of a different mode of action that could be confirmed by experimental studies. Our findings can direct efforts that monitor for mutations that result in insecticide resistance given that new interacting residues were identified. These findings can enable better management of resistance when it develops.

INTRODUCTION

The fall armyworm (*Spodoptera frugiperda*) refers to a polyphagous migratory pest originally a native of the sub-tropical and tropical regions of America. It is amongst the most destructive insects of economic importance infesting maize, cotton, rice, soybean, sorghum and vegetables (Cruz 1995; Figueiredo *et al.*, 2005). The distribution of *S. frugiperda*, covers an immense geographical area even though it is limited to warmer climates due to the increased dispersal ability of adult insects that have spread rapidly to a

wide range of host species (Sparks, 1979).

The over-infestation coupled with high economic loss has promoted reliance on intensive spraying of insecticides. Consequently, the indiscriminate widespread utilization of insecticides has prompted the existence of resistant fall armyworm (FAW) populations to various classes of insecticide including benzoylureas, organophosphates, pyrethroids and carbamates [Diez and Omoto, 2001; Yu *et al.,* 1991, 1992 and 2003).

Studies by Young and McMilan, (1979) document early insecticide resistance in FAW against carbamate. Others followed suit with several resistance instances against organophosphates and pyrethroid being documented (Yu *et al.,* 1991 and 1992). In the laboratory, resistance has been recorded in populations with described resistance ratios higher than 40-times-over to a certain pyrethroid (Morillo and Notz 2001).

In Brazil, pyrethroid resistance has been reported in FAW with a record resistance ratio of 13-folds over lambda-cyhalothrin (Diez and Omoto, 2001). Pyrethroid and organophosphates resistance biochemical characterization in fall armyworm has implied that both insecticide detoxification and target-site insensitivity by enzymes confer resistance (Yu *et al.,* 2003). Furthermore, resistance genetics in the fall armyworm to both methomyl and lambda-cyhalothrin portrays multiple recessive alleles as critical (Rios and Saldamando, 2011). However, both studies do not identify the specific mutations/genes involved.

1 Structure of Voltage-gated Sodium Channels in Insects:

VGSCs are essential in generating and propagating electrical signalling. The existing comprehensive structural and functional integrity of the VGSCs is generated from extensive functional and molecular analysis of mammalian sodium channels (Rinkevich *et al.,* 2013; Catterall, 2000; Chahine, 2018). In the late 1980s, cloned 'para' VGSC of the insect nervous system from *Drosophila melanogaster* was functionally and structurally homologous to the α-subunit of sodium channels in mammals (Davies *et al.,* 2007). Sodium channels in mammals consist of several β-subunits and a pore-forming α-subunit. The latter contains four homologous repeat domains (I–IV), each consisting of six segments (S1–S6) (Silver *et al.,* 2014; Wang *et al.,* 2013). Segments1-4 in each domain constitutes the sensing module (Figure 1A). The Segment 5 -6 and the P-loops that connect them, form the pore module (Rinkevich et al., 2013; Wang *et al.,* 2015).

Each of the S4 segments harbors five to eight positive charged and evenly spaced residues lysine or arginine hence act as voltage sensors (Chahine, 2018). The ion-selectivity filter is composed of the amino acids found in the inner linings of the P-loops between Segment 5 and S6 (Chahine, 2018). In eukaryotic VGSC, the pore signature is Asparagine/Glutamine/Lysine/Alanine (D/E/K/A) present in P- loops linking Segment 5 and 6 of domains I, II, III, and IV, respectively (Dong *et al.,* 2014). Insects largely have an apparent, single sodium channel gene (Dong, 2010) unlike the mammalian nine α-subunit genes encoding different isoforms of sodium channel with varying gating properties and expression patterns (Goldin *et al.,* 2000; Wang *et al.,* 2013). The β-subunits in mammals function as auxiliary subunits to facilitate membrane localization and modulate channels (Xu *et al.,* 2019). In contrast, insects have no orthologs of mammalian β-subunits (Wang *et al.,* 2013). However, insects depend on alternative gene-splicing mechanisms and editing of ribonucleic acid (RNA) to develop variant sodium channels that have both varying pharmacological and gating characteristics (Dong, 2007, 2010; Wang *et al.,* 2013; Soderlund, 2005). It is highly likely that these variants reflect the in vivo functional diversity of VGSCs, even though, physiological roles of splicing and editing variants is yet to be determined (Silver *et al.,* 2014).

Drosophila melanogaster has non-orthologous proteins TipE and four TipE-homologs (TEH1–4), while other insect species have three to four orthologs. These serve

as sodium channels auxiliary subunits in vivo. TipE and TEH1 contain a structural intracellular N- and C-termini with double transmembrane segments linked by a huge loop on the extracellular side (Wang *et al.,* 2013). The TipE and TEH proteins optimize the sodium current amplitude in Xenopus oocytes just as the β-subunits in mammals, and alter voltage dependence and gating kinetics of the channels in D. melanogaster (DmNav), the house fly (Vssc1) and in German cockroach (BgNav) when co-expressed heterologous in the Xenopus oocytes (Wang *et al.,* 2013; Olson *et al.,* 2008; Tan *et al.,* 2002; Liu *et al.,* 2004; Song *et al.,* 2004; Smith *et al.,* 1997; Warmke *et al.,* 1997; Feng *et al.,* 1995). Additionally, house fly TipE orthologs and Aedes aegypti mosquito (Du et al., 2013) and the American cockroach TEH1 orthologs, (Bourdin *et al.,* 2013) enhance Xenopus oocytes sodium currents. It is likely that similar TipE- and TEH-ortholog effects are in other insects. Through channel gating modulation of auxiliary subunits, sodium channel sensitivity towards insecticides can be modified. If channel inactivation is enhanced to TEH1, there is a significant decrease in potency of deltamethrin on the Drosophila sodium channel (Wang *et al.,* 2013).

2- Functionality of Voltage-gated Sodium Channels (VGSCs):

VGSCs are integral transmembrane proteins vital in cellular electrical signaling by conducting sodium ions across the cell membrane of excitable cells. On cell membrane depolarization, the opening of the activation gate occurs via the outward movement of the S4 segments thereby initiating voltage-dependent activation (Rinkevich *et al.,* 2013). The channel becomes occluded by an inactivation particle a few seconds later through fast inactivation (Catterall 1980). The fast inactivation process is executed by a cytoplasmic moiety formed by residues of Isoleucine-Phyneylalanine-Methionine (IFM) in mammals and Methionine- Phyneylalanine-Methionine (MFM) in insects, in the P-loops between domain III and IV (Fig. 1B). Consequently, this particle blocks the intracellular mouth of the pore thus stopping ion conduction (Rinkevich *et al.,* 2013). This serves to terminate action potential activation and any over-depolarization of the membrane potentials an important role of the channels in cell excitation (Silver *et al.,* 2014).

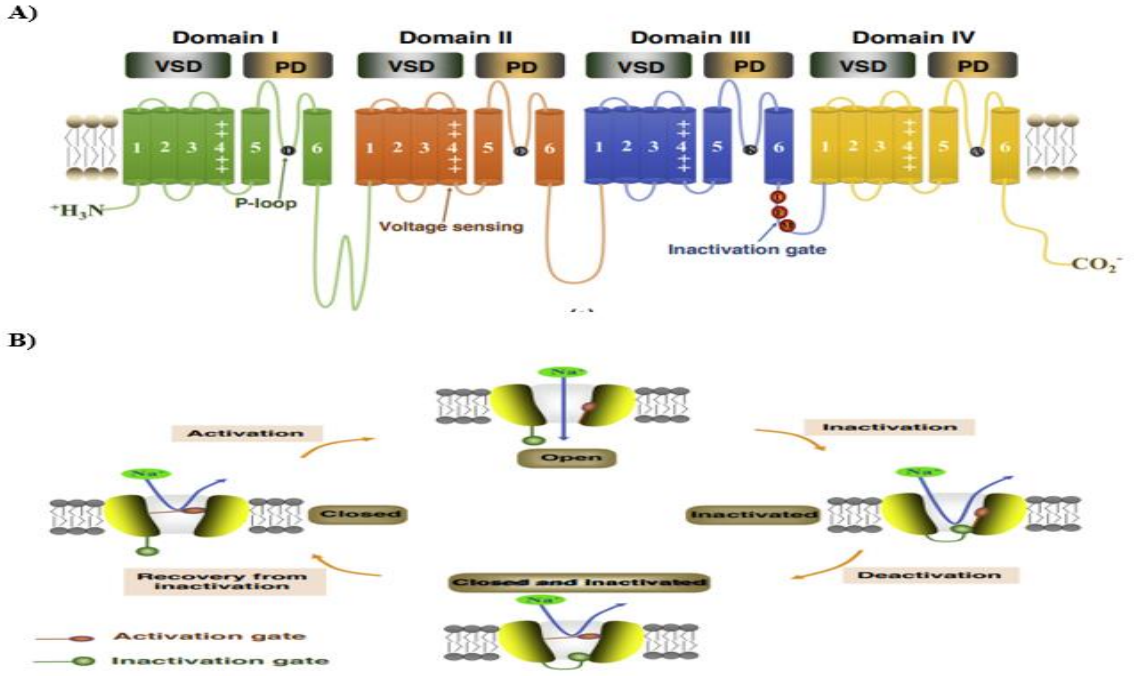

Fig. 1 (**a**) Structure of VGSCs. Segment1–4 form the voltage-sensing domain (VSD). Pore Domain (PD) constitutes the Segment5–6 and the extracellular P-loop linkers. (**b**) Transition states of VGSCs during resting, open, inactivated and closed states. The colors are to differentiate the different domains in the channel (Adopted from Xu et al., 2019).

Slow inactivation takes place to stop sodium conduction during prolonged depolarization and rapid repetitive stimulations (Chahine, 2018; Vilin and Ruben, 2001; Ulbricht, 2005). Unlike fast inactivation, the entry and recovery from the slow inactivated state take a longer time ranging from hundreds of milliseconds. This state is elucidated to be a protective mechanism against stress conditions generating accelerated repetitive stimuli (Chahine, 2018). During repolarization, the VGSCs close and Segment 4(S4) voltage sensors move backwards. Sodium channels deactivate and recover from inactivation, getting back to their resting state (Xu *et al.*, 2019).

3. Voltage-gated Sodium Channels as a Target Site:

The role of VGSCs in the nervous system makes it an ideal target region of multiple toxins in the course of evolution (Wang *et al.*, 2003; Cestele and Catterall, 2000). The binding of neurotoxins to specific receptor sites alters the functionality of VGSCs through blocking pores, altering gate channels (Du *et al.*, 2011; Tikhonov and Zhorov, 2005). This inactivation negatively alters the activation of membrane potential. Persistent transmembrane depolarization due to prolonged sodium influx induces continuous nerve firing and hyperexcitability that paralyses and kills the insect.

VGSC is primarily targeted by several synthetic insecticide classes including plant extract derivatives. For instance, pyrethrins, from flowers of pyrethrum (*Tanacetum cinerariaefolium*) extracts (Elliott, 1977), act on sodium channels (Narahashi, 1988). An additional organochlorine insecticide Dichlorodiphenyltrichloroethane (DDT), also targets sodium channels primarily. However, indoxacarb and metaflumizone target sodium channels with a different mode of action from pyrethroids (Table 1). The two-act by inhibiting sodium current (Silver *et al.*, 2010; Dong *et al.*, 2014; Wing *et al.*, 2005; Dong *et al.*,2014),

Table 1: Insecticides Mode of Action

Active Ingredient	Main Group/Class	Mode of Action
Indoxacarb	Oxadiazines	Blocks the VGSC in slow- inactivated state causing pseudopaparalysis (Soderlund, 2017)
Spinetoram	Spinosyns	Allosteric modulators of nicotinic acetylcholine receptors (Lira *et al*.,2020)
Metaflumizone	Semicarbazones	Binds to block the VGSC in slow-inactivated state causing pseudoparalysis (Soderlund, 2017)
	Pyrethroids	Promote activation, inhibit deactivation and inactivation, thus prolonged channel opening resulting to hyperexcitability (Dong *et al*.,2014)
Tebufenozide	Diacylhydrazines	Acts on 20-hydroxyecdysone molting hormone, initiating premature molting (Yu, 2008)
Cartap	Nereistoxin analogues	It is a neuromuscular blocker which leads to respiratory paralysis (Boorugu and Chrispal, 2012)
Lufenuron	Benzoylphenylurea	Inhibitors of chitin biosynthesis, causing abortive molting and hatching defects (Merzendorfer,2013)
Fipronil	Phenylpyrazoles	It antagonizes the 'calming' effect of GABA via blockage of the gamma-aminobutyric acid (GABA) regulated chloride channel in a closed state hence suppressing GABA-Induced currents in neurons (Krieger, 2010)
Chlorfenapyr	Pyrroles	Acts by Uncoupling the oxidative phosphorylation, disrupting the mitochondrial membrane's proton gradient thus inhibiting conversion of ADP to ATP eventually causing death (Nauen and Bretschneider, (2002).

4- Difference in Toxicity of Insecticides in Insects and Acarines:

Notably, *Tau-fluvalinate* (Pyrethroid) is highly toxic to mites and ticks than to insects. This is attributed to the difference between acarine VGSC and that of insects. The amino acid at the locus 933, is cysteine (C) in insects and either glycine (G), alanine (A), or valine (V) in acarines. Modelling these VGSC interactions with the specific pyrethroids implies that the Cysteine in insect channels obstructs *Tau-fluvalinate* binding, reducing the comparative efficacy of the insecticide. However, if cysteine is replaced by smaller amino acids such as in acarines, *Tau-fluvalinate* acquires the necessary room to fit hence a tighter binding, making good acaricide out of the pyrethroid (O'Reilly *et al.,* 2012).

5- Models of Knockdown Resistance (*kdr*):

A resistance mechanism to the toxins is known as knockdown resistance (kdr), which is caused by changes within the VGSC, that renders it less sensitive to the toxin in the compounds (Vais *et al.,* 2001: Soderlund and Knipple 2003). Globally, kdr has been agriculturally and medically documented as significant in arthropod pests (Soderlund, 2005, 2012; Du *et al.,* 2015; Rinkevich *et al.,* 2013). More than 50 VGSC mutations have been recorded in reference to pyrethroid resistance in various arthropods (Du et al., 2013; Li *et al.,* 2012; Xu *et al.,* 2012; Rinkevich *et al.,* 2013; Kristensen, 2005). Previous studies document that mutations conferring resistance to these insecticides are mostly common in regional domain II of channel protein (Vais *et al.,* 2001; Soderland and Knipple 2003). These are 5 including; Leu^{925}, Thr^{929} and Leu^{932} (IIS5) and Leu1014 located in IIS6 and Methionine 918 (Met^{918}) located in the linker IIS4-5. L1014F is the most common mutation, originally in houseflies (Williamson et al., 1996; Endersby *et al.,* 2011). For instance, a mutation at T929 (the binding site for DDT, deltamethrin, fenfluthrin and permethrin) confers resistance to all the four insecticide compounds, whereas mutations at M918, a distance button from fenfluthrin (pyrethroid) and DDT predicatively bind, confers resistance to deltamethrin and permethrin only (Silver *et al.,* 2014). The study shows that, the model prediction for T929I ensues resistance to all four insecticide compounds, while M918T confers resistance to permethrin and deltamethrin and not DDT or fenfluthrin (Linda *et al.,* 2017). Sun *et al.* (2016), has similarly used the same model on live insects' bioassays to support the O'Reilly model above.

Alternatively, recent studies proposed a dual-receptor site model that binds with both DDT and pyrethroids (Du *et al.,* 2015, 2016; Zhorov and Dong, 2017). In this model, the binding of two molecules to PyR1 receptor sites and PyR2 simultaneously is necessary for the sodium channel to lock in an open state (O'Reilly *et al.,* 2006). The proposed location for this binding is in domain interfaces II/III and I/II, respectively, and are arranged in a quasi-symmetrical manner. Pyrethroids attach between four helices including S5, linker-helix L45 and two S6 helices from adjacent domains (Du *et al.,* 2015).

While the original O'Reilly model L1014F affects pyrethroid binding through an indirect allosteric impact, key variances in the Du model indicate the L1014F is firmly localized within the PyR2 site. Consequently, L1014F's effect is in slowing opening of VGSC that is predicted to significantly reduce the formation of PyR1, hence limits the availability of pyrethroid for binding, conferring the kdr. An additional difference is in the orientation of pyrethroids bound within each pocket that is reversed (which begs the question of why M918T would be ineffective against toxic compounds like fenfluthrin), and that the pyrethroids sip deeper into the PyR2 protein domain.

Precise molecular markers through identification of kdr mutations rapidly aids in the assessment of resistance allele frequency in insect populations other than being important in deciphering sodium channels structural features critical in binding and action of the pyrethroids (Silver *et al.,* 2014). Studies by Amey *et al.* (2015), in aphid's genome, identified unusual properties in VGSC sequences unique to aphids only. They possess a

unique heterodimeric channel, having a characteristic ion -selectivity filter, not common in insects and who's insensitive to tetrodotoxin was high. The study implied that it is possible to design selective compounds to act on aphids while sparing other insects.

6- Homology Modelling:

Previous studies have used resolution crystal structures of bacterial potassium channels to provide structural templates for modelling of the VGSC (O'Reilly et al., 2007). The publication of a crystal structure of the electric eel sodium channel 1.4(Nav1.4) complex enables the extension of this homology modelling to encompass the VSDs. We have utilized the structure of Nav 1.4-beta1 complex, in the present study, as a template for generating a homology model for the fall armyworm VGSC in an open conformation. A combination of protein-ligand modelling and docking procedures has been adopted to describe how these ligands interact with the insect sodium channels.

MATERIALS AND METHODS

1- Homology Modelling

The crystal structure of the Nav1.4-beta1 complex from electric eel provided the structural template for a homology model of the fall armyworm sodium channel in an activated state. The Nav 1.4 –beta complex from the electric eel represents the first Nav channel to be biochemically purified and cloned. The subunits of the model are represented by the dark-blue colour in figure 5. These subunits correspond to domains I, II, III, and IV in eukaryotic sodium channels that have four domains. The model was produced using the SWISS-MODEL workspace (swissmodel.expasy.org/workspace). The chosen protein had a Global Model Quality Estimation (GMQE) score of 0.37 (the highest among the identified templates). The GMQE score is a quality estimation that combines properties from the target template alignment and the template structure. The score reflects that expected accuracy of the model built with that of the alignment and template.

The study used the ClustalW algorithm to align the amino acid sequences of the fall armyworm sodium channel (XP_035435130.1) with the Nav1.4 channels. The sequence identity of the alignments between the sequences was 33% (Fig. 2).

Fig. 2 Sequence alignments of the Nav1.4 channel and fall armyworm sodium channel.

2- Automated Ligand Docking

The crystal structure of the pyrethroids, metaflumizone, indoxocarb, benzophenylurea, cartap, fipronil, spinetoram, chlorfenapyr, and tebufenozide was obtained from the pubchem database (https://pubchem.ncbi.nlm.nih.gov/). The insecticides were downloaded in the sdf (special data file) format and converted to the pdb (protein data bank) format (compatible with Autodock Vina) via Pymol. Automated docking of the insecticides was performed using the AutoDock 4.2.6 software package (Trott and Olson, 2010). The predicted binding affinities between the VGSC and the insecticides were measured in Kcal/mol. The protein molecule (VGSC) was read into Autodock Vina and water molecules were removed. Water molecules in the binding pocket can interfere with docking. Polar hydrogens and Kollman charges were added to the protein before it was saved in pdbqt format. The protein and ligand (insecticide) were then chosen as macromolecules in the Autodock Vina. Grid maps with 40×40×40 points were constructed with a grid point spacing of 0.375Å. The grids were centered on positions 138.737, 128.216, and 125.832. A configuration file was then created to indicate the parameters for the docking process. The Iterated Local Search global optimizer algorithm with the

parameters of energy_range = 4 and exhaustiveness = 8 was used to perform docking simulations. The docking process performed using Autodock Vina was run using the command prompt. Docking predictions with the least binding free energy value (highest negative value) were deemed to be of significance. The results of the docking process were visualized using pymol which was also used to identify the specific docking points of individual insecticides.

RESULTS

The homology model of the fall armyworm VGSC is largely based on the X-ray structure of the Nav1.4-beta1 complex from electric eel (PDB accession number (5XSY). The model of the VGSC used is shown in figure 3.

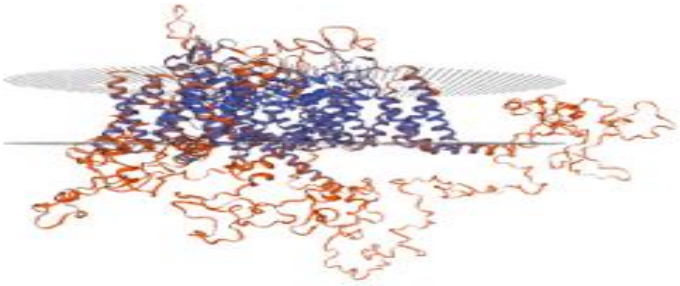

Fig. 3 Model of the VGSCs

3.1 Automated docking predictions

Insecticides have a distinct structure-activity relationship that relates to their physical properties and 3-D configuration of the entire molecule. Figure 4 illustrates the different chemical structures of the nine insecticides that were retrieved from the PubChem database. (https://pubchem.ncbi.nlm.nih.gov/).

The program Autodock was utilized in generating docking predictions for the insecticides and the modelled VGSC. The study analysed the energetically favourable docking predictions (i.e. those with negative values for binding free-energy) to determine the interactions between the insecticides and the residues in the protein model.

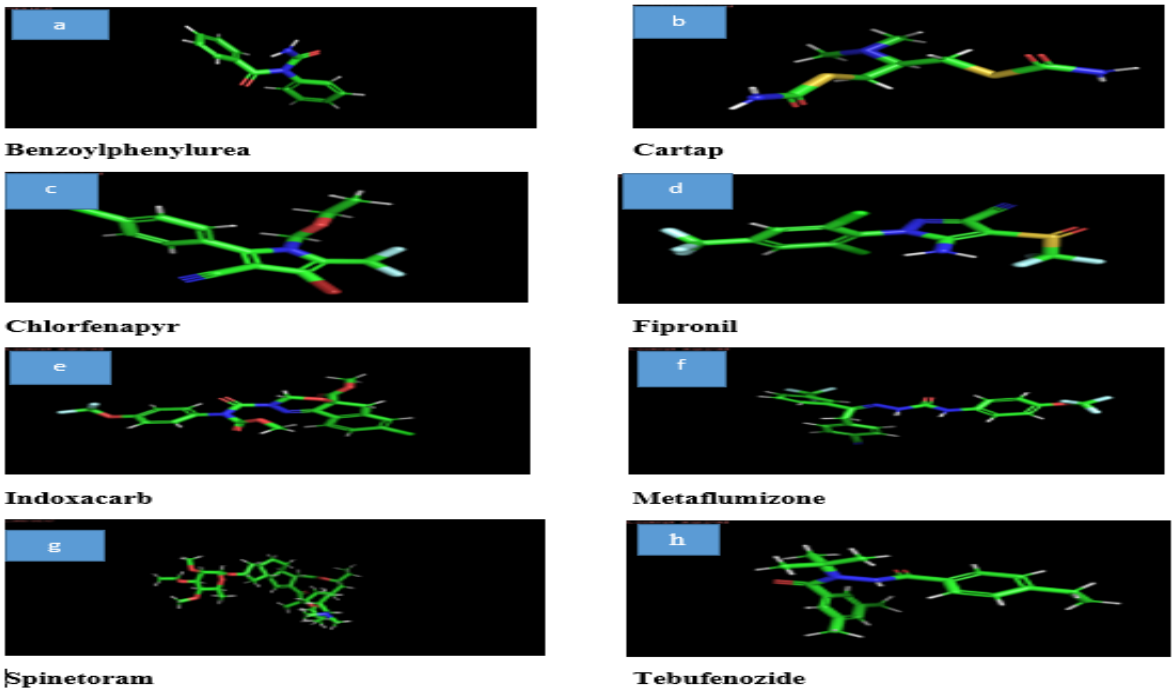

Fig. 4 Chemical structures of the insecticides that were used in the docking.

Table 2: Binding Sites Identified Through the Molecular Docking Process

Insecticide	Amino Acid	Binding Position
Benzoylphenylurea	Tyrosine	476
Cartap	Glutamine	1580
	Tyrosine	433
	Phenylalanine	1579
	Threonine	430
	Threonine	1578
Chlorfenapyr	Serine	1873
Fipronil	Serine	1873
	Tyrosine	1927
Metaflumizone	Alanine	1577
Spinetoram	Glutamine	1580
	Serine	1873
Tebufenozide	Serine	1873
Indoxacarb	Serine	1873
	Tyrosine	1927
	Asparagine	1045
Pyrethroid	Serine	1873

For each insecticide, the autodock vina software identified 9 potential binding sites. The ranking of the binding sites was based on their affinity (kcal/mol). Visualizations on the pymol software helped us identify the specific amino acids that interacted with the VGSC and the binding site where the interaction was identified (Fig. 5). The residue Ser^{1873} stood out with six of the nine insecticides indicating interactions at this position. Indoxacarb and Pyrethroids, were among the insecticides that indicated interactions with the Ser^{1873} residues. The other identified interacting residues were specific for each insecticide.

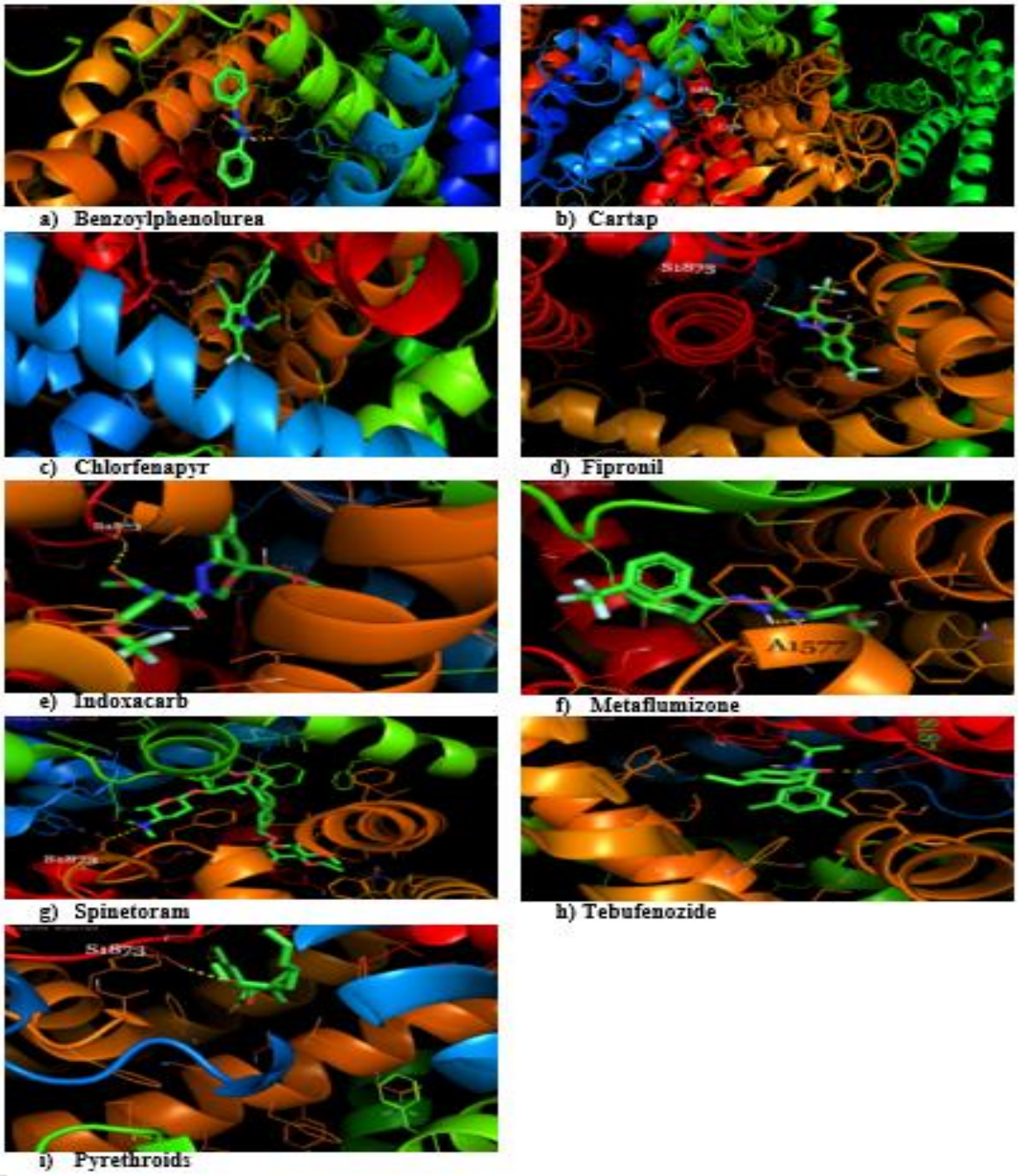

Fig. 5 (**a**) Binding site between Benzoylphenylurea and the VGSC at position Tyrosine 476 represented by a dotted yellow line. The amino acid was located at the third binding site. (**b**) The binding site between cartap and the VGSC at positions Tyrosine 433, Glutamine 1580, Tyrosine 433, Phenylalanine 1579, Threonine 430, and Threonine 1578 represented by a dotted yellow line. These amino acids were identified on the 1st, 2nd, and 3rd binding sites. (**c**) The binding site between Chlorfenapyr and the VGSC at position Serine 1873 is represented by a dotted yellow line. The amino acid was identified on the 2nd binding site. (**d**) The binding site between fipronil and the VGSC at positions Serine 1873 and Tyrosine 1927 is represented by a dotted yellow line. The protein was identified on the 1st binding site. (**e**) The binding site between indoxacarb and the VGSC at positions Serine 1873, Tyrosine 1927, and Asparagine 1045 represented by a dotted yellow line. The amino acids were identified on the 7th binding sites. (**f**) The binding site between metaflumizone and the VGSC at positions Alanine 1577 is represented by a dotted yellow line. The amino acid was identified on the 8th binding site. (**g**) The binding site between Spinetoram and the VGSC at positions Serine 1873 and Glutamine 1580 is represented by a dotted yellow line. The amino acid was identified on the 1st pose. (**h**) The binding site between Tebufenozide and the VGSC at positions Serine 1873 is represented by a dotted yellow line. The amino acid was identified on the 7th binding site.(**i**) Binding site between Pyrethroids and the VGSC at position serine 1873 identified on the 1st pose.

DISCUSSION

VGSCs are essential integral transmembrane proteins, crucial for electrical signalling in excitable cells. Their critical role in excitability has made them a target site of multiple neurotoxins. In addition, they are also the primary target of modern sodium channel binding inhibitors. The intensive insecticides application has, however, lead to resistance development against common insecticides. kdr caused by multiple mutations in the insecticide binding sites of VGSCs is a major mechanism of insecticide resistance among different insects. Insects that exhibit kdr show reduced target-site sensitivity to insecticides targeting sodium channels rising from one or more-point mutations. Understanding common insecticide binding sites for different classes of insecticides is an important step in finding a lasting solution to the growing menace of insecticide resistance. Twelve different amino acid residues that showed interactions with the insecticides under study were identified from our analyses (Table 2). Residue Ser^{1876} indicated the most frequent interactions, with 6 of the 9 insecticides used indicating interactions. The residue formed close (<4 Å) binding contacts with the analysed insecticides. Super kdr resistance has previously been attributed to Met918, Thr929, Leu925, and Leu 932 (O'Reilly *et al.,* 2006). None of these residues indicated any interactions with the two SCBIs and pyrethroid used in this study. Our results could indicate that the pyrethroid, metaflumizone, and indoxacarb have different target sites that allow them to interact with the fall army worm's VGSC. Our results also confirm that the mutations previously reported have influenced the insecticide binding affinity, resulting in insecticide resistance. This could be attributed to a lack of interactions in the previously identified binding residues. Residues Val^{410} and Leu^{1014} known for kdr-type resistance to DDT and pyrethroids were also not picked up by our study (O'Reilly et al., 2006) of the 9 insecticides analysed in the present study, cartap indicated the highest number of binding sites in the VGSC. The insecticide successfully interacted with five different amino acids, including Gln 1580, Tyr 433, Phe 1579, Thr 430, and Thr 1578. These interactions were identified in pose 4 of the docking results which had a binding affinity of -4.2kcal/mol and an rmsd value of 4.712. This insecticide is known to cause neurotoxicity among insects (Liao et al., 2003). Interactions with the VGSC have, however, not been reported by previous studies. Residue Phe^{1579} has been previously been described as an essential determinant of SCBIs binding and mode of action (von Stein *et al.,* 2013). Mutations of this residue usually interfere with the binding ability of the sodium channel inhibitors (SCIs) drugs to its receptors (Mike and Lukacs, 2010). Such results indicate the possibility of multiple modes of action for this class of insecticides. We suggest that further experimental studies be performed to look at the possibility of cartap actually binding to VGSC and affecting its toxicity through this mode of action. These studies could also be extended to the other classes of insecticides which indicate a different mode of action to that of the SCBIs. Indoxocarb, a different class of insecticides, indicated binding in three different amino acids, including Ser 1873, Ty 1927, and Asp 1045. Resistance to this insecticide has previously been attributed to Ser^{989} and Val^{1016}, both of which did not indicate any interactions from our analyses (Leticia et al., 2017). The different interacting residues in our context could probably indicate alternative binding sites of the insecticides to the VGSC. Studies on the mutant insects could help to confirm the efficaciousness of these binding sites. Serine 1873 was a highly targeted binding site with 5 of the 8 insecticides indicating binding interactions with the amino acid. This binding position has, however, not been implicated in mutations that are known to cause kdr among fall armyworm insects. The prediction of multiple binding sites and new binding sites is of great importance in informing management of the pest and monitoring resistance development. The more the binding sites of an insecticide may correlate with increased

effectiveness against the pest, that is if any of the target site has mutated there is still remaining target binding options of where the insecticide can bind to and still execute its mode of action against the pest. The model generated in this case had the highest sequence similarity with the fall armyworm's voltage-gated channel as indicated with its high Global Model Quality Estimation (GMQE) score (0.37). Future studies could work to find models and templates with higher sequence similarity matches to increase the accuracy of generated models.

CONCLUSIONS

The modelling studies reported here relied on crystal structures of homologous ion channels to model binding sites of insecticides in the VGSCs. The results in this study identified insecticide-specific binding residues on the VGSC. Our study factored in the structure-activity relationships of pyrethroids to reveal the specific binding residues. We suggest that experimental studies be carried out to identify if non-VGSCs-targeting insecticides indicate different modes of actions, especially given that they had significant interactions with the VGSCs.

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