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Modified neonicotinoid insecticide with bi-directional selective toxicity and drug resistance

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ABSTRACT

A three-dimensional quantitative structure–activity relationship (3D-QSAR) model was established based on the molecular structures and the negative logarithm of experimental lethal concentration 50 values (pLC_{50}) of neonicotinoid insecticides. Then, the mechanisms of bi-directional selective toxic effects and drug resistance were determined using homology modeling and molecular docking analyses. The results of the model showed that the 1-, 2-, 4-, and 12- positions of neonicotinoid insecticides strongly affected their toxicity, and that the introduction of bulky or electropositive groups at these positions could increase the pLC_{50} values. Using Compound 19 as a template, we designed 37 derivatives with greater toxicity (increased by 0.04–11.45%). Among them, 20 derivatives had bioconcentrations lower than that of Compound 19 (reduced by 0.38–147.88%). Further screening of Compound 19 and the 20 derivatives mentioned above by homology modeling and acetylcholine receptors (AChRs) molecular docking analyses showed that 10 derivatives had bi-directional selective toxic effects against pests and bees. Further docking analyses of Compound 19 and these 10 derivatives identified that Derivative-33 showed decreased docking with superoxide dismutase (SOD) and glutathione S transferase (GST) in pests and enhanced docking with these enzymes in bees, indicating bi-directional selective resistance for pests and bees. Accordingly, Derivative-33 was selected as a new insecticide with high toxicity to pests and low toxicity to bees (bi-directional selective toxicity), low resistance in pest populations, and high resistance in bee populations. This study provides valuable reference data and will be useful for the development of strategies to produce new environmentally friendly pesticides.

1. Introduction

After pyrethroid insecticides, neonicotinoid insecticides represent a major breakthrough in the history of insecticides (European, 2013). As nicotinic acetylcholine receptor agonists, the mode of action of neonicotinoids is relatively novel (Nagata et al., 1997). The principle is to hinder the conductance of cholinergic signals in insects, which causes the insects to become excessively excited or paralyzed, and then to lose control of their behavior (Johnson, 2015). Neonicotinoid insecticides have selective toxicity and can effectively control pests such as mammals and whitefly. However, despite their selective toxicity, these insecticides have been linked with massive declines in the global population of pollinating insects such as bees (Millar and Denholm, 2007).

In 1999, France prohibited the use of imidacloprid on sunflowers because it was toxic to bees. However, because of outdated or ineffective methods to evaluate the subacute and chronic toxicity of pesticides and their effects on the growth, development, and reproduction of bees, many countries still permit the use of these insecticides (Bonmatin et al., 2005). In 2006, Claudianos et al. (2006) found that, compared with other insects, bees had fewer genes encoding detoxification enzymes in their genomes, making them more vulnerable to pesticides. In the United States, Canada, France, Germany, Sweden, and other countries, many bee colonies decreased or disappeared within a single year in a phenomenon known as colony collapse disorder (CCD). Approximately 65.10–87.50 million bees disappeared in the United States during this period, triggering a crop pollination crisis (Johnson, 2007). To address this situation, a CCD research group was established to filter and detect 117 chemical substances in the sick bee samples. The group identified the neonicotinoid insecticides as the main cause of the crisis (Stokstad, 2007). In 2013, Tapparo et al. (2013) used liquid chromatography-mass spectrometry to analyze neonicotinoid insecticides at ultra-trace levels, and determined that these insecticides, even at nanogram levels, were strongly toxic to bees.

In addition to their effects on bees, neonicotinoid insecticides have

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also resulted in the emergence of drug resistance because of their widespread use to control pests in the fields of health and agriculture (Denholm et al., 2002). This has resulted in resistant insect populations that can tolerate dosages that would kill the majority of individuals in normal populations (Ji, 2017). Because of the selective effect of insecticides used over a long period of time, many insects have developed various degrees of resistance. The development of resistance will have many adverse effects, such as decreased crop yields, higher planting costs, and greater pesticide use (Joußen et al., 2012). Therefore, the prevention and control of resistance and the development of new insecticides are important research goals.

The earliest record of insect resistance was in 1908, when Melander et al. Anonymous (2011) found a population of *Quadraspidiotus perniciosus* (Comstock) in a Californian pear orchard that was resistant to lime sulfur. Soon afterwards, *Aonidiella aurantii* (Maskell) resistant to hydrocyanic acid and codling moth resistant to lead arsenate were discovered. By 2011, at least 600 kinds of insects and mites were known to be resistant to one or more pesticides, with the Diptera and Lepidoptera having the largest numbers of resistant members (Bruck et al., 2011). One year later, the number of resistant insect species had increased to more than 1000 (Riveron et al., 2013). Many studies have suggested that the mechanism of drug resistance is the enhanced activity of detoxification enzyme(s) in insects, which results in decreased metabolic resistance and target sensitivity (Assogba et al., 2014; Sven et al., 2010; Zhang et al., 2008).

The aim of this study was to explore the mechanism of toxicity of neonicotinoid insecticides towards bees and pests, and to evaluate the effects of various neonicotinoid insecticides to induce resistance in insects. Accordingly, Derivative-33 were screened by three-dimensional quantitative structure–activity relationship (3D-QSAR) model, homology modeling and molecular docking analyses, which has higher toxicity, lower bioconcentration effects, bi-directional selective toxicity and resistance-inducing (Chen et al., 2016). This theoretical method could be used to produce new environmentally friendly neonicotinoid insecticides with bi-directional selective toxicity and resistance-inducing effects on pests and bees.

2. Materials and methods

2.1. Data

The 3D-QSAR model was analyzed with SYBYL software (Tripos Assoc., St Louis, MO, USA) (Gu et al., 2016). The experimental data for neonicotinoid insecticides were obtained from Li et al. Tian et al. (2007a, 2007b); Shao et al. (2009, 2010); Tian et al. (2007c). We used the negative logarithm of experimental lethal concentration 50 values (pLC_{50}) as the experimental data, and selected 23 compounds as a training set and 8 compounds as a test set to establish the 3D-QSAR model. The 30 compounds were selected to represent diverse structures and universal distribution (Aouidate et al., 2017).

2.2. 3D-QSAR model for toxicity of neonicotinoid insecticides

2.2.1. Molecular structure optimization and alignment of neonicotinoid insecticides

The molecular structures were drawn using SYBYL software and then optimized to obtain the most stable conformations. To optimize compounds, we used the Tripos Force Field and Minimize programs with Gasteiger-Hückel charges, and Powell's method (maximum number of optimizations, 10,000; energy convergence gradient value, 0.005 kJ/mol, and all other parameters set to default values) (Gu et al., 2017). All of the compounds had characteristic elements in the labeled region as the common framework. We used Compound 19, which had the highest pLC_{50} value (8.74), as the target to align the other molecules (Fig. 1). All of the compounds could be well aligned.

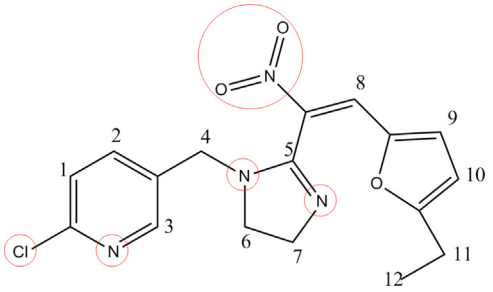

Fig. 1. Selection of target compound to align common framework.

2.2.2. Modeling of neonicotinoid insecticides with CoMFA and CoMSIA

In the QSAR module, comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) analysis methods could be chosen. The electrostatic field and steric field were calculated using the CoMFA method. The dielectric constant was related to distance (threshold, 125.4 kJ/mol; other parameters set to default values). The pLC_{50} values of 30 neonicotinoid insecticides were entered into the training table, and the parameters of the model were calculated automatically by Autofill using SYBYL. A partial least-squares regression analysis was applied to establish the relationships between the structure and the biological activity of the target compound. First, we used the leave-one-out method to cross-validate the compounds in the training set, and determined (Li et al., 2013).

Compared with the CoMFA model, the CoMSIA model increased the hydrophobic field and the hydrogen-bond donor and acceptor fields using Gaussian similarity functions. The results of the CoMSIA method were almost unaffected by the rules of compound matching, and the CoMSIA method explained the structure–activity relationship of a compound more intuitively than did the CoMFA method. Although the CoMSIA method does not have some of the drawbacks of the CoMFA method, it does not necessarily provide better results (Wang et al., 2017a). Therefore, to obtain a reliable prediction model, we used the two methods to validate and complement each other.

2.2.3. Modification of neonicotinoid insecticides based on contour maps

In the CoMFA and CoMSIA models, Compound 19 had the highest pLC_{50} value (8.74), which showed that it had the strongest toxicity effect. Therefore, we chose to modify Compound 19 to ensure that the toxicity effects of the congeners were increased significantly. In the contour maps, differently colored contours showed the influence of the different fields on the pLC_{50} values of the neonicotinoid insecticides. In the steric field, green contours showed that bulky groups increased the activities of the molecules, while yellow contours showed that bulky groups decreased the activities of the molecules. In the electrostatic field, blue contours showed that positive charges increased the activities of the molecules, and red contours showed that negative charges decreased the activities of the molecules. In the hydrophobic field, white contours showed that hydrophilic charges increased the activities of the molecules, and yellow contours showed that hydrophobic charges decreased the activities of the molecules. In the hydrogen bond donor field, blue contours showed that a hydrogen bond donor increased the activities of the molecules, and purple contours showed that a hydrogen bond donor decreased the activities of the molecules. In the hydrogen bond acceptor field, purple contours showed that a hydrogen bond acceptor increased the activities of the molecules, and red contours showed that a hydrogen bond acceptor decreased the activities of the molecules (Tong et al., 2017; Wang et al., 2017b).

2.3. Homology modeling for acetylcholine receptors (AChRs), superoxide dismutase (SOD), and glutathione S transferase (GST) docking ability

The homology modeling algorithm is a recognized method to predict the structure of a protein from its amino acid sequence. The amino

acid sequences of AChRs, SOD, and GST (GI: 347948662, 686636727, 478859730) were obtained from the National Center for Biotechnology Information and then submitted to the SWISS-MODEL automated protein modeling server (Glaxo Smith *Kl* ine, Geneva, Switzerland) to obtain the protein structures of AChRs, SOD, and GST. For each target protein, we evaluated the structural rationality of the model using the Ramachandran conformation map in PROCHECK. Generally, the sum of base percentages in the core area, allowable area, and maximum allowable area was greater than 95%, which met the quality requirements of the model.

2.4. Determination of docking scores for Compound 19 and neonicotinoid derivatives with AChRs, SOD, and GST

Compound 19 and neonicotinoid derivatives were loaded into Discovery Studio 4.0, and the selected protein was defined as the receptor molecule using the LibDock module. Possible binding sites in the receptor were found using the 'Find Sites from Receptor Cavities' function in the Define module. Binding sites were subsequently modified and defined based on the incorporation of ligand compounds into the protein binding cavity and docking with the ligand protein. The change in binding capacity was determined from the LibDock scores.

3. Results and discussion

3.1. Evaluation and analysis of CoMFA and CoMSIA models to predict neonicotinoid insecticide toxicity

Both the CoMFA and CoMSIA models had q^2 values of 0.77 (> 0.5), and their r^2 values were 0.93 and 0.99, respectively (> 0.9). This illustrated that they had reliable predictive and fitting capabilities. For the stability parameters, the CoMFA and CoMSIA models had SEP values of 0.48 and 0.57, and r^2_{pred} values of 0.81 and 0.95 (> 0.6), respectively. These results verified that the constructed 3D-QSAR model had a good external prediction ability (Salahinejad and Ghasemi, 2014).

In the CoMFA model, the contribution rates of the steric and electrostatic fields were 48.20% and 51.80%, respectively, confirming that space effects and electrostatic interactions affected the pLC_{50} values of the neonicotinoid insecticides. In the CoMSIA model, the contribution rates of the steric, electrostatic, hydrophobic, and hydrogen-bond donor and acceptor fields were 15.30%, 38.80%, 9.40%, 19.80%, and 16.70%, respectively. These results showed that all of the fields affected the pLC_{50} values of the neonicotinoid insecticides, with electrostatic interactions being the major contributor.

3.2. Determination of substituent sites and groups through contour maps

Contour maps based on the CoMFA and CoMSIA (Fig. 2) models were constructed for Compound 19.

In the CoMFA steric contour map, the contour around the 2- position of Compound 19 was green, indicating that the introduction of bulky groups to this position would increase the pLC_{50} value. The electrostatic contour map had a blue contour around the 1- and 2- positions, indicating that the introduction of electropositive groups would increase the pLC_{50} value. In the CoMSIA model, the steric field showed green contours around the 2-, 4-, and 12- positions. In the electrostatic field, hydrophobic field, hydrogen bond donor field, and hydrogen bond acceptor field maps, colored contours were not distributed around the substituents of Compound 19, indicating that introductions in these four fields would not affect the pLC_{50} value.

In summary, the contour maps produced from the CoMFA and CoMSIA models showed green and blue contours around the 2- position of Compound 19, green contours around the 4- and 12- positions, and blue contours around the 1- position. Thus, the 2- position would be affected by the steric and electrostatic fields, implying that the introduction of bulky or electropositive groups at this position could increase the pLC_{50} value. The introduction of bulky groups at the 4- and 12- positions and electropositive groups at the 1- position were also predicted to increase the pLC_{50} values. Based on this analysis, substitutions of the 1-, 2-, 4-, and 12- positions of Compound 19 were performed with eight groups (-OH, -COOH, $-OCH_3$, $-C_2H_2$, $-C_2H_4$, $-NH_2$, -NO, and -Br) to generate 37 derivatives.

3.3. Evaluation of toxicity and bioconcentration characteristics of Compound 19 and its neonicotinoid derivatives

Bioconcentration is a balanced distribution process of chemicals between aquatic organisms and water. The ratio of the concentration of chemicals in organisms (equilibrium) to their concentration in water (equilibrium) is called bioconcentration factor (BCF), which reflects the absorption and storage capacity of aquatic organisms to organic compounds in water. It is also one of the important parameters to study the environmental behavior of organic compounds.

The pLC_{50} and the logarithm of bioconcentration factor (logBCF) values were predicted for Compound 19 and its 37 derivatives, which could verify to identify compounds with increased toxicity effects and decreased accumulative capacities in the environment (Table 1).

In the CoMFA and CoMSIA models, the pLC_{50} values of all derivatives were all higher than that of compound 19 (increased by 0.04–9.75% and 0.23–11.45%, respectively). These results indicated that the 37 derivatives had greater toxicity than that of Compound 19. Although the toxicity of some derivatives was not increased significantly, the data could still provide a theoretical basis for the regeneration of neonicotinoid derivatives.

The logBCF values of 17 derivatives including Derivative-1, Derivative-5, Derivative-9, and Derivative-10 were higher than that of Compound 19 (increased by 0.87–85.29%). This indicated that the bioconcentration of these derivatives was enhanced and they would accumulate more easily in various media in the environment. Therefore, these 17 derivatives were eliminated from further analyses. The logBCF values of the other 20 derivatives were lower than that of Compound 19 (decreased by 0.38–147.88%). This indicated that the bioconcentration effect of 20 derivatives was weaker than that of Compound 19, and they would not readily accumulate in the environment. Thus, these compounds would have significantly decreased adverse effects on the environment. In summary, compared with Compound 19, these 20 neonicotinoid derivatives were predicted to

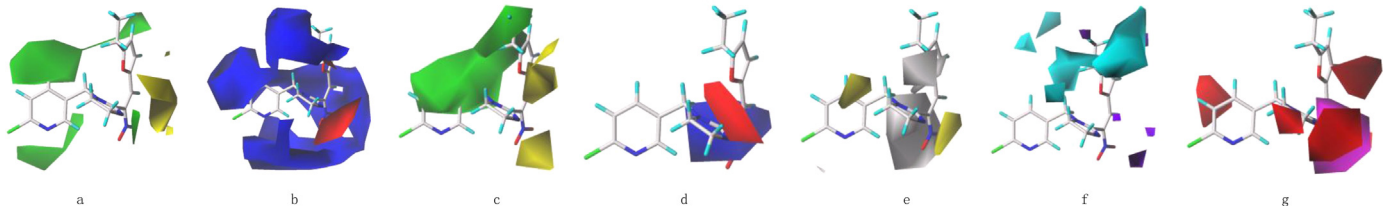

Fig. 2. Contour maps of steric field (a) and electrostatic field (b) in CoMFA model; and steric field (c), electrostatic field (d), hydrophobic field (e), hydrogen bond donor field (f), and hydrogen bond acceptor field (g) in CoMSIA model.

Table 1
Predicted pLC_{50} and logBCF values of Compound 19 and its derivatives.

No.	Compounds	The values of pLC_{50}				The values of logBCF	
		For CoMFA		For CoMSIA		For CoMSIA	
		Predicted	Residual (%)	Predicted	Residual (%)	Predicted	Residual (%)
No. 19	–	7.61		7.05		1.04	
Derivative – 1	1-Amino	8.17	7.37	7.13	1.11	1.93	85.29
Derivative – 2	1-Ethyne	7.64	0.38	7.29	3.49	0.77	− 26.44
Derivative – 3	1-Oxhydryl	7.88	3.49	7.85	11.45	0.89	− 14.13
Derivative – 4	2-Methoxy	7.99	4.93	7.27	3.21	0.79	− 24.13
Derivative – 5	2-Ethylene	7.87	3.42	7.72	9.56	1.41	35.77
Derivative – 6	2-Oxhydryl	7.77	2.10	7.54	7.05	0.07	− 92.98
Derivative – 7	4-Amino	7.76	1.98	7.54	7.04	− 0.26	− 125.00
Derivative – 8	4-Methoxy	8.16	7.21	7.67	8.80	1.02	− 1.92
Derivative – 9	12-Amino	7.83	2.93	7.45	5.70	1.65	58.37
Derivative – 10	12-Ethylene	7.75	1.79	7.23	2.63	1.41	35.29
Derivative – 11	12-Oxhydryl	8.14	6.88	7.47	5.97	1.71	64.52
Derivative – 12	1,2-$(Nitroso)_2$	7.98	4.82	7.51	6.58	0.17	− 83.85
Derivative – 13	1,2-$(Carboxyl)_2$	7.82	2.76	7.19	2.02	0.38	− 63.37
Derivative – 14	1,2-$(Bromine)_2$	8.35	9.75	7.42	5.32	1.75	68.27
Derivative – 15	1-Amino – 4-Ethylene	7.88	3.53	7.67	8.87	1.32	27.02
Derivative – 16	1-Amino – 4-Oxhydryl	7.80	2.44	7.58	7.58	0.70	− 32.31
Derivative – 17	1-Amino – 4-Carboxyl	8.16	7.15	7.10	0.74	0.31	− 70.48
Derivative – 18	1,4-$(Ethylene)_2$	7.61	0.04	7.83	11.05	1.04	− 0.38
Derivative – 19	1-Ethylene – 4-Oxhydryl	7.72	1.45	7.57	7.36	0.73	− 29.81
Derivative – 20	1,4-$(Oxhydryl)_2$	8.05	5.75	7.77	10.23	1.25	20.10
Derivative – 21	1-Carboxyl – 4-Amino	7.86	3.32	7.14	1.35	1.73	66.15
Derivative – 22	1-Carboxyl – 4-Ethylene	7.96	4.53	7.20	2.19	0.62	− 40.77
Derivative – 23	1-Carboxyl – 4-Methoxy	8.08	6.10	7.16	1.62	0.60	− 42.79
Derivative – 24	1,4-$(Carboxyl)_2$	7.95	4.51	7.14	1.38	0.60	− 42.50
Derivative – 25	2-Amino – 4-Ethyne	7.69	0.99	7.12	0.96	1.70	63.37
Derivative – 26	2-Amino – 4-Oxhydryl	7.81	2.58	7.06	0.23	0.14	− 86.92
Derivative – 27	2-Ethyne – 4-Amino	7.65	0.53	7.55	7.08	1.67	60.29
Derivative – 28	2-Ethyne – 4-Oxhydryl	8.09	6.25	7.45	5.66	0.57	− 45.19
Derivative – 29	2-Ethylene – 4-Amino	8.07	6.07	7.68	9.01	1.57	51.06
Derivative – 30	2-Ethylene – 4-Ethyne	8.11	6.56	7.25	2.91	1.89	81.25
Derivative – 31	2,4-$(Ethylene)_2$	7.70	1.16	7.43	5.45	1.03	− 1.44
Derivative – 32	2-Ethylene – 4-Carboxyl	7.69	1.01	7.10	0.79	1.18	13.37
Derivative – 33	2-Methoxy – 4-Ethylene	7.79	2.40	7.57	7.36	0.87	− 16.73
Derivative – 34	2-Carboxyl – 4-Ethyne	8.06	5.93	7.35	4.26	1.67	60.48
Derivative – 35	2-Carboxyl – 4-Ethylene	7.75	1.80	7.26	2.97	1.16	11.06
Derivative – 36	2-Carboxyl – 4-Methoxy	7.62	0.09	7.41	5.12	− 0.50	− 147.88
Derivative – 37	2,4-$(Carboxyl)_2$	7.71	1.33	7.62	8.06	1.05	0.87

Table 2
Docking analysis of Compound 19 and neonicotinoid derivatives with AChRs.

Compounds	For pests		For bees	
	LibDock Scores	Residual (%)	LibDock Scores	Residual (%)
No. 19	77.90		85.67	
Derivative – 2	81.47	4.59	87.23	1.82
Derivative – 3	83.17	6.77	85.13	− 0.63
Derivative – 4	75.86	− 2.62	46.64	− 45.55
Derivative – 6	79.32	1.82	68.07	− 20.54
Derivative – 7	81.17	4.19	88.05	2.78
Derivative – 8	84.88	8.96	95.85	11.89
Derivative – 12	79.05	1.47	88.34	3.12
Derivative – 13	80.05	2.77	41.18	− 51.93
Derivative – 16	71.32	− 8.45	56.97	− 33.50
Derivative – 17	82.63	6.07	83.76	− 2.23
Derivative – 18	76.24	− 2.13	80.16	− 6.44
Derivative – 19	88.56	13.68	81.89	− 4.42
Derivative – 22	90.63	16.34	79.63	− 7.06
Derivative – 23	87.19	11.92	73.90	− 13.73
Derivative – 24	98.74	26.75	65.10	− 24.01
Derivative – 26	77.47	− 0.55	65.58	− 23.46
Derivative – 28	70.27	− 9.79	59.70	− 30.31
Derivative – 31	80.96	3.93	61.52	− 28.19
Derivative – 33	80.69	3.58	65.43	− 23.62
Derivative – 36	76.92	− 1.25	54.69	− 36.16

have higher toxicity and lower bioconcentration effects.

3.4. Bi-directional selective toxicity of neonicotinoid derivatives based on homology modeling and molecular docking to AChRs

3.4.1. Homology modeling for AChRs

Neonicotinoid insecticides have toxic effects on pests, but they also have potential chronic sublethal effects on pollinating insects such as bees. Therefore, we modified the AChRs to have bi-directional selective toxic effects on pests and bees, so that they would be more toxic to pests and less toxic to bees. Based on the contour maps, hydrophilic amino acids made the compounds more toxic, while hydrophobic amino acids did not. Accordingly, we swapped hydrophobic amino acids for hydrophilic amino acids to target pests, and hydrophilic amino acids for hydrophobic amino acids to reduce toxicity to bees (91 – 110).

According to the evaluations of the structure of the target proteins, all amino acid residues were located in permissible regions. The percentage of the base area (84.5%, 91.4%), permissible area (12.7%, 8.1%), and maximum allowed region (2.2%, 0.5%) for the two proteins was 99.4% and 100%, respectively, which exceeded the model's quality requirement (95%) (Jiang and Li, 2016).

3.4.2. Molecular docking analysis of Compound 19 and neonicotinoid derivatives with AChRs

We used Discovery Studio 4.0 to screen Compound 19 and 20 of its

derivatives with lower bioconcentration effects (Table 2) for their docking activity with AChRs in pests and bees.

The docking scores of Derivative-4, Derivative-16, Derivative-18, Derivative-26, Derivative-28, and Derivative-36 for AChRs in pests were lower than that of Compound 19. The scores of the other 14 derivatives were higher than that of Compound 19 (increased by 1.47–26.75%), indicating that they were more likely to bind to AChRs in pests and impede the transmission of cholinergic signals. For bees, the AChRs docking scores of Derivative-2, Derivative-7, Derivative-8, and Derivative-12 were higher than that of Compound 19, and the scores of the remaining 16 derivatives were lower. This indicated that the ability of these 16 derivatives to bind to AChRs was decreased (by 0.63–51.93%), which would reduce the chronic sublethal effect on the bees. In summary, after selection for reduced bioconcentration effects, increased toxicity to pests, and reduced toxicity to bees, 10 derivatives including Derivative-3, Derivative-6, Derivative-13, Derivative-17, and Derivative-19 were identified as having bi-directional selective toxic effects on AChRs in pests and bees.

3.4.3. Correlation analysis between number of substituents and bi-directional selective toxic effects of neonicotinoid derivatives

The neonicotinoid derivatives had two types of substitution sites: monosubstituted and disubstituted sites. In these analyses, the monosubstituted and disubstituted derivatives showed differences in docking scores for AChRs between pests and bees. The correlation between the number of substituents and the bi-directional selective toxic effects of the neonicotinoid derivatives was analyzed (Fig. 3).

For pests, the effect of the monosubstituted derivatives on AChRs docking was minor (increased by 1.82–8.96%), indicating that monosubstitution had little effect on the toxicity to pests. However, disubstitution had a greater effect on AChRs docking (increased by 1.47–26.75%), indicating that disubstituted derivatives had a significant toxic effect on pests. For bees, the AChRs docking scores of monosubstituted and disubstituted derivatives were decreased by 0.63–45.55% and 2.23–51.93%, respectively, compared with that of Compound 19. These results showed that both mono- and di-substitution significantly reduced the toxicity to bees.

3.5. Drug resistance of neonicotinoid derivatives as determined by homology modeling and molecular docking analyses for SOD and GST

3.5.1. Homology modeling for SOD and GST

Insect fat contains a number of oxidases, among which SOD is very common. This enzyme can catabolize chemical insecticides, which are then excreted via the excretory system. The higher the oxidase activity, the stronger the resistance (Novo and Parola, 2008). In addition to oxidases, some individual insects have resistance genes encoding other detoxifying enzymes. After the constant use of insecticides, more individuals in an insect population have higher detoxification enzyme activity (Riveron et al., 2014). Accordingly, SOD and GST enzymes in pests and bees have become modified, such that hydrophilic amino acids have been replaced by hydrophobic amino acids in the SODs and GSTs of pests, and hydrophobic amino acids have been replaced by hydrophilic amino acids in the SODs and GSTs of bees (91 − 110, 1 − 80).

We analyzed the protein structures of SOD and GST in pests and bees (four protein structures in total). According to the rational evaluation of the target protein structure, all amino acid residues of the four proteins were located in the permissible region, while the percentage of the base area (90.10%, 90.00%, 93.00%, 93.80%), permissible area (8.80%, 8.90%, 6.00%, 5.70%), and maximum allowed region (0.50%, 0.60%, 0.50%, 0.50%) equated to 99.40%, 99.50%, 99.50%, and 100%, which exceeded the model's quality requirement (> 95%).

3.5.2. Molecular docking analysis of Compound 19 and its neonicotinoid derivatives with SOD and GST in pests and bees

The insecticides with low bioconcentration effects and bi-directional selective resistance (Compound 19 and 10 neonicotinoid derivatives) were screened to determine their docking scares with SOD and GST in pests and bees (Table 3).

For SOD in pests, the docking scores of Derivative-3, Derivative-6, Derivative-13, Derivative-17, Derivative-19, Derivative-22, and Derivative-33 were lower than that of Compound 19. This indicated that the binding ability of these seven derivatives with SOD was reduced, so they would be less easily decomposed and metabolized after entering the pests, and would be more effective poisons. For SOD in bees, the scores of all 10 neonicotinoid derivatives were higher than that of Compound 19, indicating that all 10 neonicotinoid derivatives would readily bind to SOD. Thus, after these insecticides entered bees, they would be rapidly decomposed, metabolized, and excreted via the excretory system. Thus, they would have reduced harmful effects on bees. Therefore, Derivative-3, Derivative-6, Derivative-13, Derivative-17, Derivative-19, Derivative-22, and Derivative-33 were considered to be new insecticides with bi-directional selective resistance-inducing effects on pests and bees.

For GST in pests, the docking scores of Derivative-3 and Derivative-33 were lower than that of Compound 19. For GST in bees, the docking scores of Derivative-13, Derivative-17, Derivative-19, Derivative-22, Derivative-23, Derivative-24, and Derivative-33 were higher than that of Compound 19. These results indicated that Derivative-33 would be readily detoxified by bees, but not by pests. Therefore, Derivative-33 was predicted to have bi-directional selective resistance-inducing effects on pests and bees.

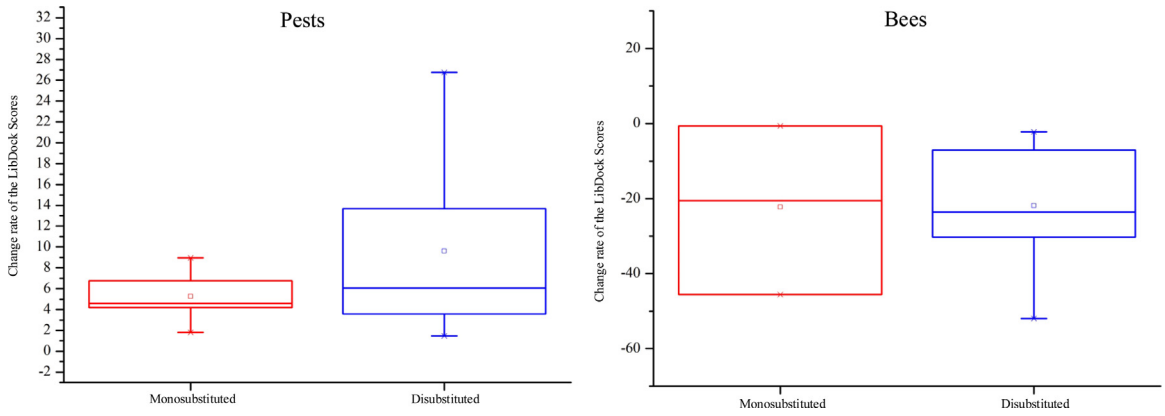

Fig. 3. Plot of substituent numbers *vs*. change rate of LibDock scores.

Table 3
Docking analysis of Compound 19 and its neonicotinoid derivatives with SOD and GST of pests and bees.

Compounds	SOD for pests		SOD for bees		GST for pests		GST for bees	
	LibDock Scores	Residual (%)	LibDock Scores	Residual (%)	LibDock Scores	Residual (%)	LibDock Scores	Residual (%)
No. 19	83.53		42.66		33.02		74.77	
Derivative－3	79.68	− 4.61	53.51	25.43	32.27	− 2.27	72.08	− 3.60
Derivative－6	79.15	− 5.25	50.51	18.40	51.73	56.65	73.48	− 1.72
Derivative－13	71.88	− 13.94	50.31	17.94	33.83	2.44	84.88	13.53
Derivative－17	81.45	− 2.48	65.03	52.43	34.10	3.26	76.67	2.54
Derivative－19	81.05	− 2.97	63.75	49.43	55.10	66.87	84.34	12.80
Derivative－22	82.13	− 1.67	75.39	76.73	50.99	54.41	88.46	18.31
Derivative－23	83.74	0.25	69.97	64.01	55.32	67.55	88.42	18.26
Derivative－24	103.88	24.37	69.15	62.09	49.68	50.44	94.78	26.76
Derivative－31	85.49	2.34	64.39	50.94	40.32	22.11	72.10	− 3.57
Derivative－33	83.08	− 0.54	45.95	7.71	21.80	− 33.98	87.88	17.54

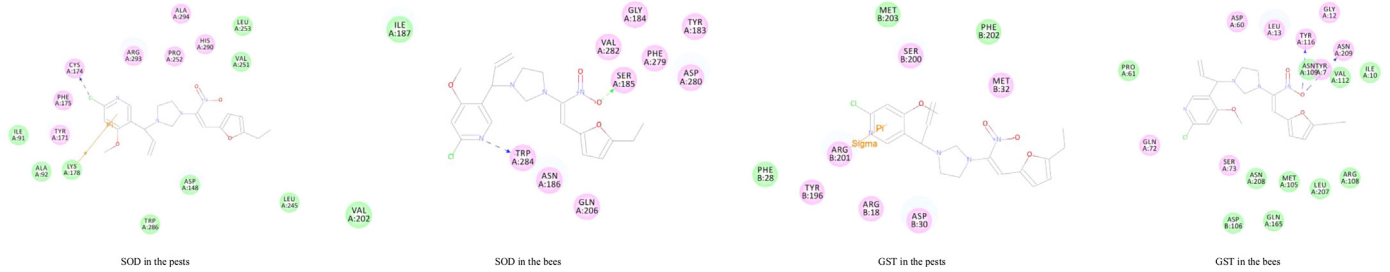

Fig. 4. Planar graph of key amino acids involved in binding of Derivative-33 with SOD and GST in pests and bees.

3.5.3. Mechanism of bi-directional selective resistance-inducing effect as determined by amino acid analysis

When Derivative-33 docked with SOD and GST, amino acid residues within a certain distance of the binding sites were predicted to play major role, and so these amino acids were identified as active residues. Therefore, the mechanism of the interaction between these amino acid residues and the binding of Derivative 33 with SOD and GST was analyzed (Fig. 4).

When Derivative-33 docked with SOD in pests, the amino acid residues involved in binding could be divided into two categories: hydrophobic amino acids (Phe175, Ala194, Leu253, Val251, Leu245, Trp286, Ala92, Ile91); and hydrophilic amino acids (Arg293, Asp148, and Lys178). When Derivative-33 docked with SOD in bees, five hydrophobic amino acids (Val202, Ile187, Trp284, Val282, and Phe279) and the same number of hydrophilic amino acids (Gln206, Asn186, and Asp280) were involved in binding.

When Derivative-33 docked with GST in pests, four hydrophobic amino acids (Phe28, Met32, Met203, and Phe202) and three hydrophilic amino acids (Arg201, Arg18, and Asp30) were involved in binding. When Derivative-33 docked with GST in bees, five hydrophobic amino acids (Met105, Leu207, Ile10, Val112, and Leu13) and eight hydrophilic amino acids (Gln72, Asp106, Gln165, Asn208, Arg108, Asn209, Asn109, and Asp60) were involved in binding.

Our analyses indicated that hydrophilic groups were beneficial for docking, while hydrophobic groups were not. The hydrophilicity of SOD and GST was stronger in pests than in bees. Therefore, the docking scores of Derivative-33 for SOD and GST in pests were lower than those of Compound 19. The SOD and GST in bees were more strongly hydrophobic, so the docking scores of Derivative-33 were higher than those of Compound 19. The difference in the docking scores of Derivative-33 for SOD and GST between pests and bees indicated that bees would be more resistant to Derivative-33, while pests would be more susceptible.

Hydrogen bonds are chemical bonds that play important roles in the structure and function of molecules, especially the binding process of protein receptors and ligands. In the interaction between Derivative-33 and SOD, the average bond length was 3.20 Å in pests and 3.00 Å in bees. In the interaction between Derivative-33 and GST, the bond length was 2.74 Å in pests, but smaller in bees (2.50 Å). Therefore, the change in bond length also contributed to the bi-directional selective resistance-inducing effects of Derivative-33 on pests and bees.

4. Conclusions

On the basis of a 3D-QSAR model established in this study, Compound 19 was modified to produce 37 neonicotinoid derivatives with higher toxicity (0.04–11.45% more toxic than Compound 19). The bioconcentration effect of 20 derivatives was decreased to varying degrees (0.38–147.88%). Ten neonicotinoid derivatives showed bi-directional selective toxicity effects and seven had bi-directional selective resistance-inducing effects on pests and bees based on homology modeling and molecular docking analyses. Among the new derivatives, Derivative-33 was selected as a new insecticide with bi-directional selective toxicity and resistance-inducing effects on pests and bees. These results provide valuable reference data and outline a method for the design of environmentally friendly neonicotinoid derivatives. These findings will accelerate the development of new pesticides with resistance-inducing effects on pest and bee populations.

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Declarations of interest

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2018.08.055.

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