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Computational insight on the binding and selectivity of target-subunit-dependent for neonicotinoid insecticides

Xiaoying Xu, Zhaokai Yang, Kai Zhu, Huilin Li, Zhaohai Qin, Hongxia Duan*

Department of Applied Chemistry, College of Science, China Agricultural University, Beijing, 100193, China

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ABSTRACT

The nicotinic acetylcholine receptor (nAChR), as an attractive target acted by neonicotinoid insecticides, was paid more and more attention in recent years. The mode of action study on neonicotinoids toward nAChR would present significant guidance on rational molecular design to further discover new insecticides. Four neonicotinoids including commercial agents imidacloprid and flupyradifurone, two previously synthesized compounds guadipyr and ethoxythiagua in our lab were docked into a putative model of aphid and honeybee nAChR, respectively, to explore the binding and selective mechanism of neonicotinoids in this study. The obtained results showed that a traditional H-bond interaction, as a dominating electrostatic driving force, always conferred the binding of four neonicotinoids not only to target aphid receptor but also to non-target honeybee one. Four neonicotinoids almost showed uniformly binding conformation into aphid receptor, namely, a vital electronegative nitro or butenolide group to be conserved to nestle in a non-selective β subunit. The bioassay study on *Aphis gossypii* also confirmed to be their excellent insecticidal activity with a lower LC_{50} value of 0.028−3.2 μg/mL. However, to non-target honeybee receptor, this special nitro or butenolide group of four neonicotinoids was no longer only binding to the non-selective β subunit of receptor. Three among four neonicotinoids like flupyradifurone, guadipyr and ethoxythiagua as a case of low bee-toxicity reported in the previous study, were happened to display an exactly inverted binding orientation, namely, an unusual electronegative group captured another selective α subunit. However, this high bee-toxicity imidacloprid remained one conserved conformation into the non-selective β subunit as that seen from aphid receptor. This unique molecular mechanism of selectively binding to honeybee receptor, particularly to different subunit, was proposed cautiously to be one of factor determined the distinctive bee-toxicity for four neonicotinoids. These findings on the diverse mode of action for neonicotinoids to target and non-target receptor would be helpful for on novel insecticides design with high bioactivity as well as good selectivity based on the structure of different insect nAChR.

1. Introduction

The nicotinic acetylcholine receptor (nAChR) plays a crucial role in the mediation of fast excitatory synaptic transmission in the central nervous system of insects [1]. Neonicotinoid insecticides targeting selectively on insect nAChR make up a major component of the global insecticide market now and are still the most important chemical class of insecticides to date [2,3]. However, the adverse effects of neonicotinoids on beneficial insect species like honeybee became increasing in recent years because of the long-term utility of neonicotinoids [4,5]. Therefore, continuous researches to develop and discover novel insecticides overcoming the question on the honeybee toxicity become more and more urgent because of the modern ecology-friendly need in agricultural sustainable development.

In recent years, a new chemical class of butenolide insecticides was found to be with a unique bioactive scaffold interacted to insect nAChR distant from the class of known neonicotinoids like imidacloprid (IMI) [6,7]. The butenolide-like flupyradifurone (FPF) became a suitable integrated pest management insecticide developed by Bayer CropScience that was a new chemical option for the control of agricultural pests, especially sucking pests of the order *Homoptera* [6]. To our excitement, FPF was indicated to be no adverse effects on actively foraging honeybees in long-term field

* Corresponding author.
E-mail address: hxduan@cau.edu.cn (H. Duan).

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trials on oilseed rape, which has been approved to use on a wide variety of crops in multiple countries [7]. More importantly, the recently synthesized compounds guadipyr (GUA) and ethoxythiagua (ETG) in our lab were also found to show not only good insecticidal activity for aphid, but also safety for honeybee with obvious low-toxicity toward bee [8,9]. However, the molecular mechanism of these neonicotinoids on bee-toxicity was an unresolved issue, which is worth to be further investigated, especially some of neonicotinoids reported with uniform good insecticidal activity but different obviously bee-toxicity.

As known to us, the crystal structure of acetylcholine-binding protein (AChBP) was available to describe the interactions between nAChR and ligands in the previous study because AChBP has the same overall architecture as the extracellular portion of nAChR [10,11]. Within the past decades, a wide variety of high-resolution crystal structures of soluble homopentameric AChBP have provided the theoretical foundation for building homology models of the corresponding nAChR-ligand binding domains, thus becoming a useful basis for virtual screening of chemical libraries and the rational design of ligands [12−14]. However, AChBP, as a widely used structure surrogate of nAChR, is eventually not the action target of neonicotinoids and maybe not the best option for the study on mode of action for neonicotinoids, especially their selectivity mechanism. To date, no crystallographic structure has been resolved for the *Apis mellifera* nAChR. The neuronal type receptors nAChR are indicated to be constituted of α and β subunits and a ligand binding site at the interface between these two subunits. Recently, the putative model of *Apis mellifera* nAChR, namely, a heteromeric functional subtype AmeIα8/ratβ2 was built based on the template of the 3D structure of *Ac*-AChBP and IMI (ID:3C79) with the aid of the full-length code sequences of *Apis mellifera* nAChR subunits isolated by Wang et al. [15]. More importantly, the study proposed that a multiple H-bond interaction, especially a unique orientation of a key nitro group binding to the putative model of AmeIα8/ratβ2, caused to *S*-(+)-dinotefuran with a higher toxicity toward *Apis mellifera* than *R*-(−)-dinotefuran confirmed by the electrophysiological and ecotoxicity studies [15]. The study could offer a believable explanation on the selective mechanism of different neonicotinoid for bee. Meanwhile, the previous electrophysiology study also indicated neonicotinoids to be good responses on a α1 subunit of *Aphis gossypii* nAChR co-assembled rat β2, namely, Agα1/ratβ2, which was consistent with their insecticidal activity [16]. Therefore, a heteromeric functional subtype Agα1/ratβ2 for *Aphis gossypii* nAChR was proposed to be an optimal structural surrogate to explore the mode of action on the binding between aphid nAChR and neonicotinoids to further clarify their insecticidal mechanism.

The study on the mode of action for target-selectivity of neonicotinoids was especially noteworthy because of their facilitating molecular design of novel selective insecticides based on nAChR structure. In the present work, four neonicotinoids including commercial agents IMI and FPF, and the recently synthesized compounds GUA and ETG, as molecular recognition detector, were docked into the putative model of nAChR functional subtype of target aphid Agα1/ratβ2 and non-target honeybee AmeIα8/ratβ2, respectively, to explore the mode of action on the binding and selectivity of neonicotinoids. It will provide a useful suggestion for novel molecule design with high bioactivity and good selectivity based on the structure of target or non-target insect nAChR.

2. Materials and methods

2.1. Materials

In our previous study, the putative model of the functional subtype AmeIα8/ratβ2 of *Apis mellifera* nAChR was indicated to offer a rational explanation on the selective mechanism on the bee-toxicity for dinotefuran enantiomers [15]. Therefore, referred to the reported model of AmeIα8/ratβ2, the putative model of a heteromeric functional subtype Agα1/ratβ2 of *Aphis gossypii* nAChR was established using the 3D structure of *Ac*-AChBP co-crystallised with imidacloprid (ID: 3C79) as a template [14] in this study. The obtained molecular modeling of Agα1/ratβ2 was authorized to explore the binding mode between neonicotinoids and aphid nAChR. Meanwhile, the selective mechanism of bee-toxicity for neonicotinoids was further investigated in the present study with the help of the reported previously model of honeybee AmeIα8/ratβ2 [15]. Neonicotinoids such as commercial ones IMI and FPF, and recently synthesized ones GUA and ETG in our lab, were selected as molecular probes labeled to explore the molecular mechanism of binding and selectivity of neonicotinoids to different species nAChR.

2.2. Methods

To clarify the binding and selective mechanism of neonicotinoids based on the structure of receptor, the putative model of Agα1/ratβ2 for *Aphis gossypii,* like the previously reported AmeIα8/ratβ2 of *Apis mellifera* [15], was generated using the 3D structure of *Ac*-AChBP with IMI (ID:3C79) as a template by the online Swiss-Model software (https://www.swissmodel. expasy.org/). Four neonicotinoid molecules including IMI, FPF, GUA, and ETG were constructed by the Sketch mode and optimized using the Tripos force field and the MMFF94 charge with other default parameters. Then they were docked into the binding pocket of the putative models of Agα1/ratβ2 and AmeIα8/ratβ2, respectively, with a low-energy conformation extracted from the obtained-above molecular mechanics optimization. Molecular docking calculations were carried out using a Surflex-dock algorithm in the Sybyl 7.3 software package on the Linux platform [17]. The suitable putative pose of ligand called protomol was generated rapidly by means of the Hammerhead scoring function with a surface-based molecular similarity method [18]. In our study, a ligand docking mode was adopted to generate an ideal protomol in the active cavity of the interface region between α and β subunits of the receptor. The molecular flexibility of all neonicotinoids, called a semi-flexible docking algorithm, was considered in the whole docking process. The Surflex-dock algorithm is well known to be an accurate, rapid, and efficient method for protein-ligand interaction simulations [19]. Surflex-dock score was used to evaluate the binding affinity between ligand and protein by a CScore parameter, which is usually correlated with the bioactivity ofligand [20]. The number of the original conformation for each ligand was set 20 and the other parameters were all in default. All molecular modeling between the putative model of Agα1/ratβ2 for *Aphis gossypii* or AmeIα8/ratβ2 for *Apis mellifera* and neonicotinoids were conducted on the Silicon Graphics® (SGI) Fuel Workstation (Silicon Graphics International Corp., CA, USA).

Insecticidal bioassays of IMI, GUA and ETG were performed on representative test organism prepared in the laboratory. Imidacloprid (95%) was purchased from Jiangsu Changlong Chemicals Co. GUA and ETG were synthesized in our lab. All compounds were dissolved in *N*,*N*-dimethylformamide solvent and diluted with 0.05% Triton X-100 to obtain a series of different concentrations samples for bioassays. The preliminary bioassay of compounds against *A. gossypii* was tested by the leaf-dipping method according to the previously reported procedures [21]. The bioassay of each compound was repeated three times at 25 ± 1 °C. The mortality rate was recorded in 48 h. The data obtained were analyzed using IBM SPSS Statistics 20 to determine the LC_{50} values.

3. Results and discussion

3.1. The binding modes between neonicotinoids and aphid nAChR

The sequence identity of α1 subunit in *Aphis gossypii* nAChR was showed to be medium of 30% with A chain of the template *Ac*-AChBP by the pairwise sequence alignment, which is high enough as a suitable template for generating a reasonable model. Although the QMEAN value, as a measure for the description of the quality of theoretical model [22], is a slightly higher deviation of −3.16 for the generated putative model Agα1/ratβ2, its 3D structure was superposed well with the template protein *Ac*-AChBP only a minimal deviation as shown in Fig. 1A. As the putative model of AmeIα8/ratβ2 reported in our previous study [15], every individual 3D model of aphid α1 subunit and rat β2 subunit, generated based on A chain and B chain in the crystal structure of *Ac*-AChBP, respectively, were co-assembled together with IMI to obtain a complex putative model of aphid nAChR, namely, a heteromeric functional subtype Agα1/ratβ2, indicated in Fig. 1B. As shown in Fig. 1C, the binding cavity of Agα1/ratβ2 was divided into two regions according to its different surrounding residues with a top polar part and a bottom hydrophobic one. It is mostly composed of some polar or aromatic residues such as Arg173, Thr177, Tyr119, Trp113, Trp176 and other hydrophobic residues like Ala166, Met175, Val167 and so on.

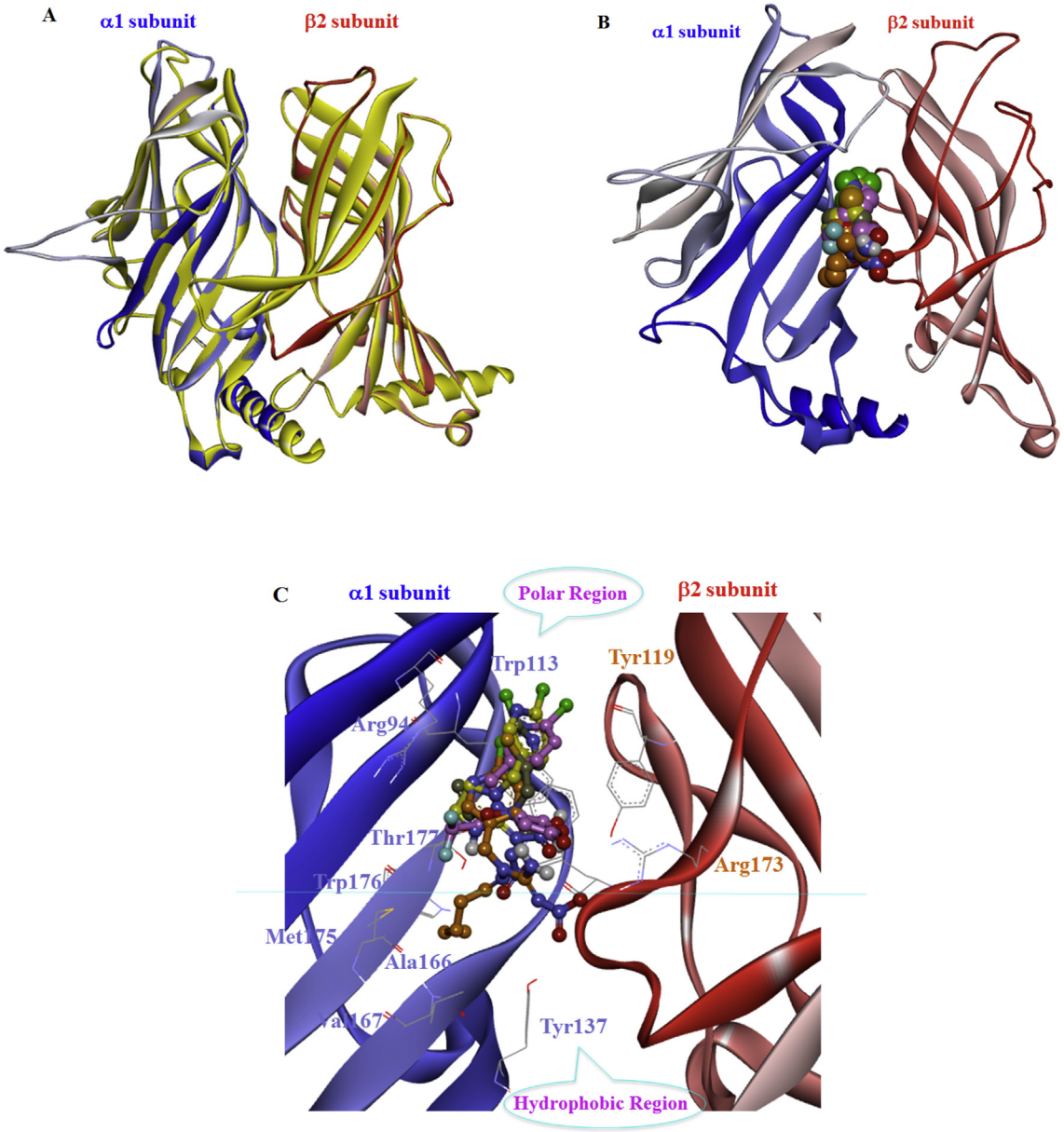


Fig. 1. The putative model and the interface binding cavity of a heteromeric functional subtype Agα1/ratβ2 for *Aphis gossypii* nAChR.
(A) The secondary structure of the Agα1/β2 model (blue and red) aligned with the template of *Ac*-AChBP (3C79) (yellow); (B) The putative model of the Agα1/β2 structure labeled with the binding cavity at the interface of α1 and β2 subunits; (C) The interface binding cavity surrounding by diverse amino acid residues in the putative model of Agα1/β2; The binding cavity of Agα1/ratβ2 was divided into two regions by labeled with a blue line namely with a top polar part and a bottom hydrophobic one. (The α and β subunit of aphid nAChR was indicated by a different color of blue and red, respectively. The α and β subunit of the template protein *Ac*-AChBP was indicated by yellow as control. The binding cavity of aphid nAChR was indicated by a ball-and-stick mode of all ligands. All corresponding residues in the binding pocket of aphid nAChR were indicated in line mode labeled by a blue and red color for α subunit and a β subunit, respectively).

Besides, it is noteworthy that the nature and position for some of residues Arg173, Tyr119 and Trp175 are conserved in the binding pocket of the putative model of Agα1/ratβ2 like those of AmeIα8/ratβ2 because of them belonging to a non-selective β subunit.

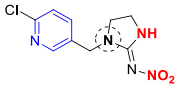
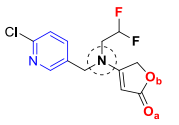
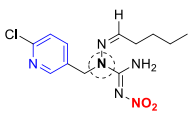
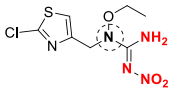
Four neonicotinoids including commercial agents IMI, FPF and the previously synthesized compounds GUA and ETG in our lab were all docked into the binding cavity of the putative model of Agα1/ratβ2 in this study. As shown in Table 1, four neonicotinoids were all with reasonable binding scores to Agα1/ratβ2, which are good agreement with their insecticidal activity for *Aphis gossypii* confirmed by a much low LC_{50} value for IMI of 0.028 μg/mL, GUA of 0.080 μg/mL, ETG of 0.357 μg/mL, respectively, in this study, and the reported previously LC_{50} value of 3.2 μg/mL for FPF by Nauen et al. [6]. As a previous report on the 3D structure of *Ac*-AChBP and IMI [17], the docked results indicated that four neonicotinoids were expected to be almost uniform each other and all located in the interface cavity of α and β subunits shown in Fig. 1C. A popular aromatic ring group not only of commercial neonicotinoids but also of the synthesized ones, such as pyridine or thiazole, both mainly stacked with its adjacent aromatic residue Trp113 of α subunit by a π effect, which made the whole molecule of neonicotinoids be located tightly into the central axis of the binding cavity between α and β subunit. As known to us [14], neonicotinoids was a tridentate type 3D conformation by a N atom as center marked with a dotted circle on in Table 1. As displayed in Fig. 2A–B, the other group of the commercial neonicotinoids, namely, an imidazolidine scaffold for IMI or a diflurinated chain for FPF was observed to only be captured by its neighboring polar residue Thr177 of α subunit. There was a moderate H-bond effect to be found with a distance of about 2.8–3.2 Å for these commercial neonicotinoids. The hydrogen bond was reported by Rocher et al. to be an important factor for the toxicity of compounds [23]. However, it is a very special issue for our previously synthesized compounds GUA and ETG because this corresponding group is a flexible long chain dragged by a polar atom N or O linking to the central atom N in Fig. 2C–D. There are different binding modes to be found for our synthesized GUA shown in Fig. 2C. Because of its larger molecular volume, GUA crossed through the top polar region, which was once occupied by the above-mentioned commercial agents, into the bottom hydrophobic one of the binding cavity of aphid nAChR. It was especially for GUA indicated that the whole molecule was shifted down the bottom region of the binding cavity because the flexible long chain dragged by a polar atom N was buried into the narrow groove of α subunit. As indicated in Fig. 2C, the binding mode on GUA was obviously determined by a diverse molecular force of the hydrophobic contact to its surrounding non-polar or aromatic residues like Ala166, Val167, Met175 and Trp176. However, ETG almost showed no evident movement to the interior binding cavity because of its smaller molecular volume and shorter hydrophobic chain than that of GUA. ETG, more like the commercial agents, only situated into the top polar region of the interface binding cavity shown in Fig. 2D.

To a known nitro or a novel butenolide group of neonicotinoids, it was more worthy to note for us that a special positively charged residue Arg173 of β subunit firmly captured this critical group via a strong H-bond interaction with a distance of less than 3.0 Å as indicated in Table 1. Meanwhile, the other two aromatic residues of β subunit Tyr119 or Trp175 exactly surrounding the two sides of Arg173 also interacted helpfully with this critical group by a powerful H-bond effect displayed in Fig. 2A–D and Table 1. It is particular for ETG that these two assistant residues Tyr119 or Trp175 replaced perfectly the positively charged residue Arg173 and contributed to the binding ability due to it binding conformation rotation via a slightly weak π-stack effect. While for the special GUA synthesized by our lab, the interior aromatic residue Tyr137 of α subunit as a complementary force also grabbed the vital electronegative nitro group by an additional H-bond network showed in Fig. 2C. Compared to the only polar residue Thr177 located the side of α subunit, it was obvious that the positively charged one Arg173 situated the other β subunit was more powerful to capture firmly the vital electronegative nitro or butenolide group of neonicotinoids. It was concluded that the positively charged Arg173 of β subunit, assistant with its two adjacent residues Tyr119 or Trp175, turned to the binding to the critical electronegative group and dominantly conferred their binding affinity to aphid nAChR further resulted in their good aphicidal activity.

3.2. The diverse mode of action for commercial neonicotinoids and honeybee nAChR

To further explore the molecular mechanism of different neonicotinoids to honeybee, commercial agents including IMI with a high bee-toxicity, FPF and our previously synthesized compounds GUA and ETG, as a case of low bee-toxicity, were all docked into the previously reported putative model of honeybee AmeIα8/ratβ2 [15]. As shown in Fig. 3A and B, the binding environment of

Table 1
The insecticidal activity against *Aphis gossypii* of four neonicotinoids, the docking score and their H-bond interaction to *Aphis gossypii* nAChR subtype Agα1/ratβ2.

Name	Structure	LC_{50} (μg/mL)	Score	H-bond(Å)	No. (H-bond)	No. (Residue)
IMI		0.028	4.05	NO_{2a}-HN (Arg173/β) 2.782 NO_{2b}-HO (Tyr119/β) 2.032 NH-O (Thr177/α) 2.865	3	3
FPF		3.2 [6]	3.48	O_a-HN (Arg173/β) 2.145 O_b-HO (Tyr119/β) 1.964 F-HO (Thr177/α) 3.194	3	3
GUA		0.080	3.68	NO_{2a}-H_bN (Arg173/β) 2.060 NO_{2b}-HO (Tyr137/α) 2.086	2	2
ETG		0.357	3.76	NO_2-NH (Trp175/β) 3.244 = N-OH (Thr177/α) 2.899 NH_{2a}-O (Tyr119/β) 2.722 NH_{2b}-O=C (Trp175/β) 2.020	4	3

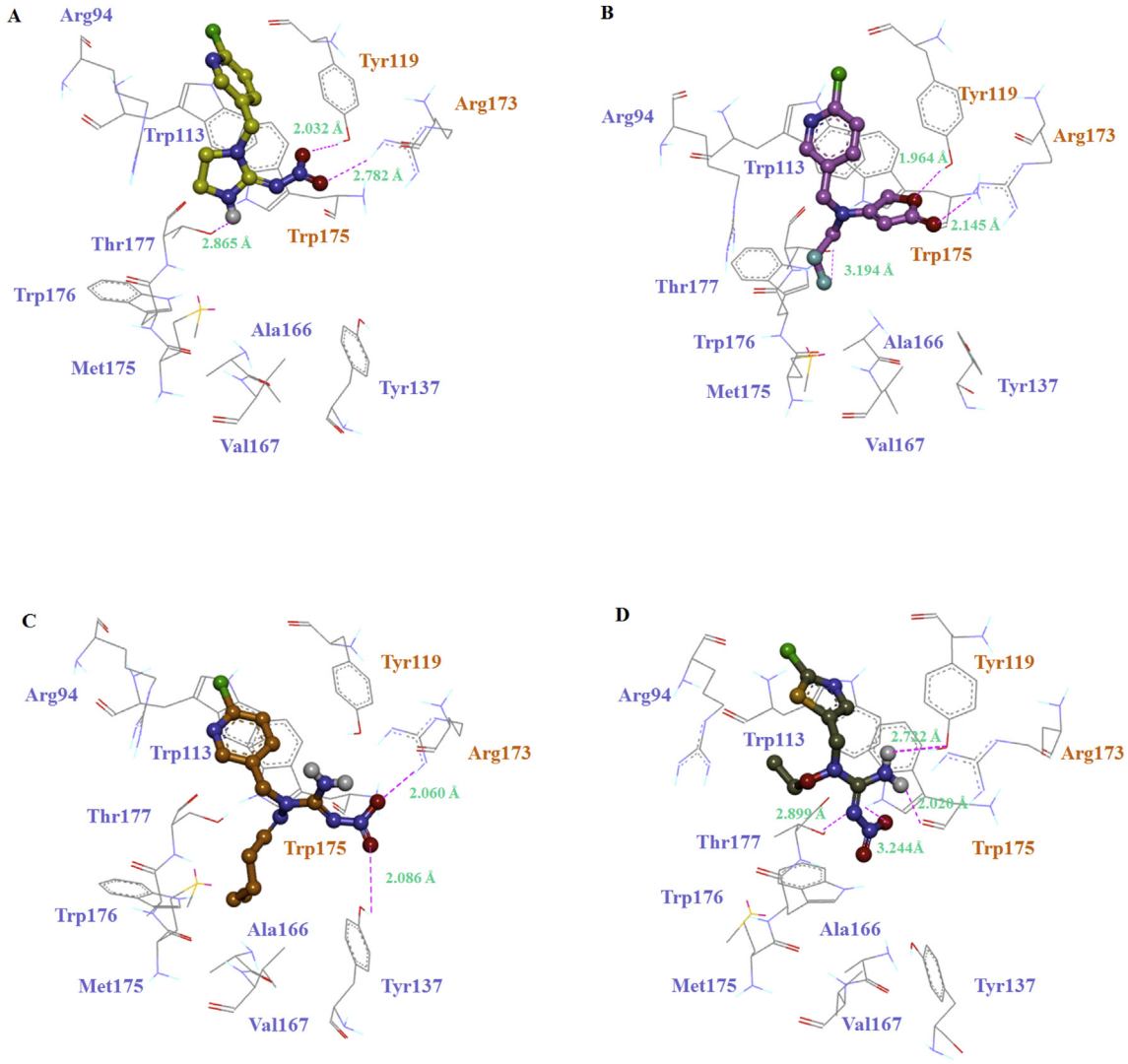


Fig. 2. The binding modes of four neonicotinoids and aphid nAChR subtype Agα1/ratβ2.
(All corresponding residue in the binding pocket of aphid nAChR was indicated in line mode labeled by a blue and brown color for α subunit and a β subunit, respectively. Ligand was indicated in ball-and-stick mode with different colors for IMI (yellow) (A), FPF (pink) (B), GUA (brown) (C), ETG (green) (D). H-bond interaction was labeled by pink line between H-bond donor and H-bond acceptor). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

AmeIα8/ratβ2 was indicated to be diverse from aphid Agα1/ratβ2 because of a different and selective α subunit each other. It was worthy to note for AmeIα8/ratβ2 that the binding cavity was separated symmetrically to be two sections by parallelled to its interface of α subunit and β subunit. It was composed of a special α region marked by a positively charged residue Lys149 and polar one Ser147 and its complementary β region with the corresponding residues Arg173 and Thr176, respectively. Compared to the binding cavity of aphid nAChR, the two residues Arg173 and Tyr119 are both conserved in the binding pocket of the putative model of AmeIα8/ratβ2 because of them belonging to the non-selective β subunit. Therefore, it was speculated that the selective bee-toxicity of neonicotinoids should be attributed to a special α subunit, indicating their disparate binding state due to the diversity of the binding environment of aphid and honeybee receptor.

Although four neonicotinoids were also occupied the interface binding site of α and β subunit for the putative model of honeybee nAChR showed in Fig. 3A, there were several diverse binding orientation from that described-above in aphid nAChR. As indicated in Table 2, IMI was reported to be high-toxicity toward honeybee with a much low LD_{50} value of 0.0325 μg/bee in the previous study [9]. The docking score of IMI was also higher to honeybee AmeIα8/ratβ2 than other three neonicotinoids in the present study, indicating it to explain reasonably its high toxicity to honeybee via the binding mode. As shown in Fig. 4A, a conventional heterocyclic group of IMI, namely, pyridine rotated slightly even to more close to α subunit of honeybee nAChR so as to make the original π effect in aphid nAChR disappeared. A following polar H-bond effect was formed with a distance of 3.085 Å to pyridine interacted with a neighboring residue Lys149 of α subunit of honeybee nAChR. It was clear for us that the special α subunit of honeybee nAChR, particularly this positively charged residue Lys149, was so strong enough as to capture firmly this critical electrostatic heterocyclic group. More obviously, this nitro group formed a strong double H-bond network to β subunit for AmeIα8/ratβ2 with an adjacent positively charged residue Arg173 and an aromatic one Tyr119, which is much similar to its binding mode of aphid Agα1/ratβ2. As proposed by Rocher et al., the hydrogen bonds is sometimes able to distinguish molecule with low LD_{50} value from those with high LD_{50} one [23]. Therefore, the double H-bond network around the nitro group was

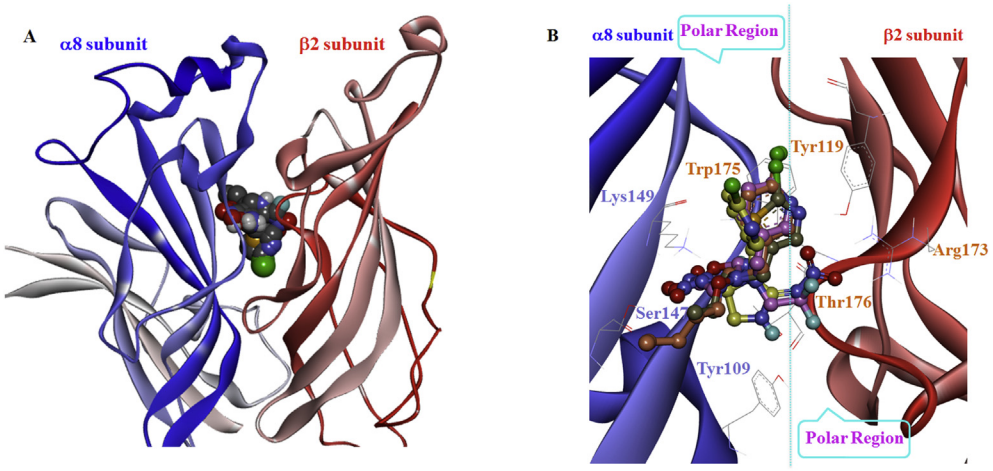
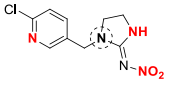
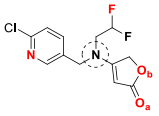
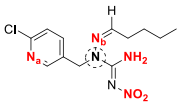
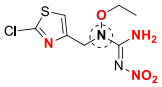


Fig. 3. The putative model and the interface binding cavity of a heteromeric functional subtype AmelIα8/β2 for *Apis mellifera* nAChR.
(A) The putative model of the AmelIα8/β2 model labeled with the binding cavity at the interface of α8 and β2 subunits; (B) The interface binding cavity surrounding by diverse amino acid residues in the putative model of AmelIα8/β2; The binding cavity of AmelIα8/β2 was separated to be symmetrical two sections by parallelled to its interface of α subunit marked by a positively charged residue Lys149 and polar one Ser147 and its complementary β subunit with corresponding residues Arg173 and Thr176, respectively. (The α and β subunit of honeybee nAChR was indicated by a different color of blue and red, respectively. The binding cavity of honeybee nAChR was indicated by a ball-and-stick mode of all ligands. All corresponding residues in the binding pocket of honeybee nAChR were indicated in line mode labeled by a blue and red color for α subunit and a β subunit, respectively). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

Table 2
The toxicity to *Apis melliferal*, docking scores and the H-bond interaction of four neonicotinoids and *Apis melliferal* nAChR subtype AmelIα8/ratβ2.

Name	Structure	Bee-toxicity [9] LD_{50} (μg/bee)	Docking Score	H-bond(Å)	No. (H-bond)	No. (Residue)
IMI		0.0325	5.33	NO_{2a}-H_aN (Arg173/β) 1.886 NO_{2b}-HO (Tyr119/β) 3.251 NH-O=C (Thr176/β) 2.126 N-HN (Lys149/α) 3.085	4	4
FPF		1.2	4.62	O_a-HN (Lys149/α) 3.188 O_b-HN(Trp175/β) 3.289 F-H_{2b}N (Arg173/β) 3.108 N-HO (Tyr119/β) 2.622	4	4
GUA		5.19	4.54	NO_{2a}-HN (Lys149/α) 2.817 NO_{2b}-HO (Ser147/α) 1.951 N_b-HN (Lys149/α) 2.154 NH_2-O=C (Trp175/β) 1.905 N_a-HO (Tyr119/β) 3.331	5	4
4–07		5.56	4.24	NO_{2a}-NH (Lys149/α) 2.760 NO_{2b}-NH (Trp175/β) 3.278 O-HN (Lys149/α) 1.979 NH_{2a}-O=C (Thr176/β) 1.960 N-HO (Tyr119/β) 2.166	5	4

speculated to mainly contribute to the binding force between IMI and its honeybee nAChR, like that in aphid nAChR, conferred its higher binding score. As reported in our previous study [15], it was believable that this nitro group was as important as a superior binding orientation to stretch the β subunit indicated in Fig. 3B. It was just this strong electrostatic effect resulted in IMI with higher binding ability to honeybee nAChR to further thus be its high-toxicity to bee. In addition, a polar imidazolidine scaffold of IMI as an H-bond donor linked to its surrounding polar residue Thr176 of the β subunit, which was very similar to its binding effect to the residue Thr177 of α subunit of aphid nAChR. In short, IMI with a smaller molecular volume was showed to be nestled into a unique electrostatic and polar groove of the binding cavity of honeybee nAChR. It was obvious that the strong multiple H-bond interaction mainly contribute to the binding effect between IMI and the corresponding residues originated from different α and β chain of honeybee nAChR, which was used to explain the reason of IMI with a high-toxicity to bee.

A recently discovered commercial product FPF was indicated to be a low toxicity toward honeybee with a LD_{50} value of only 1.2 μg/bee for *Apis melliferal* [6]. The docking score of FPF to the putative model of honeybee AmelIα8/ratβ2 was also showed to be slightly lower than that of IMI in our study, indicating a lower bee-toxicity listed in Table 2. As shown in Fig. 4B, the binding mode of FPF to honeybee nAChR was believed to be obviously different from that of high bee-toxicity IMI. The pyridine ring of FPF interacted with an aromatic residue Tyr119 of β chain by an H-bond effect with a distance of 2.622 Å, which was clearly different from the binding mode of IMI with its acting to an electrostatic residue Lys149 of α chain. To our more surprise, it was different obviously to be found that the electronegative butenolide group of FPF, as a surrogate of the nitro group in IMI, was with a totally inverse orientation to stretch α chain, not β chain for IMI, then interacted with the corresponding positively charged residue Lys149 by a H-bond effect of

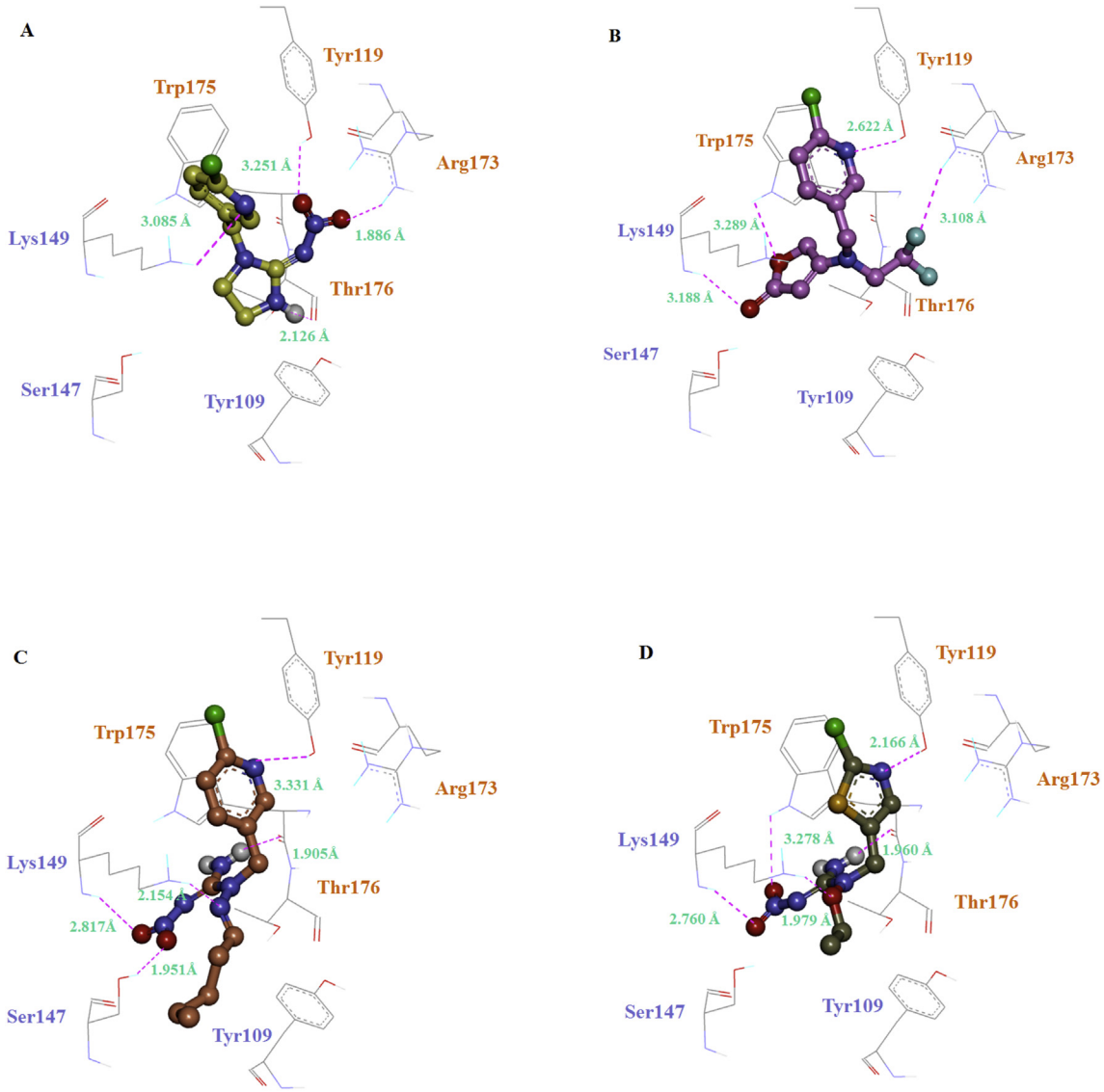


Fig. 4. The molecular interaction of four neonicotinoids and honeybee nAChR subtype AmelΑ8/ratβ2
(All corresponding residues in the binding pocket of honeybee nAChR were indicated in line mode labeled by a blue and brown color for α subunit and a β subunit, respectively. Ligand was indicated in a ball-and-stick mode with different colors for IMI (yellow) (A), FPF (pink) (B), GUA (brown) (C), ETG (green) (D). H-bond interaction was labeled by pink line between H-bond donor and H-bond acceptor). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article).

α chain. The neighboring aromatic residue Trp175 of β chain also formed H-bond effect to another polar atom O of butenolide with a distance of 3.289 Å to further fix this superior orientation of butenolide. This medium double H-bond effect was found to fix the orientation of butenolide of FPF, possibly resulting in its weaker binding ability to honeybee nAChR than that of IMI with the strong double H-bond effect. Compared to the electropositive residue Arg173 of β chain binding to IMI, it was very clear that the complementary electropositive residue Lys149 of α chain could competitively bind to the electronegative butenolide group of FPF. As reported in our previous study on the pharmacological response of dinotefuran enantiomers to honeybee nAChR [15], *S*-(+)-dinotefuran with higher acute toxicity to *Apis mellifera* was indicated to bind selectively to AmelΑ8/ratβ2 with a higher pEC_{50} value than a *R*-(−)-dinotefuran. It was found by the binding mode that a key nitro group of *S*-(+)-dinotefuran, as a case of a higher docking score, was also showed to point to an electropositive residue Arg173 of β chain, whereas its enantiomer *R*-(−)-dinotefuran showed an exactly inverse conformation with a nitro group to another electropositive residue Lys149 of α chain. As indicated by the previously reported results of Liu. et al., the different affinity may be caused from the difference of the key amino acid at the agonist binding site of nAChR subunits [24]. Therefore, it was proposed to be a key factor for different electropositive residue Arg173 or Lys149 binding to its corresponding electronegative group in commercial neonicotinoids, which resulted in different binding affinity to honeybee nAChR further to show diverse bee-toxicity. Compared to a bigger butenolide group, the third group of smaller diflurinated chain was pushed to β chain and substituted the binding site of the nitro group of IMI in aphid receptor, only acting as an H-bond acceptor to interact to the free electropositive residue Arg173 with a distance of 3.108 Å.

In summary, there is different binding cavity in honeybee nAChR from that of in aphid nAChR because of their selectively different α chain. The positively charged residues Arg173 of β chain and its complementary one Lys149 of α chain in honeybee nAChR located

on two sides of commercial neonicotinoids, like a molecular clip, both locked tightly ligand into the binding interface to determine their binding ability. The orientation of the nitro or butenolide group of neonicotinoids was concluded to be a key factor with different H-bond effect to determine their diverse binding affinity to honeybee AmeIα8/ratβ2. It was clear to be a good choice for us that various molecular interaction mode was used to explain the binding ability selectively of neonicotinoids to honeybee nAChR to further clarify different toxicity mechanism on the selective molecular design.

3.3. The unique binding mode of synthesized neonicotinoids to honeybee nAChR

To our surprised, the previously synthesized compounds GUA and ETG by our lab were indicated to be not only high insecticidal activity but also low bee-toxicity. The LD_{50} value of GUA and ETG was reported to be 5.19 and 5.56 μg/bee, respectively, in our previous study [9]. The docking scores of GUA and ETG listed in Table 2 were also found to be lower than that of IMI in the present study, indicating their low bee-toxicity. The binding mode study on GUA showed a different one to IMI as a case of a high bee-toxicity, especially an orientation of the electronegative group. As shown in Fig. 4C, an identical electronegative nitro group of GUA stretched to α chain to interact with a complementary electropositive residue Lys149 with an H-bond interaction of distance of 2.817 Å. It was obviously different from that of IMI pointed to an electropositive residue Arg173 of β chain, but same to that of low bee-toxicity FPF. This nitro group of GUA was also locked by a new next polar one Ser147 of α subunit with a H-bond effect of 1.951 Å in Table 2. It was clear that the α subunit residues offered a double H-bond force for GUA, which was different from another double H-bond effect of IMI derived from the β chain residues. Therefore, the binding ability to honeybee nAChR for GUA was exactly decreased with a lower docking score to further show a lower bee-toxicity than IMI. It was obvious that the cooperative effect of diverse multiple polar force could induce this key nitro group of neonicotinoids to rotate by an obvious different binding conformation to honeybee nAChR.

Moreover, its next group -NH_2 to nitro of GUA was pulled to an aromatic residue Trp175 by an effective H-bond interaction with a distance of 1.905 Å in Fig. 4C and Table 2. And the aromatic pyridine ring showed a similar binding mode to FPF with an H-bond interaction to its neighboring aromatic residue Tyr119 of β chain with a distance of 3.331 Å. Lastly, a polar atom N of a long flexible chain was also dragged tightly by an H-bond from Lys149 of α subunit and limited to unmove to be further helpful its selectively binding to honeybee subunit. The H-bond effect was found between the polar atom N and its neighboring residue Lys149 with a distance of 2.154 Å, which made the long chain anchor a non-electronic environment surrounding by residues Tyr109 and Thr176.

Compound ETG showed a very similar binding mode to that of GUA in the binding interface of α and β chain of honeybee nAChR shown in Fig. 4D. The aromatic thiazole ring of ETG also formed a H-bond effect to the same residue Tyr119 with a distance of 2.166 Å in Table 2. The electronegative nitro group also formed a double H-bond of a distance of 2.760 Å and 3.278 Å to the electropositive residue Lys149 of α subunit and another one Trp175 of β subunit, respectively. Moreover, its next group -NH_2 to nitro was pulled to an aromatic residue Thr176 by an effective H-bond interaction with a distance of 1.960 Å. Lastly, a polar atom O of a long flexible chain was also dragged tightly to the electropositively charged Lys149 of α subunit with an H-bond of 1.979 Å to aid its selectively binding to honeybee subunit. It was summarized that the electropositive charged residue Lys149 with the aids of its neighboring one Ser147 or Trp175 competitively bind to the key nitro group of GUA and ETG, like that of FPF, conferred to the double H-bond effect to be a low-toxicity to honeybee.

4. Conclusion

To explore the binding and selective mechanism of neonicotinoids, four ligands including commercial agents IMI and FPF and previously synthesized compounds GUA and ETG in our lab were docked into the putative model of aphid nAChR, namely, the functional subtype Agα1/β2 of *Aphis gossypii* and honeybee nAChR, namely, the functional subtype AmeIα8/β2 of *Apis mellifera*, respectively, in this study. The docking scores of all neonicotinoids to targeted aphid and non-targeted honeybee nAChR, respectively, showed good agreement with the bioactivity of their aphid-insecticide and bee-toxicity, which indicated the binding mode of neonicotinoids on different insect nAChR was believable to explain their molecular mechanism on target aphid and non-target honeybee. In our study, the binding cavity of Agα1/ratβ2 was divided into two regions according to its different surrounding residues, namely, with a top polar part and a bottom hydrophobic one. All neonicotinoids displayed identical binding conformations and aligned well into the binding cavity on the interface of the α1 and β2 subunit of aphid nAChR. More significantly, a key electronegative nitro or butenolide group of neonicotinoids was almost conserved to form a classical double H-bond network in the top polar region with a specific positively charged residue Arg173 and/or an aromatic residue Tyr119 of the non-selective β subunit. Therefore, the identical binding mechanism of all neonicotinoids to aphid nAChR was proposed to be explained their similar insecticidal activity on aphid. However, it was worthy to note for AmeIα8/ratβ2 that the binding cavity was special to be separated symmetrically to two sections by parallelled to its interface of α subunit marked by a positively charged residue Lys149 and polar one Ser147 and its complementary β subunit with the corresponding residues Arg173 and Thr176, respectively. It was observed surprisedly for four neonicotinoids that there was remarkably different binding mode to AmeIα8/β2 to determinate their diverse toxicity to honeybee. The conserved nitro group of IMI as a case of a high bee-toxicity was still pointed to the positively charged residue Arg173 of the non-selective β chain with a double H-bond effect. However, this butenolide or nitro group of three other neonicotinoids FPF, GUA, ETG, as the low bee-toxicity example, interacted with the selective α subunit and further formed a double H-bond network with a competitive positively charged residue Lys149 simultaneously with the aids of a polar residue Ser147 or Trp175. It was clear that there was almost the complementary electropositive residue for Arg173 and Lys149 at the two sides of the binding cavity, which could be powerful enough to competitively induce the key electronegative nitro or butenolide group to be reversed for the active binding conformation of neonicotinoids. These results could be reasonable to explain the selective mechanism of different neonicotinoids on bee-toxicity. It was obvious that electrostatic interaction is a main driver not only for molecular binding but also for its selective recognition mechanism of neonicotinoids to different species nAChR. The study will be helpful to design and discover novel neonicotinoids with high-bioactivity to target and low-toxicity to non-target based on the interaction mode of ligand-receptor.

Author contributions

X.Y. Xu, H.X. Duan and Z.H. Qin conceived and designed the experiments. X.Y. Xu, Z.K. Yang, K. Zhu, H.L. Li, Z.H. Qin and H.X. Duan completed the experiment and discussion. X.Y. Xu, H.X. Duan and Z.H. Q in wrote the first draft of the manuscript and H.X. Duan

and Z.H. Q in made critical revisions and approved the final manuscript. All the authors reviewed and approved the final manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmgm.2020.107586.

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