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ORIGINAL PAPER

A novel halogen bond and a better-known hydrogen bond cooperation of neonicotinoid and insect nicotinic acetylcholine receptor recognition

Hongxia Duan · Weiwei Zhang · Jin Zhao · Desheng Liang · Xinling Yang · Shuhui Jin

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Abstract Neonicotinoid insecticides target the insect nicotinic acetylcholine receptor (nAChR) and are highly effective against the piercing-sucking pests. To explore the molecular interaction mechanism between the neonicotinoids and the insect nAChR, some key neonicotinoid compounds were docked into *Aplysia californica* acetylcholine binding protein (*Ac*-AChBP), which serves as a suitable structural surrogate of the insect nAChR. The binding mode study showed that the hydrogen bond force between the electronegative pharmacophore of the neonicotinoids and Cys190NH of the target binding pocket is crucial to the high efficiency of the neonicotinoids. Increasing the coplanarity between the guanidine or amidine and the electronegative pharmacophore of the neonicotinoids could increase the Π-Π stacking effect with Tyr188 of the *Ac*-AChBP and thus enhance the insecticidal potency. The introduction of an azide group to the chloropyridine ring of the neonicotinoids would reduce its binding ability due to the disappearance of a novel halogen bonding interaction. A series of novel neonicotinoid molecules were designed based on the halogen bonding interaction and two compounds with 6-bromopyridine-3-yl and 6-(trifluoromethyl)-3-pyridinyl were found to be with potential insecticidal activities.

H. Duan (✉) · W. Zhang · J. Zhao · X. Yang · S. Jin (✉)
Department of Applied Chemistry, College of Science, Key Laboratory of Pesticide Chemistry and Application Technology, China Agricultural University,
Beijing 100193, People's Republic of China
e-mail: hxduan@cau.edu.cn
e-mail: shuhuij@cau.edu.cn

D. Liang
College of Chemistry and Chemical Engineering,
Graduate University of Chinese Academy of Sciences,
Beijing 100049, People's Republic of China

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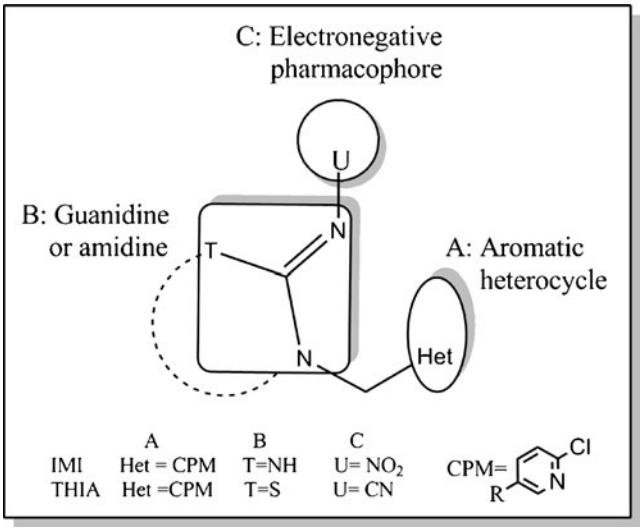
Introduction

Neonicotinoid insecticides are increasingly used for the systemic control of plant-sucking insects because of their effectiveness against pests while remaining safe for humans and wildlife. The neonicotinoids imidacloprid (IMI) and thiacloprid (THIA) share many common features: an aromatic heterocycle 6-chloropyridin-3-yl (CPM) coupled through a methylene flexible linkage to guanidine or amidine moiety, which is coplanar with the electronegative pharmacophore nitro ($-NO_2$) or cyano (−CN) (Scheme 1) [1]. The electronegative pharmacophore of the neonicotinoids is nonprotonated at physiological pH and plays a crucial role in the high affinity and selectivity for the target of the insect [2]. The coplanar segment between guanidine or amidine and pharmacophore in the neonicotinoids could create an electronic conjugation to facilitate the partial negative charge flow toward the tip atom and increase the binding affinity to the insect target [3].

Many attempts have been made to explore neonicotinoid compounds with novel chemical structures and high insecticidal activities. Tomizawa *et al*. synthesized some neonicotinoid analogues with different electronegative pharmacophores and found that the -NO analogues essentially retained the high binding affinity of the $-NO_2$ compounds, while the isosteric -C(O)H congeners diminished the potency [4]. However, the potential reason was not further explained. Zhang *et al.* synthesized the neonicotinoids with an azido substituent group at the 5-position of the CPM as a photoaffinity probe to identify the insecticide-binding



Scheme 1 General structure of neonicotinoids

subunits interaction. It was reported that the *K*i value of the azidoneonicotinoids was indecreased compared with the nonazido parent compounds because of the spatial effect of the substituent group [5]. As Tomizawa and Zhang reported, increasing the coplanar system between the guanidine or amidine and the key pharmacophore could contribute to enhance the affinity of the neonicotinoids [4, 5]. Their essential molecular mechanism was not clear yet.

The insect nicotinic acetylcholine receptor (nAChR) is identified as the target of the neonicotinoid insecticides [6]. Each nAChR consists of five subunits usually including α and non-α subunits and the ligand-binding site is located at the interface between the two adjacent subunits [7, 8]. The α-subunit residues contribute to the principal part or (+)-face, and the neighboring subunit residues form the more variable complementary part or (−)-face of the binding pocket [9]. However, experiments have not yet to produce some X-ray crystallography structural data with sufficient resolution of nAChR, which leads to a less well-defined view of the receptor-ligand binding site until now [10]. The acetylcholine binding protein (AChBP) was found to share 20-24% sequence identity with the nAChR α subunits and contain all the conserved residues of the ligand binding pocket for nAChR [6, 11]. Moreover AChBP was reported to bind some known nAChR agonists acetylcholine, imidacloprid and competitive antagonists such as β-tubocurarine and α-bungarotoxin [6, 12, 13]. Therefore, AChBP is believed to be the optimal structure surrogate of the extracellular ligand-binding domain of nAChR at present. Fortunately, Sixma *et al.* obtained a high resolution X-ray crystallography structure of a freshwater snail *Lymnaea stagnalis* AChBP (*Ls*-AChBP) [6], which greatly facilitates the understanding on the ligand binding mechanism of nAChR. Subsequently, the X-ray crystal structures of *Aplysia californica* (*Ac*-) AChBP and its complexes with epibatidine (EPI) and IMI, and *Bulinus truncates (Bt*-) AChBP were reported successively [14–16]. Recently, it was found that the *Ls*-AChBP has a lower affinity for the neonicotinoids than the nicotinoids whereas the *Ac*-AChBP is highly sensitive to the neonicotinoids [3]. Therefore, *Ac*-AChBP served as a plausible structural surrogate of the insect nAChR for exploring the molecular interaction mechanism between the neonicotinoid insecticide and its target nAChR.

As a template of the *Ac*-AChBP structure, some molecular interaction studies between the nAChR binding site and its ligands were reported in recent years. The crystallography structure of the *Ac*-AChBP with a methyllycaconitine (PDB ID: 2BYR) was used to explore the alkaloid recognition mode. The results showed that all compounds fit well in the protein binding pocket and exhibited a good correlation between their ED_{50} and the docking binding free energy [17]. Molecular docking of the neonicotinoids with different aromatic heterocycle into *Ac*-AChBP (PDB ID: 3C79) was completed using the Auto Dock Vina docking program. The reported results indicated that all of the aromatic heterocycles of the neonicotinoid compounds form an important water bridge interaction with the amino acid residue Ile118 of the *Ac*-AChBP binding pocket [18]. Therefore, the crystal structure of *Ac*-AChBP bound with IMI (PDB ID: 3C79) was selected as a template in our study and the neonicotinoid compounds shown in Fig. 1 were docked into the *Ac*-AChBP to explore the molecular mechanism between the neonicotinoid compounds and the target of the insect nAChR. Unless otherwise indicated, the following AChBP referred to *Ac*-AChBP.

Materials and methods

Materials

As shown in Fig. 1, neonicotinoid derivatives were reported by Tomizawa *et al.* as the docking ligands in this study [4, 19–21]. The potencies of all the compounds were determined using the same method as an inhibitor of [^{3}H] IMI binding to the native *Drosophila* brain nAChR [22–24]. *Ac*-AChBP co-crystallized structure with IMI was retrieved from RCSB Protein Data Bank (PDB ID: 3C79, resolution: 2.48 Å) as a docking template [14].

Methods

The Surflex-Dock algorithm, a fully automatic flexible molecular docking algorithm, combines an empirically derived scoring function and a surface-based molecular similarity method to dock a ligand into the protein binding site. This

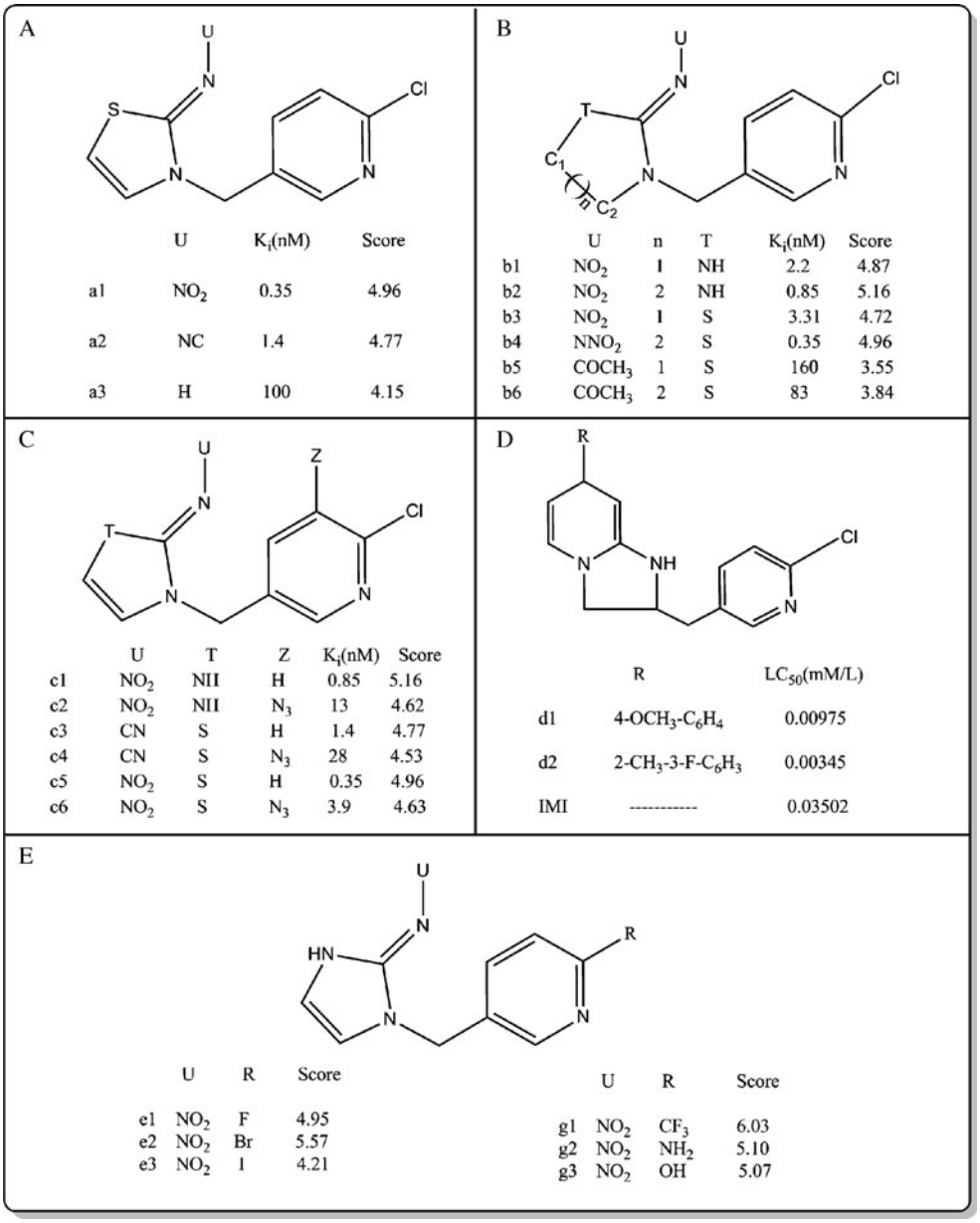


Fig. 1 The chemical structures and the insecticidal activity of the neonicotinoid compounds. **a**, **b**, **c**: The insecticidal activity to the *Drosophila* nAChR. **d**: The insecticidal activity to Pea Aphid (*Aphis craccivora*). **e**: New designed compounds

algorithm performance was significantly better with a true positive rate of greater than 80% and a false positive rate of less than 1% [25]. The scoring function (score) was used to predict the binding affinities of protein/ligand complexes, with its output being represented in units of -log(*K*d) [26]. In our study, the Surflex-Dock algorithm of the SYBYL molecular modeling package was applied to explore the molecular binding mode between the neonicotinoid insecticides and the insect target nAChR [27]. The crystal structure AChBP complexed with IMI was modified by removing all ligands, solvent molecules and some water molecules and then adding the polar hydrogen and the MMFF94 charges. Ligand docking mode and other default parameters were employed to generate the binding pocket of the target AChBP. The low energy conformation of each neonicotinoid analogue was optimized by a MMFF94 force field and MMFF94 charges as an initial docking conformation. The electrostatic potential on the halogen surface was analyzed using the molecular electrostatic potential, *EP(i)*, defined by Eq. 1:

$$EP(i) = \sum_{i=1}^{N} \frac{q_j}{r_{ij}} \tag{1}$$

EP(i) is the potential at the surface point *i*; q_j is the partial charge of atom *j*; r_{ij} is the distance between point *i* and atom *j*. No shifted force potential or cutoff radius were applied [28]. All calculations were performed by the Sybyl7.3 software package on the Linux platform.

Results and discussion

The binding mode of the neonicotinoid compounds

The binding mode and the binding affinity between the neonicotinoids and the AChBP were studied by the molecular simulation technique. The docked results exhibited a good correlation between the predicted binding affinity (score) and the insecticidal potency (*Ki*) of neonicotinoids with a correlation coefficient 0.873. As Fig. 2 shows, the hydrogen bond force, the Π–Π stacking effect, and the halogen bonding interaction were found to be greatly important for the molecular recognition of the neonicotinoids and the AChBP. The tip one O atom of the pharmacophore -NO_2 in the neonicotinoid made a hydrogen bond to Cys190NH on the (+)-face of AChBP. However, a bridge ring amine in the nicotinoid compound EPI targeted with the aromatic side chain of the residue Trp147 of AChBP and formed a cation–Π interaction in a reverse direction [15]. This difference of the molecular interaction was a critical reason that the neonicotinoid compounds showed a higher selectivity than the nicotinoid compounds. The conjugate guanidine or amidine plane formed a Π–Π stacking effect with the aromatic side chain of Tyr188 on the (+)-face of AChBP, which contributed to their stable binding conformation. This kind of Π–Π stacking interaction was not present between the nicotinoid insecticide and its target because most of the nicotinoid compounds lacked an aromatic ring. It was reported that the CPM N atom in the neonicotinoid formed a water bridge with the backbone of the residue Ile118 of the (−)-face of AChBP and the CPM Cl atom interacted with Met116O by the van der Waals force. Interestingly, most of the distances between the CPM Cl atom in these key neonicotinoid compounds and the O atom of the residue Met116 of AChBP were obtained close to 3.10 Å in our study, which were shorter than the sum of their van der Waals radius (3.27 Å). This molecular interaction force looked similar to the recently reported halogen bonding interaction [29]. Halogen bond, in biomolecules, is defined as a short C-X···O-Y interaction, where X is a chlorine, bromine or iodine. The character of the halogen bond is that the X···O distance is less than or equal to the sum of their van der Waals radii (3.27 Å for Cl···O, 3.37 Å for Br···O, and 3.50 Å for I···O, respectively) with the C-X···O angle (θ_1)≈165° and the X···O-Y angle (θ_2)≈120° [30]. Therefore, it was believed that the interaction between the CPM Cl atom of neonicotinoids and Met116O of the target AChBP was a novel halogen bond interaction, instead of a general van der Waals force in our study. The CPM Cl atom of the nicotinoid compound EPI interacted with the residue Met116O of AChBP only by the traditional van der Waals force with a longer distance 3.43 Å. To sum up, the binding mode between the neonicotinoids and the AChBP should be very important to guide and design new neonicotinoid compounds with the high insecticide activity.

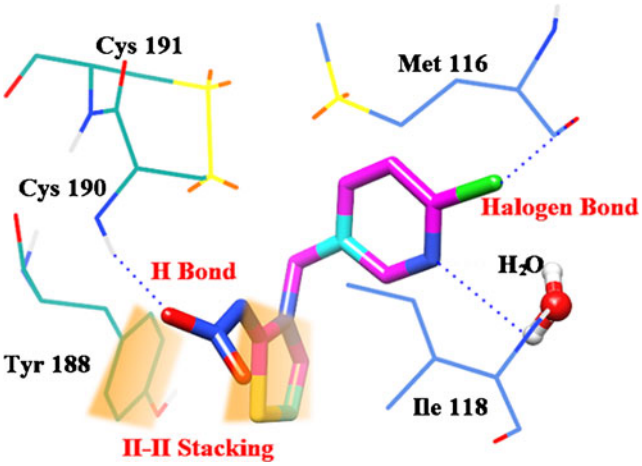


Fig. 2 The binding mode of neonicotinoids and the binding pocket of *Ac*-AChBP as an insect nAChR surrogate. Neonicotinoid is buried in the interfacial agonist binding pocket between the primary or (+)-face (light sea green) and complementary or (−)-face (cornflower blue) subunits. In each ligand and amino acid residue, chlorine, nitrogen, oxygen and sulfur atoms are colored green, blue, red and yellow, respectively

Hydrogen bond force of the electronegative pharmacophore

The electronegative pharmacophore of the neonicotinoid plays a crucial role in the high affinity and selectivity for the insect nAChR [2]. Two typical neonicotinoid analogues were docking in the target AChBP to explore the molecule mechanism between the neonicotinoid pharmacophore and the insect nAChR. For comparison, nicotinoid compound a3 was also docked in the AChBP pocket. As shown in Fig. 3, the electronegative pharmacophore of neonicotinoid compounds a1 and a2 both nestled near the amino acid residue Cys190 of the AChBP binding pocket in a similar orientation, but there was a reverse direction to be located for the tip cationic functional group =NH of the nicotinoid compound a3. The two binding modes were in accordance with those of the reported crystal structure of AChBP with IMI and EPI, respectively [14, 15]. It was obvious that the tip atoms O and N of the electronegative pharmacophore -NO_2 and -CN for the neonicotinoid compounds a1 and a2 both formed the hydrogen bond to Cys190NH of the (+)-face for AChBP, respectively. However the N atom of the tip =NH in the nicotinoid compound a3 had a partial positive charge (δ^+) and only formed a cation-Π interaction to another residue Trp147 of AChBP. As a result, the affinity of the nicotinoid compound a3 was markedly lower than those of the neonicotinoid compounds a1 and a2. As shown in Fig. 3, the potency of the neonicotinoid compound a2 was lower than that of compound a1 also due to the orientation

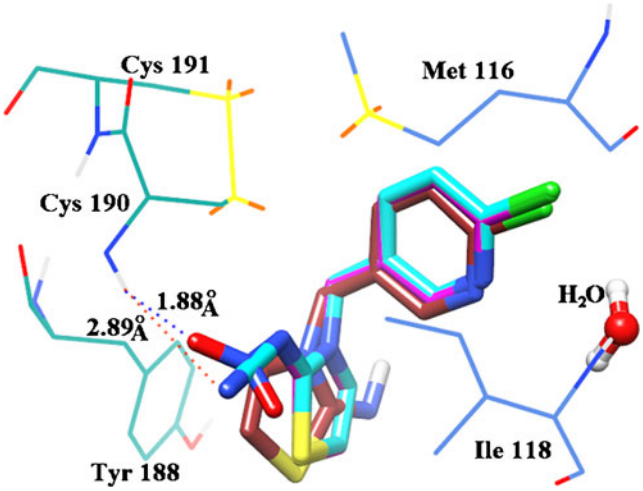


Fig. 3 The hydrogen bond force between the electronegative pharmacophore of neonicotinoids and the amino acid residue Cys190 on the (+)-face subunit of *Ac*-AChBP. The pharmacophore of compounds a1 (magenta), a2 (cyan), a3 (brown) are -NO_2, -CN, =NH, respectively. The = NH group of compound a3 does not form hydrogen bond with Cys190NH of *Ac*-AChBP

difference of the pharmacophore -NO_2 and -CN. The hydrogen bond length (1.88 Å) between the electronegative pharmacophore of the compound a1 and Cys190NH of AChBP pocket was found to be much shorter than that of compound a2 (2.89 Å) in Fig. 3. It was clear that the hydrogen bond force is very important for the binding ability between the neonicotinoid compounds and the nAChR target, especially the orientation of the electronegative pharmacophore. To further clarify the fact, two other neonicotinoid compounds with different pharmacophores -NO and -C(O)H were also docked in the AChBP binding pocket in our study. The calculated results indicated that the O atom of the compound with the -NO pharmacophore maintained the same orientation to interact with its target AChBP compared with that of the compound with the -NO_2 pharmacophore. It was in agreement with the QSAR results of cis-neonicotinoid derivatives that only one O atom of the -NO_2 pharmacophore was a great contribution to the bioactivity reported by Duan *et al*. [31], which revealed the essential reason that the -NO analogues had the same binding affinity of the -NO_2 compounds [4]. The O atom orientation of the -C(O)H compound was found to be slightly different from that of the -NO_2 compound by the docking simulation, which caused a slightly lower bioactivity of the -C(O)H neonicotinoid analogue than those of the -NO_2 and the -NO neonicotinoid compounds [4]. Thus it is confirmed that the orientation of the electronegative pharmacophore for the neonicotinoids would greatly influence the strength of the hydrogen binding interaction with insect target nAChR to further lead to the insecticide activity difference of the neonicotinoids to a great extent.

Π–Π stacking effect of the conjugation guanidine or amidine group

The Π–Π stacking effect also contributed to a more stable binding conformation between the neonicotinoids and its target nAChR. To explore the conjugate area influence by the guanidine or amidine plane on the binding affinity between the neonicotinoids and AChBP, six typical neonicotinoid compounds were docked in the target AChBP binding pocket. As Fig. 4 shows, the conjugate guanidine or amidine plane of the neonicotinoids was found to interact with an aromatic side chain of the residue Tyr188 on the (+)-face of AChBP through a Π–Π stacking effect. The binding conformations of compounds b3 and b4 overlapped very well in the AChBP pocket expected for the different C_1 and C_2 moiety. The compound b4 with the C_1=C_2 moiety showed a larger coplanar area than the compound b3 with the C_1-C_2 moiety in the amidine plane, which increased the whole ring conjugation. The bigger conjugated system shortened the centroid distance of two stacked planes to 4.00 Å in the compound b4 from 4.26 Å in the compound b3 to further enhance the Π-Π stacking effect. The binding affinities of compounds b2 (*Ki*=0.85 nM), b4 (*Ki*=0.35 nM) and b6 (*Ki*=83 nM) with the C_1=C_2 moiety were reported to be markedly higher than those of the corresponding

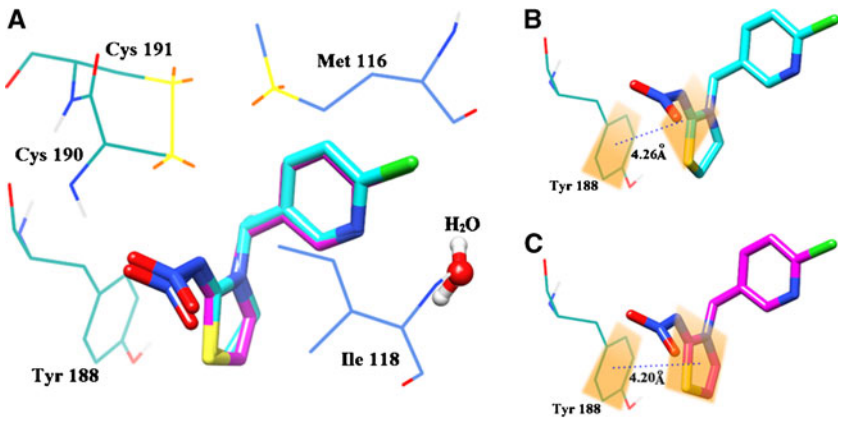


Fig. 4 The Π-Π stacking effect mode between neonicotinoids and the amino acid residue Tyr188 on the (+)-face subunit of *Ac*-AChBP. **A**:The superposition conformation of compounds b3 (cyan) and b4 (magenta) in the binding pocket of *Ac*-AChBP. **B**/**C**:The Π-Π stacking area and distance between compound b3/b4 and the amino acid residue Tyr188 of *Ac*-AChBP

compounds with the C_1-C_2 moiety (b1 *Ki*=2.2 nM, b3 *Ki*= 3.31 nM and b5 *Ki*=160 nM), respectively. Recently, the neonicotinoid analogues bearing a 1, 4-dihydropyridine scaffold were designed to enhance the Π–Π stacking effect of the guanidine group and their bioassay tests showed that most of the compounds with a larger coplanar area exhibited high insecticidal activities [32]. Therefore, it was deduced that the guanidine or amidine plane with a larger conjugation area for the neonicotinoids should enhance the Π-Π stacking effect with the aromatic residue Tyr188 of the AChBP pocket and then increase the binding affinity of the neonicotinoid compounds. Moreover, the coplanarity and conjugation of the amidine moiety were also reported by Qian *et al.* to favorably increase the hydrogen bonding strength between the electronegative pharmacophore of the neonicotinoids and Cys190NH of the (+)-face for AChBP [33]. In short, it was proposed that a more aligned guanidine or aminide plane conjugation should be considered to improve the insecticidal activity in further structural modification of the neonicotinoid compounds.

Halogen bonding interaction of the CPM

The CPM is an important functional group with the insecticide activity not only in neonicotinoids but also in nicotinoids. 5-azido-CPM neonicotinoids were usually synthesized as the photoaffinity probe to label the critical amino acid residues in the ligand-binding site of nAChR [5]. However, the introduction of the azide group was reported to reduce the binding affinity compared with the nonazido parent compounds. To clarify the essential molecular mechanism, the binding interaction of the nonazido and the 5-azido-CPM neonicotinoid compounds with the target AChBP pocket were studied by a Surflex-Docking algorithm. For three nonazido neonicotinoids, it was found that the distances between the CPM Cl atom and Met116O of the AChBP pocket were 3.18, 3.16 and 3.15 Å for c1, c2 and c3, respectively, which were all shorter than the sum of their van der Waals radius 3.27 Å. Meanwhile, the C-Cl···O angles were reported to approximately 160º and the Cl···O-Y angles were more or less 110°. Therefore, the molecular interaction between the CPM Cl atom of nonazido neonicotinoids and Met116O of the target AChBP should be attributed to a novel halogen bonding interaction in our study. This kind of the novel halogen bonding interaction is equivalent to the better-known hydrogen bond force and stronger than the traditional van der Waals force in strength. In addition, as shown in Fig. 5, the CPM N atom of the nonazido neonicotinoid c1 formed a water bridge with the backbone of the residue Ile118 on the (−)-face of AChBP. However, the introduction of an azido substituent group caused the binding conformation of the pyridine moiety to be markedly rotated for the 5-azido-CPM neonicotinoids c2. The distance between the CPM Cl atom and

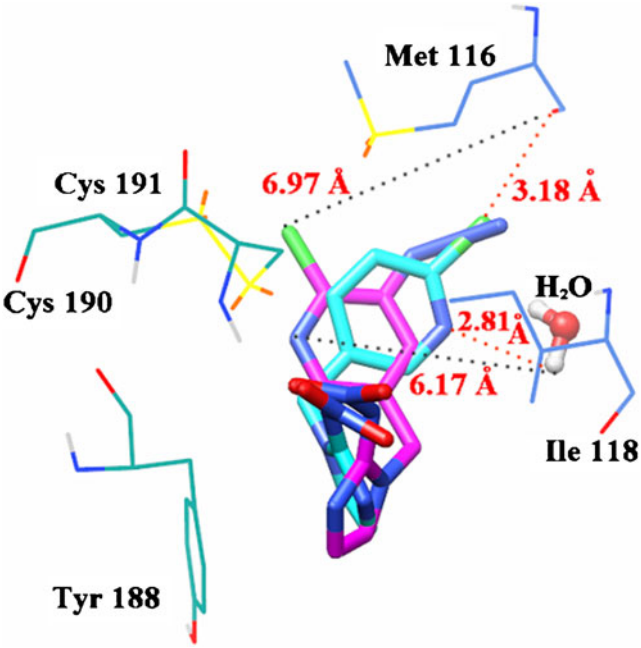


Fig. 5 The conformation superposition of 5-azido-CPM neonicotinoid c2 and its nonazido parent neonicotinoid c1 bound to *Ac*-AChBP. Neonicotinoid compounds c2 and c1 are colored magenta and cyan, respectively

the Met116O of the AChBP pocket was increased to 6.97 Å in the compound c2 from 3.18 Å in the compound c1 in Fig. 5. It was obvious that the halogen bonding interaction between the Cl atom of the 5-azido-CPM neonicotinoids and Met116O of the target AChBP already disappeared completely because of the larger spatial effect of the azide group. As Fig. 5 shows, the distance between the pyridine N and the water molecule also became much longer to 6.17 Å in compound c2 from 2.81 Å in compound c1. It indicated that the hydrogen bond force becomes markedly weak in the 5-azido-CPM neonicotinoid due to the introduction of the azide group. It was clear that the introduction of the azide group to the aromatic heterocycle CPM in the neonicotinoids would weaken the binding ability to the target nAChR because of the disappearance of the halogen bond and the water bridge interaction. In sum, the CPM as an essential functional group, particularly the halogen bond character, was favorable to the insecticide activity for neonicotinoid compounds and should be maintained in the later neonicotinoid molecular design.

New molecule design based on the halogen bonding interaction

It is understood that the binding mode between ligands and the receptor can provide a great significant guidance to design new compounds with high potency. As a novel molecular interaction in biomolecules, the halogen bonding force was

found to play an important part in the binding of the neonicotinoids and the AChBP in our study. Therefore, a series of compounds were designed based on the halogen bond interaction to discovery some novel neonicotinoid compounds. All the new designed compounds were docked into the AChBP receptor to predict their potential bioactivities. As shown in section E of Fig. 1, e1, e2 and e3 with 6-fluoropyridine-3-yl, 6-bromopyridine-3-yl and 6-iodopyridine-3-yl, respectively, were design based on b2 with a high insecticidal activity. It was found that the Br atom of e2 interacted with Met116O of AChBP with $d_{Br\text{-}O}$=3.10 Å, θ_1=157°, and θ_2=117° by a novel halogen bonding interaction. And e2 was predicted to exhibit a higher potency with score 5.57 than b2 with score 5.16. It is reported that Br···O halogen bond is not only very significant in the ligand binding and recognition, [34, 35], but also is the strength of the Br halogen bond usually much stronger than that of the Cl halogen bond [29, 36]. As one of the recently reported σ-hole bonding, the halogen-bonding interaction was confirmed to increase with the halogen atom X from Cl to I because they are less electronegative and more polarizable [37, 38]. Therefore, it was believed that e2 with Br substituted group is a great potential neonicotinoid candidate with a high bioactivity owing to a strong Br···O halogen bonding interaction. e3 with 6-iodopyridine-3-yl showed a relatively low potency with score 4.25. It may be that the steric effect of the iodine atom led to the disappearance of the halogen bond with $d_{I\text{-}O}$=6.13 Å in our study. In addition, it may also be that a larger desolvation penalty of the C–I bond resulted in the weakening of the halogen bond as Murray *et al.* reported [39].

e1 with 6-fluoropyridine-3-yl was also predicated to be a relatively high potency with score 4.95. Surprisingly, it was a hydrogen bond force to be found between the F atom of e1 and H_2O of AChBP, instead of a halogen bonding interaction. To further verify the truth, g1, g2 and g3 were designed based on b2 with 6-(trifluoromethyl)-3-pyridinyl, 6-aminopyridine-3-yl, and 6-hydroxypyridine-3-yl, respectively. g1 with -CF_3 was found to exhibit a much higher predicted potency with score 6.03 than b2, because of a strong hydrogen bond force between the F atom of g1 and Ile118NH of AChBP. Recently, sulfoxaflor with 6-(trifluoromethyl)- 3-pyridinyl was firstly reported to exhibit a higher insecticidal activity than the sulfoxaflor analogue with 6-chloropyridin-3-yl on the sap-feeding insects [40]. It was further confirmed that the neonicotinoids g1 with -CF_3 is likely to be an excellent insecticide candidate in the future. In addition, the potencies of g2 with score 5.10 and g3 with score 5.02 were both equal to that of b2. In fact, -NH_2 of g2, as a hydrogen bond donor, interacted with Met116O of AChBP and –OH of g3 as a hydrogen bond accepter formed a hydrogen bond with Ile118NH of AChBP. It was believed that introducing hydrogen bond donor/accepter into the pyridine-3-yl was a good strategy as well as forming a halogen bond for the neonicotinoid molecular design. Briefly, to design a new type of neonicotinoids with high insecticidal activity, a halogen atom or a hydrogen bond acceptor/donor is a top priority to be introduced into the pyridine moiety of neonicotinoids.

Conclusions

Aplysia californica AChBP served as a suitable structural surrogate for insect nAChR at present. The molecular binding mode of neonicotinoids and its target surrogate *Ac*-AChBP has been studied using the Surflex-Dock algorithm. The electronegative pharmacophore of the neonicotinoids interacted with the Cys190NH of the (+)-face for AChBP by the hydrogen bond force. The orientation of the tip atom O or N of the important pharmacophore would influence the neonicotinoid binding affinity to insect nAChR. The conjugate guanidine or amidine plane of the neonicotinoids showed a Π–Π stacking effect with the aromatic side chain of the amino residue Tyr188 of the AChBP pocket. Increased the coplanarity and conjugation not only improved this Π–Π stacking effect but also increased indirectly the hydrogen bonding strength between the electronegative pharmacophore and Cys190NH of the (+)-face of AChBP. In addition, the CPM N atom of the neonicotinoids formed a hydrogen bond force by a water bridge with the backbone of the residue Ile118 of AChBP. It was clear that the important functional groups of the neonicotinoids including the electronegative pharmacophore, the coplanar guanidine or amidine and the CPM all directly or indirectly contributed to the hydrogen binding interaction. Surprisingly, a novel halogen bonding interaction was firstly found between the CPM Cl atom of the neonicotinoids and Met116O for AChBP, which greatly contributes to the binding interaction between the neonicotinoids and its target. In general, a novel halogen bonding interaction and the crucial hydrogen bond force coexist and cooperate in the molecular recognition process of the neonicotinoids and its target nAChR. New compounds were designed based on the binding mode between the neonicotinoids and the target nAChR. Compunds e2 and g1 with 6-bromopyridine-3-yl and 6-(trifluoromethyl)-3-pyridinyl, respectively, were found to be good candidates with high activities as a result of the strong halogen bonding interaction and the hydrogen bond force. This research should provide some significant guidance and reference for the design and optimization of the neonicotinoid compounds to further enhance their insecticide activity.

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