JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Receptor Structure-Guided Neonicotinoid Design

Motohiro Tomizawa,*,§ Shinzo Kagabu,§ and John E. Casida#

§Faculty of Education, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

#Environmental Chemistry and Toxicology Laboratory, Department of Environmental Science, Policy and Management, University of California, Berkeley, California 94720-3112

ABSTRACT: Neonicotinoid agonists with a nitroimino pharmacophore are used worldwide for crop protection and animal health care. Chemical and structural biology investigations on the nicotinic acetylcholine receptor structure in the neonicotinoid-bound state revealed a unique niche beyond the nitro oxygen tip toward the loop D subsite. The nitroimino pharmacophore can be replaced to suitably fit the newly recognized cavity by acylimino [=NC(O)R] and phenoxycarbonylmino [=NC(O)OPh] variants. The =NC(O)R analogues, where R is a hydrogen acceptor pyridine, pyrazine, or trifluoromethyl, showed high receptor potency, suggesting that the extended pharmacophore undergoes hydrogen bonding with the loop D Arg basic residue. The =NC(O)OPh analogues had appreciably higher affinity with an electron-donating substituent on the phenyl ring than with an electron-withdrawing group, predicting that the benzene plane and loop D Trp indole form a face-to-edge aromatic interaction. These studies illustrate strategic ligand design combining the chemorational approach with the three-dimensional receptor structure.

KEYWORDS: neonicotinoid, neonicotinoid pharmacophore, nicotinic acetylcholine receptor, receptor structure-guided insecticide design

INTRODUCTION

Neonicotinoids, represented by imidacloprid (IMI) and thiacloprid (THIA) bearing nitro- and cyanoimino pharmacophores, respectively (Figure 1), are extensively utilized as systemic insecticides for crop protection against piercing−sucking insect pests, currently accounting for over one-fifth of the world insecticide market.[1, 2] Neonicotinoids act as selective agonists at the insect nicotinic acetylcholine receptor (nAChR), combining excellent insecticidal effectiveness with minimal risk to people and wildlife.[3, 4] Neonicotinoid binding site interactions at the chemical or atomic scale have been resolved by photoaffinity labeling and X-ray crystallography approaches using mollusk acetylcholine-binding protein, which is an appropriate structural surrogate of the extracellular ligand-binding domain of the nAChRs.[5−10] The defined binding site structure in the neonicotinoid-bound state consequently facilitates the molecular design of novel insecticidal nicotinic compounds with unique pharmacophore(s), undergoing distinct binding mechanism(s), which may expand the insecticidal spectrum of the present neonicotinoids and circumvent the possible resistance caused by activated detoxification systems [11] and modified target sites.[12] The present paper describes our studies on nicotinic ligands with novel chemotype pharmacophores based on combining the chemorational approach with the receptor binding site structure. We have identified several candidate compounds with excellent insecticidal effectiveness rivaling those of the present neonicotinoids and other classes of commercial insecticides.

STRATEGY FOR PHARMACOPHORE MODIFICATION

Prospect for Neonicotinoid Pharmacophore Modification. The distinctive molecular feature of neonicotinoids is an electronegative nitro- or cyanoimino moiety (Figure 1), which is coplanar with the guanidine or amidine plane, yielding electronic

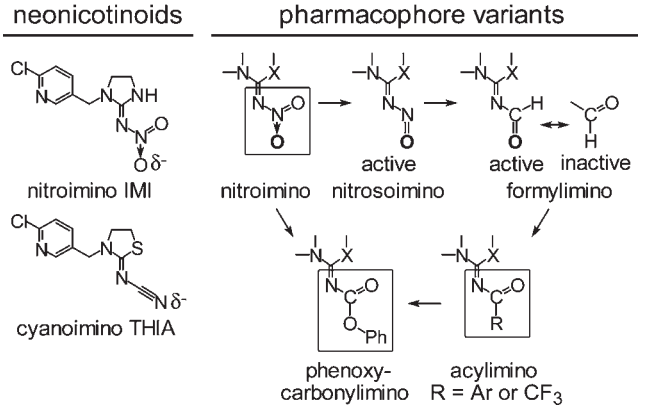

Figure 1. Neonicotinoid insecticides IMI and THIA with nitro- and cyanoimino moieties, respectively, and systematic variants of the electronegative pharmacophore. The nitroso oxygen tip has a predominantly stable conformation, enabling H-bonding with the receptor subsite (active),[15] whereas the formyl moiety possibly takes two contradictory conformations in the direction of the oxygen tip (active and inactive). Thus, the flexibility of the tip oxygen orientations in the binding pocket may determine the final binding constant as an average.[16]

conjugation to facilitate partial negative charge (δ^-) flow toward the tip, in this manner enabling hydrogen-bonding and π-stacking with the receptor subsites.[5,8,13−15] Interestingly, the nitrosoimino (=NNO) analogues retain the receptor potency of

Special Issue: Strategic Molecular Design of Neonicotinoid Insecticides

Received: June 30, 2010
Revised: September 2, 2010
Accepted: September 8, 2010
Published: September 27, 2010

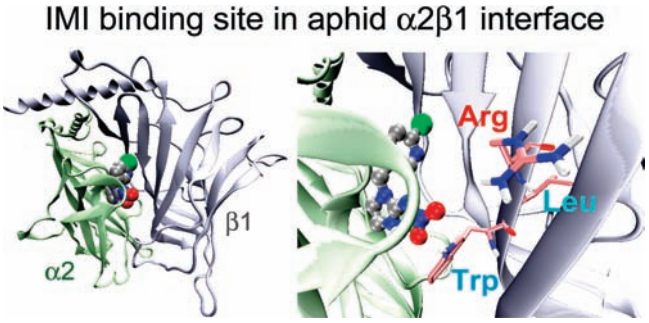

Figure 2. Aphid nAChR structural model in IMI-bound state reveals a unique niche extending from the bound IMI nitro tip oxygen toward the loop D Arg. IMI is docked into the aphid (*Myzus persicae*) α2β1 interfacial agonist-binding pocket[7] (left). Three amino acid residues (Trp, Leu, and Arg) on the β1 subunit make up the loop D cavity (right). The loop D Arg on the aphid β1 subunit is spatially equivalent to Thr with a shorter side chain on the vertebrate β2 subunit.

the nitroimino compounds, thereby defining the functional tip oxygen. However, the formylimino [=NC(O)H] congeners showed greatly reduced potency. The clear potency difference between the two functional groups [=NNO versus =NC(O)H] can be attributable to their pharmacophore orientations. The =NNO tip oxygen substantially faces the descending direction (active form) for H-bonding formation with the subsite. In contrast, the =NC(O)H oxygen possibly takes two flexible directions under biological conditions, that is, the alternative upward oxygen orientation (inactive form) and the active one as with the =NNO tip (Figure 1).[15,16] Thus, the direction of the oxygen tip presumably determines the binding constant of these pharmacophore types. However, the =NC(O)H moiety can be replaced by extended and hydrophobic substituents, conferring an incentive to explore novel chemotype pharmacophores.

Neonicotinoid-Bound Receptor Structure Reveals a Unique Niche. The IMI binding site is located at an interfacial region between α and β subunits of the insect nAChR (Figure 2). The neonicotinoid nitro oxygen or cyano nitrogen tip H-bonds to the loop C Cys (and/or adjacent one) backbone on the α subunit face (not shown).[5,7,8,10] The IMI-receptor structure, intriguingly, reveals a unique niche extending from the bound IMI nitro tip oxygen toward the loop D Arg on the β subunit. This space provides room for one aromatic ring structure. Moreover, the loop D Arg on the insect β subunit is spatially equivalent to Thr with a short side chain on the β2 subunit of the vertebrate neuronal nAChR, suggesting a difference between insect and vertebrate loop D cavities in their depths and functional residues. Therefore, these observations in the chemorational aspect and the receptor binding site structure mutually lead to designing prototype analogues with extended acylimine and phenoxycarbonylimine functional groups proposed to specifically bind to the loop D region of the insect nAChRs.[17]

■ ACYLIMINO PHARMACOPHORE

Structure−Activity Relationship (SAR). Replacement of the nitro- or cyanoimino pharmacophore by extended substituents may provide point(s) for hydrogen acceptance and/or van der Waals contacts at the targeted binding domain (loop D). According to this hypothesis, prototype benzoylimino, pyridinoylimino, and pyrazinoylimino derivatives (Figure 3) were prepared to evaluate the binding potency.[17] The benzoyl compound showed moderate potency, thus indicating that an aromatic ring can be accommodated into the target niche. The 3-pyridinoyl analogue with an H-acceptor nitrogen atom had a much higher affinity than that of the benzoyl compound. This enhancement was accentuated with the pyrazinoyl analogue with two H-acceptor nitrogen atoms.[17] Therefore, these observations clearly underscore that H-accepting nitrogen atoms play a crucial role in recognition by the amino acid(s) at the target subsite. Astonishingly, the trifluoroacetylimino analogue, providing both van der Waals contacting and H-bonding abilities, exhibited high affinity comparable to those of IMI and THIA, and the illustrative acylimino compounds with pyrazine and CF_3 substituents showed high intrinsic insecticidal activity rivaling those of commercial neonicotinoids.[17] Moreover, the trifluoroacetyl neonicotinoid has unique biological properties attributable to the enhanced hydrophobicity (discussed later).[18]

Target Site Selectivity. Selectivity of the pyrazinoyl and trifluoroacetyl analogues was determined by comparing receptor potency at the insect and vertebrate nAChRs and toxicity to flies and mice (Table 1).[17] As with IMI and THIA, the two illustrative compounds had low potency at the vertebrate α4β2 receptor and low toxicity to mice. In addition, the binding affinities of the pyrazinoyl and trifluoroacetyl analogues to a hybrid receptor, consisting of the aphid (*Myzus*) α2 subunit and the rat β2 subunit, were much lower than those of IMI and THIA,[17,19] strongly indicating that the insect α subunit plays a critical role in the recognition of IMI and THIA with nitro- and cyanoimino moieties. In sharp contrast, the insect β subunit with loop D Arg (or functionally analogous amino acid) is clearly important for embracing the extended pyrazinoylimine or trifluoroacetylimine moiety. Accordingly, the difference between insect and vertebrate loop D niches in their functional amino acid residues presumably serves as a determinant for the target site selectivity of the acylimino neonicotinoids, although the selectivity mechanism for IMI and THIA depends on the multiple binding conformations.[7]

Binding Site Interactions. In a molecular dynamics simulation of the insect nAChR structural model liganded with the pyrazinoylimino analogue, two pyrazine nitrogen atoms and the =NC(O) oxygen undergo H-bonding with the loop D Arg guanidine NH_2 and Trp indole NH (Figure 3). Similarly, the trifluoroacetylimino compound variously interacts with loop C and D regions: that is, the fluorine atoms hydrogen bond to loop D Arg and Trp (directly or possibly via water bridges) and to loop C Cys and also make van der Waals contact with the Trp side chain; the NC(O) oxygen H-bonds with the Trp indole NH.[17] Binding mechanisms of the chloropyridine and amidine moieties are identical to those of IMI and THIA.[5,7,10] Accordingly, the in silico binding site interactions are unambiguously consistent with the observed SAR.

■ PHENOXYCARBONYLIMINO VARIANT BINDING MECHANISM

Neonicotinoids with the phenoxycarbonylimino variant may take a dissimilar binding conformation to that of acylimino analogues due to the flexibility of the *O*-hinged (rotatable) phenyl ring. This suggests that the biologically active geometry of the phenoxy moiety could ultimately be determined by the binding subsite.[16] The substituted-phenoxycarbonylimino analogues (Figure 3) in the thiazoline series were thus synthesized to define the SAR and subsequently predict the binding site

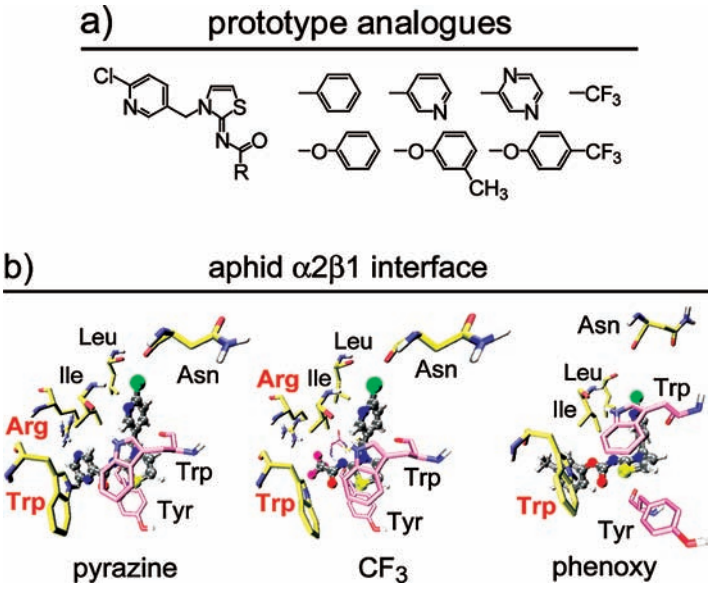

Figure 3. Prototypical neonicotinoid ligands proposed to fit the loop D niche of the insect nAChR and the predictive binding site interactions: (a) illustrative analogues with acylimino and phenoxycarbonylimino pharmacophores; (b) structural models for binding site interactions of neonicotinoids with pryazine, CF_3, and phenoxy (with 3-methyl substituent) moieties with the α2-β1 subunit interfacial agonist-binding pocket of the aphid (*Myzus*) nicotinic receptor.[17,20] Amino acids in pink or yellow are from the α2 or β1 subunit, respectively. In the model for the CF_3 compound, two Cys residues near the CF_3 head are not labeled because this would visually obscure the other atoms. The chloropyridine chlorine undergoes van der Waals interactions with the loop E Asn and Leu, and the nitrogen atom forms a water bridge to the loop E Ile and Asn. The amidine plane primarily π-stacks with the loop C Tyr and secondarily with the loop B Trp.

Table 1. Selectivity of Neonicotinoids with Nitro- or Cyanoimino and Acylimino Pharmacophores between Insects and Vertebrates

	binding to nAChRs, IC_{50} (nM)			toxicity, LD_{50} (mg/kg)	
compound	*Drosophila* native[a]	hybrid aphid α2/rat β2[a]	chick α4β2[b]	housefly[c]	mouse[d]
IMI	4.3	3.6	2600	0.021	45
THIA	2.7	10	860	0.032	30
pyrazine	1.5	720	900	0.035	>24 (0%)[e]
CF_3	3.1	280	500	0.027	>36 (0%)[e]

[a] Assayed with [^{3}H]IMI. [b] Assayed with [^{3}H]nicotine. [c] Intrathoracic injection into houseflies (*Musca domestica*) pretreated with a cytochrome P450 inhibitor [*O*-propyl *O*-(2-propynyl) phenylphosphonate] (defined as intrinsic insecticidal activity). [d] Intraperitoneal administration. [e] Percent lethality at the indicated dose (maximal dose administered due to solubility limitations in the vehicle).

Table 2. Summarized SAR of Trifluoroacetyl Neonicotinoid Insecticides[a]

	binding to nAChR	toxicity to houseflies, LD_{50} (μg/g, female)		hydrophobicity
pharmacophore	K_i (nM)[b]	injection (synergist)[c]	topical (alone)[d]	log P[e]
NNO_2 and NCN	2.2−25	0.02−0.1	>100	0.5−1.2
$NC(O)CF_3$	2.5−31	0.02−0.2	1.1−17	1.9−3.1
$CHC(O)CF_3$	0.5−1.5	0.004−0.01	0.8−3.8	1.7−2.1

[a] Based on Ohno et al.[18] [b] Housefly brain nAChR assayed with [^{3}H]IMI. [c] Intrinsic insecticidal activity evaluated by intrathoracic injection route with a cytochrome P450 inhibitor (synergist) pretreatment. [d] Administered by topical application in the absence of a synergist. Chlorpyrifos as a standard insecticide had a LD_{50} value of 1.9 μg/g, female, as compared with those of other important insecticides propoxur, dieldrin, and DDT of 23, 0.7, and 14 μg/g, female, respectively. [e] Log *P* coefficients between 1-octanol and water.

interactions at the insect nAChR. The unsubstituted phenoxy compound showed high receptor potency, which was reinforced by introducing an electron-donating 3-methyl substituent and greatly reduced by substitution with an electron-withdrawing 4-CF_3. Therefore, the π-electron density on the benzene ring plays a key role in the interaction with a regional binding domain.[16,20] The receptor potency of the 3-methylphenoxy compound was equal to that of IMI, although it was much less toxic to flies, conceivably due to metabolic lability (hydrolysis). Finally, the SAR results predict binding site interactions featuring the phenoxy ring of the neonicotinoid and the receptor loop D Trp indole plane forming a T-shaped aromatic interaction[16,20] (Figure 3).

The face-to-edge aromatic interaction can provide as much stabilization as the more standard π-stacking.[21,22]

TRIFLUOROACETYL ANALOGUES WITH ENHANCED HYDROPHOBICITY AND EFFECTIVENESS

Neonicotinoids generally have low log *P* values (from −0.7 to 1.3), leading to exceptional plant-systemic properties.[2,4] An exhaustive SAR study of neonicotinoid analogues with a $=NC(O)CF_3$ or $=CHC(O)CF_3$ substituent was conducted to examine the hypothesis that the trifluoroacetyl pharmacophore enhances the hydrophobicity and thereby confers the improved insecticidal effectiveness relative to those of the standard nitro or cyano neonicotinoids.[18] The $=NC(O)CF_3$ analogues were highly active both in receptor binding and in intrinsic insecticidal potencies comparable to those of the $=NNO_2$ and =NCN compounds[15,17,18] (Table 2). The $=CHC(O)CF_3$ analogues showed outstanding insecticidal activity associated with superior receptor potency. Surprisingly, the compounds with $=NC(O)CF_3$ and $=CHC(O)CF_3$ pharmacophores were excellent insecticides on topical application without synergist treatment, rivaling the potency of other standard insecticides. In contrast, the corresponding $=NNO_2$ or =NCN neonicotinoids were entirely inactive in the same condition. The $=NC(O)CF_3$ and $=CHC(O)CF_3$ substituents greatly enhance the hydrophobicity of nitro- or cyanoimino neonicotinoids.[18] Therefore, the increased hydrophobicity of trifluoroacetyl neonicotinoids presumably improves the penetrability of the compound through the insect integument and insecticidal effectiveness. In addition, the $=NC(O)CF_3$ neoncotinoid appears to retain adequate photostability, yet the $=CHC(O)CF_3$ analogue is conceivably photolabile.[18]

CONCLUDING REMARKS

Several insecticidal acylimino and $=CHC(O)CF_3$ analogues of the types discussed in this review were known prior to the current study and are used here to illustrate the approach of receptor structure-guided neonicotinoid design. Outstandingly potent and selective nicotinic insecticides can be achieved by pharmacophore modification of neonicotinoids providing extended and/or hydrophobic substituents specifically fitting the unique niche of the insect nAChR in a very different way from the nitroimino IMI or cyanoimino THIA. Consequently, the strategy illustrated may expedite receptor structure-guided ligand design for discovery of novel insecticides with high performance and unique biological properties.

AUTHOR INFORMATION

Corresponding Author
*E-mail: mtomizaw@gifu-u.ac.jp.

ACKNOWLEDGMENT

M.T. and S.K. are deeply indebted to Dr. Satoru Kumazawa of Kureha Corp. for his support. J.E.C. holds the William Muriece Hoskins Chair in Chemical and Molecular Entomology of the University of California at Berkeley.

ABBREVIATIONS USED

IMI, imidacloprid; log *P*, logarithm of the partition coefficient of the compound between 1-octanol and water; nAChR, nicotinic acetylcholine receptor; SAR, structure−activity relationship; THIA, thiacloprid.

REFERENCES

(1) Kagabu, S. Molecular design of neonicotinoids: past, present, and future. In *Chemistry of Crop Protection*; Voss, A., Ramos, G., Eds.; Wiley-VCH: Weinheim, Germany, 2003; pp 193−212.

(2) Jeschke, P.; Nauen, R. Neonicotinoids − from zero to hero in insecticide chemistry. *Pest Manag. Sci.* **2008**, *64*, 1084–1098.

(3) Matsuda, K.; Shimomura, M.; Ihara, M.; Akamatsu, M.; Sattelle, D. B. Neonicotinoids show selective and diverse actions on their nicotinic receptor targets: electrophysiology, molecular biology, and receptor modeling studies. *Biosci., Biotechnol., Biochem.* **2005**, *69*, 1442–1452.

(4) Tomizawa, M.; Casida, J. E. Neonicotinoid insecticide toxicology: mechanisms of selective action. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 247–268.

(5) Tomizawa, M.; Talley, T. T.; Maltby, D.; Durkin, K. A.; Medzihradszky, K. F.; Burlingame, A. L.; Taylor, P.; Casida, J. E. Mapping the elusive neonicotinoid binding site. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 9075–9080.

(6) Tomizawa, M.; Maltby, D.; Medzihradszky, K. F.; Zhang, N.; Durkin, K. A.; Presley, J.; Talley, T. T.; Taylor, P.; Burlingame, A. L.; Casida, J. E. Defining nicotinic agonist binding surfaces through photoaffinity labeling. *Biochemistry* **2007**, *46*, 8798–8806.

(7) Tomizawa, M.; Maltby, D.; Talley, T. T.; Durkin, K. A.; Medzihradszky, K. F.; Burlingame, A. L.; Taylor, P.; Casida, J. E. Atypical nicotinic agonist bound conformations conferring subtype selectivity. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 1728–1732.

(8) Talley, T. T.; Harel, M.; Hibbs, R. E.; Radić, Z.; Tomizawa, M.; Casida, J. E.; Taylor, P. Atomic interactions of neonicotinoid agonists with AChBP: molecular recognition of the distinctive electronegative pharmacophore. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 7606–7611.

(9) Tomizawa, M.; Talley, T. T.; Park, J. F.; Maltby, D.; Medzihradszky, K. F.; Durkin, K. A.; Cornejo-Bravo, J. M.; Burlingame, A. L.; Casida, J. E.; Taylor, P. Nicotinic agonist binding site mapped by methionine- and tyrosine-scanning coupled with azidochloropyridinyl photoaffinity labeling. *J. Med. Chem.* **2009**, *52*, 3735–3741.

(10) Tomizawa, M.; Casida, J. E. Molecular recognition of neonicotinoid insecticides: the determinants of life or death. *Acc. Chem. Res.* **2009**, *42*, 260–269.

(11) Karunker, I.; Morou, E.; Nikou, D.; Nauen, R.; Sertchook, R.; Stevenson, B. J.; Paine, M. J. I.; Morin, S.; Vontas, J. Structural model and functional characterization of the *Bemisia tabaci* CYP6CM1vQ, a cytochrome P450 associated with high levels of imidacloprid resistance. *Insect Biochem. Mol. Biol.* **2009**, *39*, 697–706.

(12) Liu, Z.; Williamson, M. S.; Lansdell, S. J.; Denholm, I.; Han, Z.; Millar, N. S. A nicotinic acetylcholine receptor mutation conferring target-site resistance to imidacloprid in *Nilaparvata lugens* (brown planthopper). *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 8420–8425.

(13) Kagabu, S.; Matsuno, H. Chloronicotinyl insecticides. 8. Crystal and molecular structures of imidacloprid and analogous compounds. *J. Agric. Food Chem.* **1997**, *45*, 276–281.

(14) Tomizawa, M.; Lee, D. L.; Casida, J. E. Neonicotinoid insecticides: molecular features conferring selectivity for insect versus mammalian nicotinic receptors. *J. Agric. Food Chem.* **2000**, *48*, 6016–6024.

(15) Tomizawa, M.; Zhang, N.; Durkin, K. A.; Olmstead, M. M.; Casida, J. E. The neonicotinoid electronegative pharmacophore plays the crucial role in the high affinity and selectivity for the *Drosophila* nicotinic receptor: an anomaly for the nicotinoid cation−π interaction model. *Biochemistry* **2003**, *42*, 7819–7827.

(16) Ohno, I.; Tomizawa, M.; Durkin, K. A.; Naruse, Y.; Casida, J. E.; Kagabu, S. Molecular features of neonicotinoid pharmacophore variants interacting with the insect nicotinic receptor. *Chem. Res. Toxicol.* **2009**, *22*, 476–482.

(17) Tomizawa, M.; Kagabu, S.; Ohno, I.; Durkin, K. A.; Casida, J. E. Potency and selectivity of trifluoroacetylimino and pyrazinoylimino

nicotinic insecticides and their fit at a unique binding site niche. *J. Med. Chem.* **2008**, *51*, 4213–4218.

(18) Ohno, I.; Tomizawa, M.; Aoshima, A.; Kumazawa, S.; Kagabu, S. Trifluoroacetyl neonicotinoid insecticides with enhanced hydrophobicity and effectiveness. *J. Agric. Food Chem.* **2010**, *58*, 4999–5003.

(19) Tomizawa, M.; Millar, N. S.; Casida, J. E. Pharmacological profiles of recombinant and native insect nicotinic acetylcholine receptors. *Insect Biochem. Mol. Biol.* **2005**, *35*, 1347–1355.

(20) Ohno, I.; Tomizawa, M.; Miyazu, N.; Kushibiki, G.; Noda, K.; Hasebe, Y.; Durkin, K. A.; Miyake, T.; Kagabu, S. Structural features of phenoxycarbonylimino neonicotinoids acting at the insect nicotinic receptor. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5933–5935.

(21) McGaughey, G. B.; Gagné, M.; Rappé, A. K. π-Staking interactions. Alive and well in proteins. *J. Biol. Chem.* **1998**, *273*, 15458–15463.

(22) Bissantz, C.; Kuhn, B.; Stahl, M. A medicinal chemist's guide to molecular interactions. *J. Med. Chem.* **2010**, *53*, 5061–5084.