Bioorganic & Medicinal Chemistry Letters 20 (2010) 5933–5935

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structural features of phenoxycarbonylimino neonicotinoids acting at the insect nicotinic receptor

Ikuya Ohno [a], Motohiro Tomizawa [b], Nozomi Miyazu [b], Gohito Kushibiki [b], Kumiko Noda [b], Yasunori Hasebe [b], Kathleen A. Durkin [c], Taiji Miyake [d], Shinzo Kagabu [b,*]

[a] The United Graduate School of Agricultural Science, Gifu University, Gifu 501-1193, Japan
[b] Department of Chemistry, Faculty of Education, Gifu University, Gifu 501-1193, Japan
[c] Molecular Graphics and Computational Facility, College of Chemistry, University of California, Berkeley, CA 94720-1460, USA
[d] Research Center, Kureha Corporation, 16 Ochiai, Nishiki-machi, Iwaki, Fukushima 974-8686, Japan

ARTICLE INFO

Article history:
Received 6 May 2010
Revised 12 July 2010
Accepted 13 July 2010
Available online 16 July 2010

Keywords:
Imidacloprid
Neonicotinoid insecticides
Nicotinic receptor
Phenoxycarbonylimino pharmacophore

ABSTRACT

Substituted-phenoxycarbonylimino neonicotinoid ligands with an electron-donating group showed significantly higher affinity to the insect nicotinic receptor relative to that of the analogue with an electron-withdrawing substituent, thereby establishing in silico binding site interaction model featuring that the phenoxy ring of neonicotinoids and the receptor loop D tryptophan indole plane form a face-to-edge aromatic interaction.

© 2010 Elsevier Ltd. All rights reserved.

Neonicotinoid insecticides, represented by imidacloprid (IMI) (Fig. 1), are agonists of the nicotinic acetylcholine receptor (nAChR) and are broadly used for crop protection accounting for more than 20% of global insecticide market.[1–4] High neonicotinoid potency and selectivity toward the insect nAChR are ultimately attributable to the disparate binding site interactions which have been deciphered in chemical or atomic scale using mollusc acetylcholine binding protein (AChBP) as structural homologue of the extracellular ligand-binding domain of the nAChR.[5–9] Our illustrative studies in the nAChR structure-guided insecticide design led to identify some highly potent compounds with acylimino and aryloxycarbonylimino pharmacophores (Fig. 1), which may confer dissimilar binding mechanisms to that of IMI with the nitroimino functional group.[10–12] The present investigation discusses extensive structure–activity relationships (SARs) of the substituted-phenoxycarbonylimino neonicotinoid analogues in terms of insect nAChR binding affinity and insecticidal activity. The observed SAR findings consequently lead to predict the unique binding site interaction of the phenoxycarbonylimino pharmacophore with the loop D subsite of the receptor.

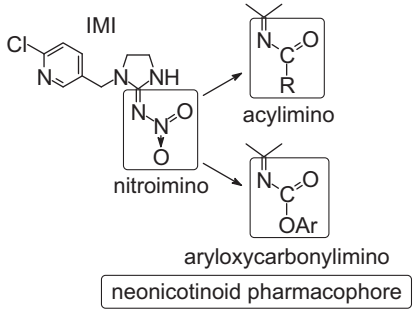


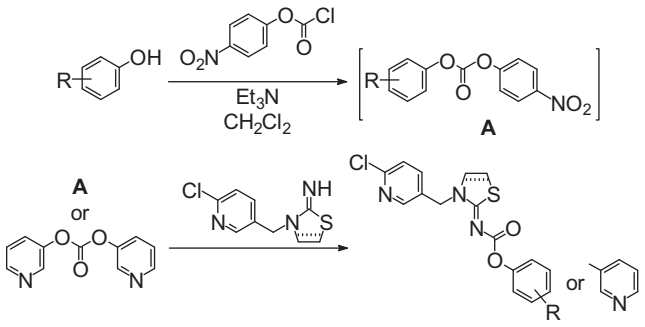
Figure 1. Neonicotinoid insecticide IMI and the pharmacophore modifications.

Compounds **1** and **16** were prepared according to the published procedure.[11] The other compounds were available by adding of 3-(6-chloropyridin-3-ylmethyl)-2-imino-1,3-thiazolidine to the *in situ* prepared carbonate by reaction of substituted phenol with 4-nitrophenyl chloroformate or di-3-pyridinyl carbonate in the presence of triethylamine (Scheme 1 and Supplementary data). The 1,3-thiazoline derivatives were similarly synthesized.

Binding affinities of phenoxycarbonylimino neonicotinoid analogues were evaluated with housefly (*Musca domestica*) brain nAChR using [^{3}H]IMI radioligand.[13,14] Among the thiazolidine analogues, unsubstituted phenoxy compound **1** had a binding affinity (K_i) 46 nM (Table 1). However, the pyridin-3-oxycarbonylimino compound (**2**) was a threefold less potent (140 nM), conceivably due to the decreased π-electron density of the pyridine. Hence the aromatic ring π-electron density may play an important role on the interaction with the regional subsite.

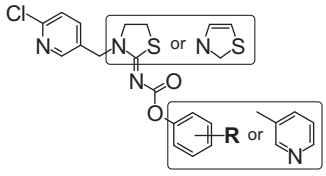
* Corresponding author. Tel.: +81 58 293 2253; fax: +81 58 293 2207.
E-mail address: kagabus@gifu-u.ac.jp (S. Kagabu).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.07.049



Scheme 1. Preparations of *N*-aryloxycarbonylimino neonicotinoid analogues.

Table 1
SARs of phenoxycarbonylimino neonicotinoid analogues in terms of insect nAChR affinity and insecticidal activity



Compound		*Musca* nAChR	Toxicity to *Musca*[b] (μg/g female)	
No.	Substituent (R)	$K_i \pm SD$[a] (nM, $n = 3$)	KD_{50} (6 h)	LD_{50} (24 h)
Thiazolidines				
1	H	46 ± 5.5	1.0	>1 (7%)[c]
2	Pyridin-3-yl	140 ± 23	>1 (13%)[c]	>1 (20%)[c]
3	2-F	150 ± 10	>1 (20%)[c]	>1 (13%)[c]
4	3-F	150 ± 14	>1 (0%)[c]	>1 (13%)[c]
5	4-F	210 ± 22	>1 (20%)[c]	>1 (20%)[c]
6	4-Cl	240 ± 45	>1 (0%)[c]	>1 (7%)[c]
7	4-NO_2	320 ± 27	>1 (0%)[c]	>1 (0%)[c]
8	4-CF_3	520 ± 25	>1 (0%)[c]	>1 (0%)[c]
9	2-CH_3	40 ± 2.2	0.12	0.63
10	3-CH_3	18 ± 2.3	0.60	>1 (20%)[c]
11	4-CH_3	44 ± 4.6	0.90	>1 (13%)[c]
12	2,4-$(CH_3)_2$	32 ± 4.0	0.16	1.0
13	2-OCH_3	33 ± 3.7	0.11	>1 (40%)[c]
14	3-OCH_3	19 ± 1.1	1.0	>1 (7%)[c]
15	4-OCH_3	57 ± 3.0	>1 (20%)[c]	>1 (18%)[c]
Thiazolines				
16	H	8.9 ± 0.1	0.08	0.52
17	3-CH_3	4.7 ± 0.2	0.08	0.22
18	3-OCH_3	5.6 ± 0.6	0.08	1.0
19	4-CF_3	180 ± 7.2	>1 (0%)[c]	>1 (20%)[c]
Control	**IMI**	4.5 ± 0.2	0.01	0.02

[a] Assayed with [^{3}H]IMI. $K_i = IC_{50}/(1 + [L]/K_d)$ with [L] 5 nM and K_d 5.4 nM.[13,14]
[b] Fifty percent knockdown (KD_{50}) and lethality (LD_{50}) doses were evaluated for 6 and 24 h, respectively, after toxicant administration via intrathoracic injection into flies, which were pretreated with a cytochrome P450 inhibitor [*O*-propyl *O*-(2-propynyl) phenylphosphonate].[15,16]
[c] Knockdown or mortality % at the indicated dose.

This hypothesis is then examined by the present SAR approach with phenoxylcarbonylimino probes with electron-withdrawing and electron-donating substituents clarifying the function of the aromatic π-electron system. First, an electron-withdrawing fluorine atom (similar in size to hydrogen atom) was introduced on 2-, 3- or 4-position, and expectedly the fluorophenoxy compounds **3**–**5** clearly lost the affinity of compound **1** by 3.3- to 4.6-fold. Compounds with 4-chloro, 4-nitro and 4-trifluoromethyl groups (**6**–**8**) also resulted in diminished affinity to a large extent presumably associated with electronic circumstance and/or possibly bulk of substituent on the phenyl ring. In contrast, substitution on 2- or 4-position by an electron-donating methyl group (**9** or **11**) had similar potency to that of unsubstituted **1**. Interestingly, 3-methylphenoxy analogue (**10**) showed a 2.6-fold higher potency than that of **1**. The 2,4-dimethyl analogue (**12**) had similar potency. The three methoxy isomers (**13**–**15**) showed the identical activity pattern to that of methyl analogues (**9**–**11**). Substitution at the 4-position and/or possibly also at the 2-position appears to affect activity perhaps due to steric clashes. On the other hand, the thiazoline analogue with an unsubstituted phenoxy (**16**) was 5.2-fold more potent relative to the thiazolidine compound (**1**), indicating that an additional π-electron system on the heterocyclic ring possibly makes a favourable interaction to the subsite. Alternatively, aromatisation of thiazoline ring may affect the conjugated electron system of the carbonylamidine moiety, leading to a better hydrogen-accepting ability of the carbonyl oxygen. The 3-methyl or 3-methoxy analogue (**17** or **18**) gave optimal affinity, rivalling that of control neonicotinoid IMI. As expected, compound **19** with a strong electron-withdrawing 4-trifluoromethyl group had a largely reduced potency. Therefore, the π-electron density on the benzene ring is decisively responsible for the interaction with a regional subsite of the receptor.

Subsequently insecticidal activity of phenoxycarbonylimino neonicotinoids to adult female flies was determined by intrathoracic injection route (Table 1).[15,16] Knockdown activity of compound is a sign of the intrinsic neurotoxicity triggered by receptor interactions, yet lethal potency is largely influenced by metabolic detoxification phase. Compounds (**1, 9–14 and 16–18**) with high nAChR affinity showed apparent knockdown effect (KD_{50}). However, the phenoxycarbonylimino analogues generally

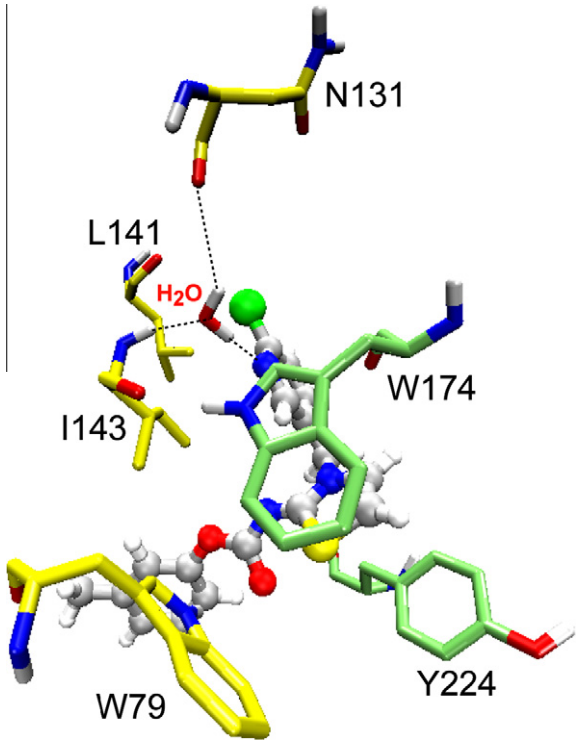


Figure 2. Structural model for binding site interactions of compound **17** with the α–β subunit interfacial agonist-binding domain of insect nAChR. The compound **17** is embraced, with an energy of −9.34 kcal/mol, by the ligand-binding pocket. Relevant amino acids in green (loop B W174; loop C Y224) are from α subunit and in yellow (loop D W79; loop E N131, L141, and I143) are from β subunit. The pyridine nitrogen atom forms a water bridge to the backbone NH of I143 and the carbonyl oxygen of N131. The neonicotinoid phenyl plane undergoes a T-shape aromatic interaction with the loop D W79 indole.

had inferior insecticidal potency (LD_{50}) relative to that of the nitroimino IMI. These observations suggest that the phenoxylcarbonylimino neonicotinoids may be intrinsically active but are more metabolically labile, conceivably due to the hydrolysis, relative to the analogue with nitroimino or acylimino group.[10,12]

A docking simulation for the most active compound **17**, 3-methylphenoxycarbonyliminothiazoline analogue, was performed with an aphid (*Myzus persicae*) α2β1 nAChR structural model[6,10,11] to examine the binding site interactions (Fig. 2).[17–24] The compound **17** was calculated to be docked favourably by the interfacial agonist-binding pocket between the *Myzus* α2 and β1 subunits. The chloropyridinyl chlorine atom can have favourable van der Waals interactions with the backbone of loop E N131 and L141. The pyridine nitrogen forms a water bridge to the backbone of loop E N131 and I143. The electronically conjugate amidine plane primarily π-stacks with the loop C Y224 aromatic side chain and also interacts via stacking or hydrophobic interactions with other aromatic residues like the loop B W174 indole moiety. Olefinic π-electrons of the thiazoline compound are sandwiched between Y224 and W174, conferring the enhanced affinity compared with that of saturated thiazolidine compound. The carbonyl and phenoxy oxygen atoms make hydrogen-bonds with the loop D W79 aromatic NH and/or loop C C226 backbone NH (not displayed). The neonicotinoid phenyl ring and the W79 indole side chain undergo a T-shaped aromatic interaction reinforced by 3-methyl substituent on the phenyl ring. The face-to-edge aromatic interaction can provide about as much stabilization as the more standard π-stacking.[25] Accordingly, the present calculation result is fully reflective of the observed SARs.

In summary, this investigation exemplifies a ligand molecular design reconciling the chemorational SAR and the receptor structure-aided approaches, stimulating further discovery of novel nicotinic insecticides with unique biological properties.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.049.

References and notes

1. Kagabu, S. In *Chemistry of Crop Protection*; Voss, A., Ramos, G., Eds.; Wiley-VCH: Weinheim, 2003; pp 193–212.
2. Matsuda, K.; Shimomura, M.; Ihara, M.; Akamatsu, M.; Sattelle, D. B. *Biosci. Biotechnol. Biochem.* **2005**, *69*, 1442.
3. Tomizawa, M.; Casida, J. E. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 247.
4. Jeschke, P.; Nauen, R. *Pest Manag. Sci.* **2008**, *64*, 1084.
5. Tomizawa, M.; Talley, T. T.; Maltby, D.; Durkin, K. A.; Medzihradszky, K. F.; Burlingame, A. L.; Taylor, P.; Casida, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 9075.
6. Tomizawa, M.; Maltby, D.; Talley, T. T.; Durkin, K. A.; Medzihradszky, K. F.; Burlingame, A. L.; Taylor, P.; Casida, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 1728.
7. Talley, T. T.; Harel, M.; Hibbs, R. E.; Radić, Z.; Tomizawa, M.; Casida, J. E.; Taylor, P. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 7606.
8. 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. *J. Med. Chem.* **2009**, *52*, 3735.
9. Tomizawa, M.; Casida, J. E. *Acc. Chem. Res.* **2009**, *42*, 260.
10. Tomizawa, M.; Kagabu, S.; Ohno, I.; Durkin, K. A.; Casida, J. E. *J. Med. Chem.* **2008**, *51*, 4213.
11. Ohno, I.; Tomizawa, M.; Durkin, K. A.; Naruse, Y.; Casida, J. E.; Kagabu, S. *Chem. Res. Toxicol.* **2009**, *22*, 476.
12. Ohno, I.; Tomizawa, M.; Aoshima, A.; Kumazawa, S.; Kagabu, S. *J. Agric. Food Chem.* **2010**, *58*, 4999.
13. Tomizawa, M.; Latli, B.; Casida, J. E. *J. Neurochem.* **1996**, *67*, 1669.
14. Zhang, A.; Kayser, H.; Maienfisch, P.; Casida, J. E. *J. Neurochem.* **2000**, *75*, 1294.
15. Liu, M.-Y.; Lanford, J.; Casida, J. E. *Pestic. Biochem. Physiol.* **1993**, *46*, 200.
16. Yamamoto, I.; Tomizawa, M.; Saito, T.; Miyamoto, T.; Walcott, E. C.; Sumikawa, K. *Arch. Insect Biochem. Physiol.* **1998**, *37*, 24.
17. We performed preliminary docking of compound **17** to an *Aplysia californica* AChBP crystal structure (protein data bank code 2WNJ, see Ref. 18) complexed with 3-(2,4-dimethyoxybenzylidene)-anabaseine which is similar in size to compound **17**. We then carried out docking of this ligand to a homology model of the *Myzus* α2β1 receptor (see Refs. 6,10,11), after first allowing the active site of the homology model to optimise and adjust to ligands of similar size. It is well known that a water molecule forms a critical hydrogen-bonding bridge between the pyridinyl nitrogen of the neonicitinoid and the receptor (see Refs. 7,19), so we placed a water molecule in the active site prior to optimisation and docking. Geometry optimisations included all residues within 5 Å of the active site while residues beyond that were either partially constrained (5 Å beyond inner shell) or frozen (remainder of the structure). Up to 5000 steps per minimisation were run to achieve a gradient of 0.5 with respect to energy using the OPLS forcefield in Macromodel (see Refs. 20,21). Docking calculations were done using Glide as implemented in Maestro (Glide 5.5, Maestro 9.0, Schrödinger, LLC, New York, NY, 2010) (see Ref. 22). The docking includes a spatial fit of the ligand to the receptor grid, followed by minimisation and scoring of hits based on a discretized ChemScore function (see Refs. 23,24). Ligands were flexibly docked using standard precision and the top hits were examined.
18. Hibbs, R. E.; Sulzenbacher, G.; Shi, J.; Talley, T. T.; Conrod, S.; Kem, W. R.; Taylor, P.; Marchot, P.; Bourne, Y. *EBMO J.* **2009**, *28*, 3040.
19. Ohno, I.; Tomizawa, M.; Durkin, K. A.; Casida, J. E.; Kagabu, S. *J. Agric. Food Chem.* **2009**, *57*, 2436.
20. Jorgensen, W. L.; Maxwell, D. S.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1996**, *118*, 11225.
21. Mohamadi, F.; Richard, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.
22. Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. *J. Med. Chem.* **2004**, *47*, 1739.
23. Eldridge, M. D.; Murray, C. W.; Auton, T. R.; Paolini, G. V.; Mee, R. P. *J. Comput. Aided Mol. Des.* **1997**, *11*, 425.
24. Baxter, C. A.; Murray, C. W.; Clark, D. E.; Westhead, D. R.; Eldridge, M. D. *Proteins* **1998**, *33*, 367.
25. McGaughey, G. B.; Gagné, M.; Rappé, A. K. *J. Biol. Chem.* **1998**, *273*, 15458.