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Note

Affinity to the Nicotinic Acetylcholine Receptor and Insecticidal Activity of Chiral Imidacloprid Derivatives with a Methylated Imidazolidine Ring

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Four imidacloprid derivatives with an asymmetrically methylated imidazolidine ring were synthesized. Their affinity to the nicotinic acetylcholine receptor of housefly *Musca domestica* and insecticidal activity against the housefly were measured. The compound with a 5*R*-methylated imidazolidine ring demonstrated intrinsic activity comparable to that of the unsubstituted compound. Most of the compounds were synergized by oxygenase inhibitors.

Key words: imidacloprid; *Musca domestica*; insecticidal activity; synergist; nicotinic acetylcholine receptor

Imidacloprid (**1**, Fig. 1)[1,2] is one of the typical neonicotinoids marketed for pest control in many countries. It acts on insect nicotinic acetylcholine receptors (nAChRs) to kill pests. Various structure-activity relationship (SAR) analyses have been performed during the last two decades to understand the structural factors influencing the insecticidal activity and affinity to the receptor.[2,3] We have quantitatively analyzed SAR for the nitromethylene analogue (**2**, Fig. 1) and its derivatives, and demonstrated that some steric and electrostatic factors around the pyridine ring and the *N*3 atom of the imidazolidine ring influenced receptor binding.[4–6] However, the effect on receptor binding of the steric structure around the ethylene moiety remains unknown. This ethylene moiety is hydroxylated in the housefly, *Musca domestica*.[7] The stereoselective introduction of a substituent to the ethylene moiety of the imidazolidine ring would probably influence the metabolism of the compounds in insects to induce changes in the insecticidal activity. We synthesized in this study four methylated derivatives with an asymmetric carbon atom in the imidazolidine ring (**3**–**6**, Fig. 2) to evaluate their affinity to housefly nAChRs and insecticidal activity against female adult houseflies.

The inhibition constant, K_i (nM), was evaluated as an indicator of the affinity to the receptor (Table 1). Compound **3** (5*R*-CH_3) was 7-fold more potent than its enantiomer (5*S*-CH_3), *i.e.*, compound **4**, and was equipotent to unsubstituted compound **2**. Stereoisomers at position 5 of the imidazolidine ring had differing affinity, suggesting that the configuration of this position would be accurately recognized by the receptor. On the other hand, the introduction of a methyl group to position 4 of the imidazolidine ring induced over 50-fold less potency, and compound **5** (4*R*-CH_3) was about 2-fold more potent than its enantiomer (4*S*-CH_3), *i.e.*, compound **6**.

The insecticidal activity against female adult houseflies of compounds **2**–**6** was measured with and without such synergists as piperonyl butoxide (PBO) and propargyl propyl phenylphosphonate (NIA) which suppressed oxidative metabolic degradation and whose synergistic effects on imidacloprid have been reported.[7–10] In the absence of a synergist, the *S*-isomers (compounds **4** and **6**) were approximately 4-fold less potent than compound **2**, whereas their enantiomers were equipotent to compound **2**. PBO synergized the insecticidal activity of unsubstituted compound **2** and compounds **3** and **4**, which have an imidazolidine ring methylated at position 5. The methylated compounds other than compound **3** were 4–11-fold less potent than compound **2** in the presence of PBO. In the presence of NIA, all compounds other than **5** were synergized over 10-fold, and compound **3** showed 4-fold less potency than compound **2**, whereas compounds **4**–**6** were approximately 10-fold less potent than compound **2**. The insecticidal activity of all compounds increased markedly in the presence of both synergists. The insecticidal activity of the four methylated compounds in the presence or absence of a synergist was affected to varying degrees, suggesting that these synergists had unknown and differing influence on the metabolism of the compounds in houseflies.

We have reported a positive correlation between their affinity to the receptor of some imidacloprid derivatives that was evaluated by using [^{3}H] imidacloprid and the insecticidal activity measured in the presence of both synergists.[11] In this study, a positive correlation was also observed between these activities ($r^2 = 0.809$, $n = 21$, including data previously reported[11]), suggesting receptor binding to be an important factor even if the ethylene moiety was modified. This study demonstrated the possibility of improving the bioactivity of imidacloprid derivatives by synthesizing asymmetrically modified compounds.

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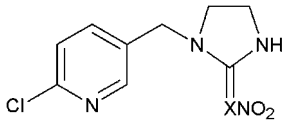
Abbreviations: CH-IMI, nitromethylene analogue of imidacloprid; IMI, imidacloprid; nAChR, nicotinic acetylcholine receptor; NIA, propargyl propyl phenylphosphonate (Niagara 16388); PBO, piperonyl butoxide; SAR, structure-activity relationship

Experimental

The synthetic scheme for the preparation of compounds **3–6** is shown in Fig. 2. The metabolic inhibitor, NIA16388, was synthesized according to a previously published method.[12] NMR data were measured by using a Jeol JNM-EX400 NMR spectrometer. The authenticity of each final compound was confirmed by an elemental analysis with a Yanako MT-5 CHN recorder. Melting point (mp) data for the compounds were measured by Yanaco melting point apparatus (Yanaco, Kyoto, Japan) and are uncorrected. Optical rotation values were evaluated by using a P-2100 polarimeter (Jasco, Tokyo, Japan).



1: Imidacloprid (X=N)

2: Nitromethylene analogue (X=CH; CH-IMI)

Fig. 1. Chemical Structures of the Imidacloprid Derivatives.

*(2′*R*)*-N-*(2′*-t-*Butoxycarbonylamino)propylphthalimide (**8**, step **A**).* To a reaction mixture of anhydrous tetrahydrofuran (THF, 100 mL) containing 3.37 g (12.9 mmol) of triphenylphosphine, 2.52 g (17.1 mmol) of phthalimide, and 1.50 g (8.6 mmol) of *N-t*-butoxycarbonyl-D-alaninol (**7**), 6.71 g (40% in toluene, 15.3 mmol) of diethylazodicarboxylate dissolved in anhydrous THF (50 mL) was added dropwise while stirring in an ice bath. After 6 h at ambient temperature, the solvent was evaporated, and the resulting residue was purified by column chromatography (hexane:ethyl acetate = 3:1) to afford **8** (2.23 g, 85%). Mp 122–123 °C, $[\alpha]_D^{25}$ −20.5 (c 0.27, $CHCl_3$). NMR δ_H ($CDCl_3$): 1.21 (3H, d, $J = 7$ Hz, CH_3), 1.25 (9H, s, $(CH_3)_3C$), 3.68 (2H, d, $J = 9$ Hz, CH_2), 4.10 (1H, m, CH_3CH), 4.71 (1H, br, NH), 7.71 (2H, m, Ph), 7.84 (2H, m, Ph).

*(2′*R*)*-N-*(2′-(6-Chloro-3-pyridyl)methylamino)propylphthalimide (**10**, steps **B**-i and -ii).* A concentrated HCl solution (15 mL) was added dropwise to THF (50 mL) containing **8** (1.79 g, 5.9 mmol) while stirring at ambient temperature. After continuing stirring overnight, the solvent was evaporated to afford **9** which was used in the subsequent reaction without further purification. The product was dissolved in 20 mL of acetonitrile, to which 1.68 mL of triethylamine and 0.97 g (6 mmol) of 2-chloro-5-chloromethylpyridine hydrochloride were added, and the mixture was refluxed overnight. The solvent was

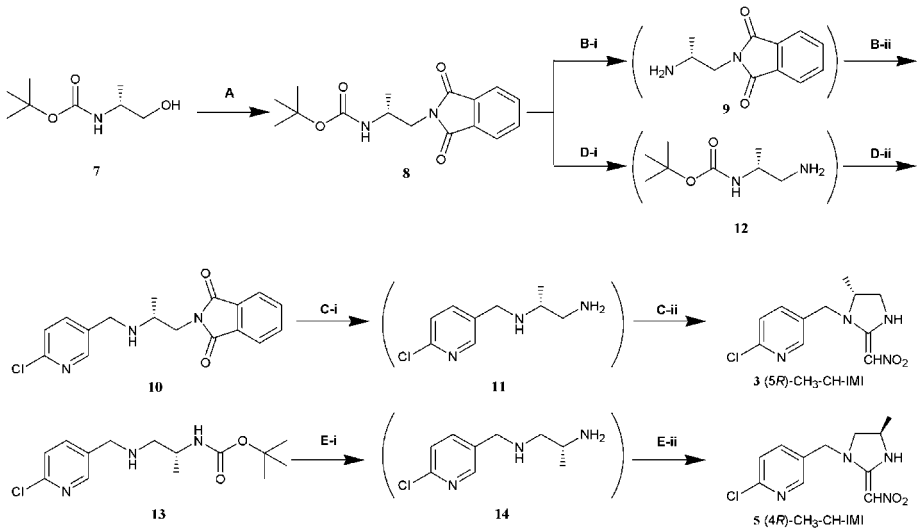


Fig. 2. Synthetic Route to the Imidacloprid Derivatives, *i.e.*, Compounds **3** ((5*R*)-CH_3-CH-IMI) and **5** ((4*R*)-CH_3-CH-IMI).

Their enantiomers, *i.e.*, compounds **4** ((5*S*)-CH_3-CH-IMI) and **6** ((4*S*)-CH_3-CH-IMI), were synthesized by the same method, using *N-t*-butoxycarbonyl-L-alaninol as the starting material. A, Phthalimide, diethyl azodicarboxylate, triphenylphosphine/anhydrous THF; B-I, concentrated HCl/THF; B-ii, 2-chloro-5-chloromethylpyridine, Et_3N/acetonitrile; C-i, hydrazine hydrate/EtOH; C-ii, 1,1-bis(methylthio)-2-nitroethylene, K_2CO_3/EtOH; D-i, hydrazine hydrate/EtOH; D-ii, 2-chloro-5-chloromethylpyridine, Et_3N/acetonitrile; E-i, concentrated HCl/THF; E-ii, 1,1-bis(methylthio)-2-nitroethylene, K_2CO_3/EtOH.

Table 1. Inhibition Constants for the Imidacloprid Derivatives for [^{3}H]imidacloprid Binding to Housefly Head Membranes and Their Insecticidal Activity against Houseflies

Compound no.	Config.[a]	K_i (nM)[b]	ED_{50} (pmol/insect)[c]						
			None (A)	PBO (B)	Ratio (A)/(B)	NIA (C)	Ratio (A)/(C)	PBO + NIA (D)	Ratio (A)/(D)
2	H	0.0367 ± 0.0063	4.18[d]	1.00 ± 0.22 (3)	4.2	0.128 ± 0.013 (3)	33	0.136 ± 0.019 (3)	31
3	5*R*	0.0428 ± 0.0079	5.82 ± 1.06 (4)	1.72 ± 0.35 (3)	3.4	0.474 ± 0.032 (3)	12	0.0854 ± 0.0228 (3)	68
4	5*S*	0.313 ± 0.190	21.0 ± 4.02 (3)	4.85 ± 1.75 (3)	4.3	0.829 ± 0.406 (3)	25	0.643 ± 0.306 (3)	33
5	4*R*	2.07 ± 0.28	6.30 ± 2.35 (3)	7.99 ± 1.59 (3)	0.8	1.12 ± 0.55 (3)	5.6	0.774 ± 0.348 (3)	8
6	4*S*	5.64 ± 1.07	16.1 ± 3.15 (3)	11.4 ± 6.44 (3)	1.4	0.943 ± 0.494 (3)	17	1.00 ± 0.46 (3)	16

[a]Position of the imidazolidine ring attached by a methyl group and its configuration for compounds **3–6**. Compound **2** is unsubstituted.

[b]Inhibition constant of each test compound for [^{3}H]imidacloprid binding to nAChR. The K_i value was calculated according to the following equation by using PRISM software (Graphpad): $K_i = IC_{50}/1 + ([L]/K_d)$, where [L] is the final concentration of the [^{3}H]imidacloprid (0.63 TBq/mmol, 20 nM (GE Healthcare, UK)) and K_d (2.65 nM) is the dissociation constant of [^{3}H]imidacloprid for the receptor fraction, which was newly measured in this study and is consistent with previous studies.[11,13] K_i values for the test compounds were obtained in three separate experiments. Data are presented as the mean and standard error of the mean. See detailed method in Ref. 11.

[c]Effective dose for inducing paralysis or death 1 h after injection in 50% of the female adult houseflies. The value was calculated by using the probit transformation.[14] See details of the method in Ref. 11. Data are presented as the mean and standard error of the mean (the figure in parentheses is the number of experiments).

[d]Calculated from the data cited in Ref. 15.

evaporated, and the resulting residue was purified by column chromatography (hexane:ethyl acetate = 1:1) to afford **10** (0.39 g, 20% in 2 steps). $[\alpha]_D^{25}$ −29.1 (c 1.1, $CHCl_3$). NMR δ_H ($CDCl_3$): 1.04 (3H, d, $J = 7$ Hz, CH_3), 2.94 (1H, m, $CH_3C\underline{H}$), 3.52 (2H, m, PhthalC$\underline{H_2}$), 3.67 (1H, d, $J = 12$ Hz, PyC$\underline{H_2}$), 3.76 (1H, d, $J = 12$ Hz, PyC$\underline{H_2}$), 7.01 (1H, d, $J = 8$ Hz, Py), 7.46 (1H, dd, $J_1 = 8$ Hz, $J_2 = 2$ Hz, Py), 7.60 (2H, m, Ph), 7.69 (2H, m, Ph), 8.13 (1H, d, $J = 2$ Hz, Py).

*(5*R*)-1-(6-Chloro-3-pyridylmethyl)-5-methyl-2-nitromethylene-imidazolidine (**3**, steps **C**-i and -ii).* To an ethanol solution containing 0.39 g (1.2 mmol) of **10**, 0.33 mL (5.8 mmol) of hydrazine monohydrate was added, and the mixture was refluxed for 6 h while stirring. After the insoluble residue had been filtered, the resulting filtrate was evaporated to afford **11** which was used for the subsequent reaction without further purification. After diaminopropane **11** had been dissolved in 20 mL of ethanol, 0.20 g (1.2 mmol) of 1,1-bis(methylthio)-2-nitroethylene and 0.16 g (1.2 mmol) of K_2CO_3 were added to the solution, and the mixture was refluxed overnight. After removing K_2CO_3 by filtration, the filtrate was evaporated *in vacuo*, and the resulting residue was purified by column chromatography (ethyl acetate) to afford **3** (0.22 g, 67% in two steps). Mp 139–141 °C. Elemental analysis. Calcd. for $C_{11}H_{13}N_4O_2Cl$: C, 49.17; H, 4.88; N, 20.85. Found: C, 48.94; H, 4.91; N, 20.66. $[\alpha]_D^{25}$ +9.9 (c 1.54, $CHCl_3$). NMR δ_H ($CDCl_3$): 1.33 (3H, d, $J = 6$ Hz, CH_3), 3.39 (1H, m, CH), 3.93 (2H, m, CH_2), 4.30 (1H, d, $J = 17$ Hz, PyC$\underline{H_2}$), 4.37 (1H, d, $J = 17$ Hz, PyC$\underline{H_2}$), 6.56 (1H, s, $CHNO_2$), 7.35 (1H, d, $J = 8$ Hz, Py), 7.55 (1H, dd, $J = 8$ Hz, $J = 2$ Hz, Py), 8.29 (1H, d, $J = 2$ Hz, Py), 8.70 (1H, br, NH). NMR δ_C ($CDCl_3$): 18, 44, 50, 56, 97, 125, 130, 138, 148, 152, 159. >99% ee [confirmed by a ^{1}H-NMR chemical shift of the $CHNO_2$ group by the shift reagent, Chirabite].

*(5*S*)-1-(6-Chloro-3-pyridylmethyl)-5-methyl-2-nitromethylene-imidazolidine (**4**).* Mp 140–142 °C. Elemental analysis. Calcd. for $C_{11}H_{13}N_4O_2Cl$: C, 49.17; H, 4.88; N, 20.85. Found: C, 49.12; H, 4.86; N, 20.85. $[\alpha]_D^{25}$ −9.5 (c 1.58, $CHCl_3$). The NMR spectral data agreed with those of its enantiomer. >99% ee (confirmed by the same procedure as that for determining the chirality of **3**).

*(2*R*)-*N*-(6-Chloro-3-pyridylmethyl)-*N*′-t-butoxycarbonyl-1,2-diaminopropane (**13**, steps **D**-i and -ii).* Hydrazine monohydrate (3 mL, 53 mmol) was added dropwise to ethanol containing **8** (3.23 g, 10 mmol) while stirring at ambient temperature. After refluxing for 3 h, the reactant was filtered, and the resulting filtrate was evaporated to afford **12**. The residue was dissolved in 20 mL of acetonitrile, to which 5 mL of triethylamine and 1.26 g (8 mmol) of 2-chloro-5-chloromethylpyridine hydrochloride was added, and the mixture was refluxed overnight. After the solvent had been evaporated, the residue was dissolved in distilled water, and the pH was adjusted to 8.0 with 1 M of aqueous NaOH. The solution was extracted three times with dichloromethane, and the organic phase was dried over sodium sulfate. The solvent was evaporated, and the resulting residue was purified by column chromatography (ethyl acetate:ethanol = 9:1) to afford **13** (1.07 g, 36% in two steps). $[\alpha]_D^{25}$ −61.1 (c 1.8, $CHCl_3$). NMR δ_H ($CDCl_3$): 1.08 (3H, d, $J = 7$ Hz, CH_3), 1.39 (9H, s, $(CH_3)_3C$), 2.12 (1H, m, CH), 3.69 (2H, m, CH_2), 4.74 (1H, br, NH), 7.22 (1H, d, $J = 8$ Hz, Py), 7.62 (1H, d, $J = 8$ Hz, Py), 8.27 (1H, s, Py).

*(4*R*)-1-(6-Chloro-3-pyridylmethyl)-4-methyl-2-nitromethylene-imidazolidine (**5**, steps **E**-i and -ii).* After deprotecting the Boc group of 1.07 g (3.6 mmol) of **13** by concentrated HCl to obtain **14**, 2.31 g (14 mmol) of 1,1-bis(methylthio)-2-nitroethylene and 1.93 g (14 mmol) of K_2CO_3 were added to 20 mL of an ethanol solution containing **14**, and the mixture was refluxed overnight. After filtering to remove K_2CO_3, the resulting filtrate was evaporated, and the residue was purified by column chromatography (ethyl acetate:ethanol = 9:1) to afford **5** (0.13 g, 13% in two steps). Mp 139–141 °C. Elemental analysis. Calcd. for $C_{11}H_{13}N_4O_2Cl$; C, 49.17; H, 4.88; N, 20.85. Found: C, 49.11; H, 4.58; N, 20.76. $[\alpha]_D^{25}$ −7.5 (c 1.18, $CHCl_3$). NMR δ_H ($CDCl_3$): 1.36 (3H, d, $J = 6$ Hz, CH_3), 3.12 (1H, dd, $J_1 = 10$ Hz, $J_2 = 7$ Hz, CH), 3.67 (1H, t, $J = 10$ Hz, CH), 4.21 (1H, m, CH), 4.31 (2H, d, $J = 4$ Hz, PyC$\underline{H_2}$), 6.64 (1H, s, $CHNO_2$), 7.36 (1H, d, $J = 8$ Hz, Py), 7.56 (1H, dd, $J_1 = 8$ Hz, $J_2 = 3$ Hz, Py), 8.29 (1H, d, $J = 3$ Hz, Py), 8.77 (1H, br, NH). NMR δ_C ($CDCl_3$): 21, 47, 50, 51, 55, 96, 125, 129, 138, 149, 152, 159. >99% ee (DAICEL chiral column OD-H, 254 nm, mobile phase of hexane:2-propanol = 1:1, $t_R = 94$ min).

*(4*S*)-1-(6-Chloro-3-pyridylmethyl)-4-methyl-2-nitromethylene-imidazolidine (**6**).* Mp 139–141 °C. Elemental analysis. Calcd. for $C_{11}H_{13}N_4O_2Cl$: C, 49.17; H, 4.88; N, 20.85. Found: C, 49.09; H, 4.83; N, 20.83. $[\alpha]_D^{25}$ +7.8 (c 1.14, $CHCl_3$). >99% ee (DAICEL OD-H, 254 nm, mobile phase hexane:2-propanol = 1:1, $t_R = 102$ min).

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References

1) Elbert A, Overbeck H, Iwaya K, and Tsuboi S, "Proc Brighton Crop Protect Conf—Pests & Diseases," BCPC, Farnham, pp. 21–28 (1990).
2) Kagabu S, *J. Agric. Food Chem.*, **59**, 2887–2896 (2011).
3) Sun C, Yang D, Xing J, Wang H, Jin J, and Zhu J, *J. Agric. Food Chem.*, **58**, 3415–3421 (2010).
4) Nishiwaki H, Nakagawa Y, Takeda DY, Okazawa A, Akamatsu M, Miyagawa H, Ueno T, and Nishimura K, *Pest Manag. Sci.*, **56**, 875–881 (2000).
5) Nishiwaki H, Nakagawa Y, Ueno T, Kagabu S, and Nishimura K, *Pest Manag. Sci.*, **57**, 810–814 (2001).
6) Kagabu S, Nishiwaki H, Sato K, Hibi M, Yamaoka N, and Nakagawa Y, *Pest Manag. Sci.*, **58**, 483–490 (2002).
7) Nishiwaki H, Sato K, Nakagawa Y, Miyashita M, and Miyagawa H, *J. Pestic. Sci.*, **29**, 110–116 (2004).
8) Liu M, Lanford J, and Casida JE, *Pestic. Biochem. Physiol.*, **46**, 200–206 (1993).
9) Nishimura K, Kanda Y, Okazawa A, and Ueno T, *Pestic. Biochem. Physiol.*, **50**, 51–59 (1994).
10) Yamamoto I, Tomizawa M, Saito T, Miyamoto T, Walcott EC, and Sumikawa K, *Arch. Insect Biochem. Physiol.*, **37**, 24–32 (1998).
11) Nishiwaki H, Nakagawa Y, Kuwamura M, Sato K, Akamatsu M, Matsuda K, Komai K, and Miyagawa H, *Pest Manag. Sci.*, **59**, 1023–1030 (2003).
12) Soliman SA, *J. Toxicol. Environ. Health*, **10**, 907–920 (1982).
13) Liu M, Latli B, and Casida JE, *Pestic. Biochem. Physiol.*, **50**, 171–182 (1994).
14) Finney DJ, "Probit Analysis" 2nd ed., Cambridge University Press, London (1952).
15) Nishiwaki H, Nakagawa Y, Ueno T, and Nishimura K, *J. Pestic. Sci.*, **26**, 91–92 (2001).