Pest Manag Sci 58:483–490 (online: 2002)
DOI: 10.1002/ps.488

Nicotinic acetylcholine receptor binding of imidacloprid-related diaza compounds with various ring sizes and their insecticidal activity against *Musca domestica*

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Abstract: Fifteen 5-substituted 1-(6-chloro-3-pyridylmethyl)-2-nitromethylene-1,3-diazacyclohexanes and three other related compounds having a five- or seven-membered ring were synthesized and their biological activities were measured *in vivo* and *in vitro*. The insecticidal (*in vivo*) activity was evaluated against houseflies *Musca domestica* L under synergistic conditions with propargyl propyl phenyl phosphonate and piperonyl butoxide. The binding activity of each compound to nicotinic acetylcholine receptor *in vitro* was measured using [^{125}I]α-bungarotoxin. The insecticidal activities of the unsubstituted diazacyclohexane analogues were slightly higher than those of the imidazolidine analogues, but the enlargement of ring size to diazacycloheptane lowered the activity. Substitution of 1,3-diazacyclohexane or imidazolidine rings was not generally favourable for the activity, but the unsubstituted 1,3-diazacyclohexane analogue showed the highest binding activity. Ring substitutions and ring enlargement decreased the activity 100–30 000-fold.

Keywords: insecticidal activity; *Musca domestica*; chloronicotinyl insecticides; neonicotinoids; [^{125}I]α-bungarotoxin; nicotinic acetylcholine receptor; 2-nitromethylene-1,3-diazacyclohexane

1 INTRODUCTION

Imidacloprid and related chloronicotinyl compounds (or neonicotinoids) have distinctive chemical structures, and these compounds are insecticidally very potent against various pest species.[1–7] It has been shown that these compounds have agonistic effects on insect nicotinic acetylcholine receptor (nAChR) in various bioassay systems.[8–10] The correlation analyses between insecticidal activity and the binding activity to nAChR for a set of compounds clearly indicated that the insecticidal action of these compounds is caused by their binding to nAChR of insects.[11–13] Structure–activity relationship analyses have also been performed for the *in vitro* activities.[9,11–20] In particular, three-dimensional quantitative structure–activity relationship (3-D QSAR) procedures are helpful to predict the receptor–ligand interactions.[17,18,21]

We have applied comparative molecular field analysis (CoMFA), a 3-D QSAR procedure,[22] to the binding activity of imidacloprid-related compounds.[17,18] In CoMFA each active structure of a compound is superposed onto that of a reference compound so that each structural component is as close as possible to the corresponding component of the reference in the 3-D lattice. A charge (+1) and an sp^3 carbon atom are placed at the various intersections of the 3-D lattice and electrostatic and steric interactions are calculated for all atoms, and the optimum latent variables are extracted by the partial least-square method of Wold *et al.* [23] In CoMFA the electrostatically and sterically favourable/unfavourable fields surrounding the molecules are determined, and may be helpful in drug design. In our compound set, various structures are included: (1) NNO_2 at the 2-position of the imidazolidine ring is substituted with $CHNO_2$, NCN or CHCN, (2) the N atom at the 3-position is replaced with S, O or C, (3) the 6-chloro-3-pyridylmethyl moiety is replaced with various structures such as benzyl, 6-chloro-3-pyridylethyl, etc.[17] In a further study we have combined nitenpyram derivatives which have acyclic structure in the imidazolidine moiety.[3,18] The latest CoMFA gave the favourable and unfavourable steric/electrostatic fields essential for the binding activity, but the regions surrounding the pyridine ring and the C4-C5 and N3 positions of imidazolidine ring of imidacloprid were not clearly described because the structural variations are small with respect to these moieties.[17]

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(*Received 5 October 2001; revised version received 2 January 2002; accepted 30 January 2002*)

To clarify these uncertain fields we have synthesized a series of substituted benzyl derivatives,[13] *N*3-alkylated imidacloprid analogues[14,19] and corresponding $CHNO_2$ derivatives.[14,24] In this paper we report the synthesis of a new series of compounds including 1,3-diazacyclohexane (6-membered ring) and 1,3-diazacycloheptane (7-membered ring) as well as 4- or 5-substituted imidazolidine analogues. We also introduced various substituents at the 5-position of the 1,3-diazacyclohexane ring. The insecticidal activity and the binding activity against houseflies were measured and their relationship to structure was analyzed.

2 MATERIALS AND METHODS

2.1 Chemicals

The compounds used in this study were newly synthesized by the published methods.[24,25] Representative procedures are described here for compounds **7**, **17** and **18** (see Fig 1). All melting points are uncorrected. NMR spectra were obtained by a Varian Gemini 2000 C/H instrument (400 MHz). The chemical shifts were recorded in δ (ppm) and the coupling constants in Hz. Mass spectra were recorded (EI, 70 eV) with a Shimadzu QP 1000 mass spectrometer. Log *P* values of newly synthesized compounds were measured in 1-octanol + water using the flask-shake method. α-Bungarotoxin (α-BGTX) was purchased from Sigma Chemical Co (St Louis, MI, USA), and [^{125}I]α-BGTX was obtained from Amersham Pharmacia Biotech (Buckinghamshire, UK). Propargyl propyl phenyl phosphonate (NIA16 388 or NIA) was obtained from our stock sample as prepared by Nakagawa *et al.*[26] Piperonyl butoxide (PB), an inhibitor of mixed function oxidases, was purchased from Nacalai Tesque (Kyoto, Japan).

2.2 Synthesis

2.2.1 5-n-Butyl-1-(6-chloro-3-pyridylmethyl)-2-nitromethylene-1,3-diazacyclohexane (7)

6-Chloronicotinaldehyde (1.30 g, 9.21 mmol) was added in small portions to a refluxing solution of 2-butyl-1,3-propanediamine (1.49 g, 11.5 mmol) in benzene (30 ml). Heating was continued until no water separated in an attached Dean–Stark trap (2∼3 h). The benzene was distilled off under reduced pressure and the remaining imine was used without further purification. Powdered $NaBH_4$ (1.47 g, 38.8 mmol) was added in small portions to a suspension of the imine in ethanol + water (5 + 1 by volume). The resulting mixture was stirred at room temperature for 24 h. After most of the ethanol had been distilled off under a weak vacuum, the diamine was extracted with isopropyl ether (IPE) from the water phase. Evaporation of IPE left an oily liquid, which was taken up with hydrochloric acid (1 M) and washed with IPE (2 × 10 ml). The aqueous solution was alkalized to pH 12 with solid sodium hydroxide with ice cooling, extracted with chloroform (5 × 10 ml), and dried over anhydrous magnesium sulfate. Evaporation of the solvent afforded 1.97 g of *N*-(6-chloro-3-pyridylmethyl)-2-*n*-butyl-1,3-propanediamine in 84% crude yield. A mixture of 1.94 g (7.6 mmol) of crude diamine and 1,1-bis(methylthio)-2-nitroethylene (1.25 g, 7.6 mmol) in 30 ml of ethanol was heated under reflux for 27 h. After evaporation of the ethanol, the semi-solid residue was dissolved in hot ethyl acetate after adding a small amount of activated charcoal, and filtered while hot. Colourless needles of **7** crystallized from the filtrate while standing at room temperature. The analytical sample was recrystallized twice from ethanol. The yield was 0.65 g (26%).

*2.2.2 1-(6-Chloro-3-pyridylmethyl)-4,4-dimethyl-2-nitromethyleneimidazolidine (**17**)*

A solution of 1,2-diamino-2-methylpropane (1.90 g, 22 mmol) in acetonitrile (10 ml) was added dropwise to a solution of 6-chloro-3-pyridylmethyl chloride (0.70 g, 4.3 mmol) in acetonitrile (10 ml) and then the resulting solution was refluxed overnight. After removing the insoluble solids by filtration, the filtrate was evaporated in vacuum. Distilled water was added to the residue, which was then extracted with dichloromethane. The separated organic layer was dried over anhydrous magnesium sulfate. Evaporation of the solvent afforded a mixture of *N*-(6-chloro-3-pyridylmethyl)-1,1-dimethylethylenediamine and *N*-(6-chloro-3-pyridylmethyl)-2,2-dimethylethylenediamine (0.98 g, quantitative yield). A solution of the mixture (0.92 g, 4.3 mmol) in ethanol (20 ml) was added dropwise to a suspension of 1,1-bis(methylthio)-2-nitroethylene (0.69 g, 4.2 mmol) and potassium carbonate (0.57 g, 4.2 mmol) in ethanol (20 ml), and the resulting mixture was refluxed overnight. After filtration of the precipitates, the filtrate was concentrated. The residue was recrystallized from ethanol to afford the product **17**. The yield was 0.23 g (19%).

*2.2.3 1-(6-Chloro-3-pyridylmethyl)-5,5-dimethyl-2-nitromethyleneimidazolidine (**18**)*

The filtered solution remaining after recrystallization of **17** was concentrated. The residue was subjected to silica gel column chromatography with ethanol + ethyl acetate (1 + 2 by volume) as eluate. The collected crude product was developed on HPLC (Shimadzu, LC-6A; COSMOSIL 5C18-AR, 20 × 250 mm) with water + methanol (65 + 35 by volume) as mobile phase. An analytical pure sample was obtained from the fractions at a retention time of 24.2 min. The yield was 0.07 g (5%).

2.3 Bioassay

2.3.1 Insecticidal activity

The method for the insecticidal test against houseflies was that previously reported.[15] In brief, a methanol solution (1 μl) containing NIA (0.2%) and PB (0.2%) was topically applied to the abdomen of female houseflies anaesthetized using carbon dioxide. After 1 h at 25 °C, solutions (0.24 μl) of test compound in

ethanol+water (50+50 by volume) at various concentrations were injected into the dorsal side of the thorax. After 1 h at 25 °C, the number of dead and paralyzed flies was counted. The 50% effective concentration, EC_{50} (M), was evaluated from the dose–response relationship using probit methods.[27,28] The reciprocal logarithm of the EC_{50} value, pEC_{50}, was defined as the insecticidal activity.

2.3.2 *Binding activity*

The procedure for the receptor binding assay was that previously reported.[13,17] Briefly, the membrane preparation obtained from housefly heads was incubated with test compounds and [^{125}I]α-BGTX (0.2 nM) at 24 °C for 60 min. The reaction was terminated by rapid filtration through a Unifilter GF/B (Packard Instrument Co, Meriden, CT, USA), which had been treated with polyethyleneimine (0.1%). The filters were rinsed three times with sodium phosphate (10 mM; pH 7.4) containing sodium chloride (50 mM), followed by methanol. After adding Microscinti-O (Packard Instrument Co, Meriden, CT, USA) as a scintillation cocktail, the radioactivity was measured using a Topcount instrument (Packard Instrument Co, Meriden, CT, USA). The molar concentration required for 50% inhibition of the specific binding of [^{125}I]α-BGTX, IC_{50} (M), was determined by a non-linear regression analysis using PRISM (Graphpad Software Inc, San Diego, CA, USA). The binding activity was expressed as the K_i (M) value calculated by

$$K_i = IC_{50}/(1 + [L]/K_d),$$

where [L] is the concentration of [^{125}I]α-BGTX and K_d is the dissociation constant of α-BGTX. The K_d value of α-BGTX was determined in each membrane preparation. The mean K_d of α-BGTX binding to membrane preparations used to estimate the K_i value of test compounds was 0.31 (±0.15) nM (n = 4). This K_d value was consistent with the value (0.29 nM) that we previously estimated.[14] The reciprocal logarithm of the K_i (M) value, pK_i, was calculated for each compound.

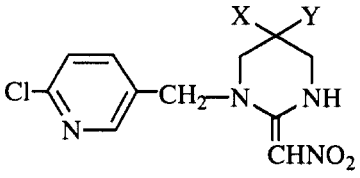
3 RESULTS

3.1 Chemistry

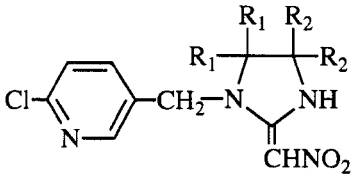
The structures of test compounds are shown in Fig 1. The NMR assignments of the protons and the main MS fragments of the newly prepared compounds are given in Tables 1 and 2, respectively. The physical and spectral data for the prepared compounds are given in Tables 1 and 2. The new compounds gave satisfactory elemental analyses for C, H and N. Log P values of the compounds range from −0.80 to 1.55 and are listed in Table 3.

3.2 Insecticidal activity

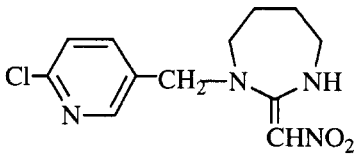
The activity values varied from 3.99 (compound **14**) to 6.12 (compound **1**) as listed in Table 3, showing an activity change of more than 100-fold as a result of imidazolidine ring modifications. Notably, the unsub-



1 X=H, Y=H
2 X=H, Y=CH_3
3 X=CH_3, Y=CH_3
4 X=H, Y=CH_2CH_3
5 X=H, Y=*n*-C_3H_7
6 X=H, Y=*i*-C_3H_7
7 X=H, Y=*n*-C_4H_9
8 X=H, Y=*i*-C_4H_9
9 X=H, Y=*s*-C_4H_9
10 X=H, Y=*t*-C_4H_9
11 X=H, Y=C_6H_5
12 X=H, Y=$CH_2C_6H_5$
13 X=H, Y=OH
14 X=H, Y=OCH_3
15 X=H, Y=OC_6H_5



16 R_1=R_2=H
17 R_1=H, R_2=CH_3
18 R_1=CH_3, R_2=H



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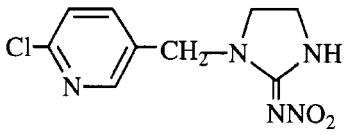


20 (imidacloprid)

Figure 1. Imidacloprid and related chloronicotinyl compounds.

Table 1. [1]H spectral data of newly prepared compounds[a]

Compound no	Chemical shifts (ppm)				
	$NCH_2C(XY)CH_2N$[b]	CHX	CH_2	$=CHNO_2$	Pyridine and others
1	3.44 (m), 3.50 (m)	2.11 (m)	4.43	6.23	7.37 (d, *J*=8.4), 7.53 (dd, *J*=8.4/2.6), 8.25 (d, *J*=2.6), 10.82 (NH, bs)
2	3.05 (2H, m) 3.32 (1H, m) 3.45 (1H, m)	2.20 (m)	4.40 (d, *J*=17.2), 4.46 (d, *J*=17.2)	6.64	1.07 (3H, d, *J*=6.6), 7.36 (d, *J*=8.0), 7.54 (dd, *J*=8.0/2.5), 8.25 (d, *J*=2.5), 10.8 (NH, bs)
3	3.06 (s), 3.17 (s)		4.43	6.71	1.08 (6H, s), 7.38 (d, =7.7) 7.57 (dd, *J*=7.7/1.8), 8.29 (d, *J*=1.8) 10.9 (NH, bs)
4	3.10 (2H, m) 3.36 (1H, m) 3.58 (1H, m)	1.98 (m)	4.45	6.62	0.96 (3H, t, *J*=7.7), 1.98 (2H, m), 7.36 (d, *J*=8.1), 7.54 (dd, *J*=8.1/2.2), 8.24 (d, *J*=2.2), 10.8 (NH, bs)
5	3.08 (2H, m), 3.34 (1H, m), 3.57 (1H, m)	2.06 (m)	4.46	6.63	0.92 (3H, m), 1.34 (4H, m), 7.36 (d, *J*=8.1), 7.54 (dd, *J*=8.1/1.8), 8.24 (d, *J*=1.8), 10.8 (NH, bs)
6	3.16 (2H, m), 3.35 (1H, ddd, *J*=12.5/4.6/2.2), 3.60 (1H, m, *J*=13.2)	1.81 (m)	4.43 (d, *J*=17.2) 4.48 (d, *J*=17.2)	6.61	0.94 (3H, d, *J*=6.6), 0.98 (3H, d, *J*=8.0), 1.58 (1H,m), 7.35 (d, *J*=8.0), 7.53 (dd, *J*=8.0/2.6), 10.8 (NH, bs)
7	3.08 (2H, m), 3.35 (1H, m), 3.57 (1H, m)	2.05 (m)	4.44	6.63	0.89 (3H, t, *J*=6.6), 1.31 (6H, m), 7.36 (d, *J*=8.4), 7.54 (dd, *J*=8.4/2.6), 8.24 (d, *J*=2.6), 10.8 (NH, bs)
8	3.06 (2H, m), 3.31 (1H, m), 3.56 (1H, m)	2.14 (m)	4.43	6.64	0.91 (6H, d, *J*=6.6), 1.20 (2H, m), 1.58 (1H, m), 7.37 (d, *J*=8.0), 7.53 (dd, *J*=8.0, 2.6), 8.25 (d, *J*=2.6), 10.8 (NH, bs)
9	3.18 (2H, m), 3.31 (1H, m), 3.57 (1H, m)	1.94 (m)	4.41 (d, *J*=17.2), 4.47 (d, *J*=17.2)	6.62	0.86–0.95 (3H+3H, m), 1.22 (1H,m), 1.44 (2H, m), 7.37 (d, *J*=8.1), 7.52 (dd, *J*=8.1/2.2), 8.24 (d, *J*=2.2), 10.8 (NH, bs)
10	3.13–3.29 (3H, m), 3.57 (1H, m)	1.85 (m)	4.41 (d, *J*=17.2), 4.47 (d, *J*=17.2)	6.61	0.95 (9H, s), 7.37 (d, *J*=8.4), 7.51 (dd, *J*=8.4/2.6), 8.24 (d, *J*=2.6), 10.8 (NH, bs)
11	3.46–3.61 (3H, m), 3.74 (1H, m)	3.30 (m)	4.47	6.70	7.20 (C_6H_5, 2H, m), 7.30–7.39 (C_6H_5, 4H, m) 7.48 (dd, *J*=8.1/2.6), 8.23 (d, *J*=2.6), 11.0 (NH, bs)
12	3.16 (2H, m), 3.30 (1H,,m), 3.50 (1H, m)	2.39 (m)	4.39	6.64	2.61–2.73 ($C_6H_5CH_2$, m), 7.50 (dd, *J*=8.4/2.5 8.21 (d, *J*=2.5), 10.8 (NH, bs)
13[c]	3.25 (1H, d, *J*=13.2), 3.34 (1H, d, *J*=12.1), 3.45 (1H, d, *J*=12.1), 3.62 (1H, d, *J*=13.2)	4.14 (m)	4.55 (d, *J*=18.0), 4.64 (d, *J*=18.0)	6.51	5.50 (OH, d, *J*=3.2), 7.54 (d, *J*=8.5), 7.80 (dd, *J*=8.5/2.2), 8.39 (d, *J*=2.2), 10.4 (NH, bs)
14[c]	3.40 (1H, d, *J*=14.2), 3.45 (1H, d, *J*=14.2) 3.63 (1H, d, *J*=12.1) 3.66 (1H, d, *J*=12.1)	3.85 (m)	4.56 (d, *J*=17.6), 4.65 (d, *J*=17.6)	6.52	3.29 (OCH_3), 7.55 (d, *J*=8.4), 7.75 (dd, *J*=8.4/2.2), 8.33 (d, *J*=2.2), 10.4 (NH, bs)
15[c]	3.55 (1H, d, *J*=13.9), 3.62 (1H, d, *J*=13.9), 3.71 (1H, d, *J*=13.2), 3.86 (1H, d, *J*=13.2)	5.07 (m)	4.55 (d, *J*=17.6), 4.70 (d, *J*=17.6)	6.61	6.95–7.01 (C_6H_5, 3H,m), 7.31 (C_6H_5, dd, *J*=8.3/8.4), 7.52 (d, *J*=8.4), 7.76 (dd, *J*=8.4/2.6), 8.36 (d, *J*=2.6), 10.5(NH)
16[c]	3.59 (2H, m), 3.81 (2H, m)		4.33	6.66	7.37 (1H, d, *J*=8.1), 7.59 (1H, dd, *J*=8.1/2.5) 8.31 (1H, d, *J*=1.8), 8.73 (NH, bs)
17	3.28 (2H, s)		4.29	6.61	1.42 (6H, s), 7.38 (1H, d, *J*= 8.1), 7.55 (1H, dd, *J*= 8.4 /2.4), 8.28 (1H, d, *J*= 2.4), 8.70 (NH, bs)
18	3.62 (2H, s)		4.49	6.33	1.37 (6H, s), 7.34 (1H, d, *J*= 8.1), 7.54 (1H, dd, *J*= 8.4/2.4), 8.30 (1H, d, *J*= 2.4), 8.71 (NH, bs)
19	1.63 (2H, m), 1.75 (2H, m), 3.26 (2H, m), 3.48 (2H, dd, *J*=10.5/5.0)		4.41	6.52	7.37 (1H, d, *J*=8.1), 7.55 (1H, dd, *J*=8.4/2.4) 8.30 (1H, d, *J*=2.4), 10.06 (1H, NH, bs)

[a] In deuterochloroform unless otherwise stated; coupling constant *J* (Hz) for H–H.

[b] CH_2CH_2 for compound **16**, $CH_2C(CH_3)_2$ for compound **17**, $C(CH_3)_2CH_2$ for compound **18**, $CH_2CH_2CH_2$ for compound **19**.

[c] In hexadeuterodimethyl sulfoxide.

Table 2. Melting points and mass spectra of newly prepared compounds

Compound no	Mp (°C)	m/z (Intensity)
1	186	268 (M^+, 22%), 223 (39%), 222 (76%), 128 (40%), 126 (100%)
2	198	282 (M^+, 15%), 252 (11%), 236 (100%), 200 (19%), 195 (14%), 126 (73%)
3	223	296 (M^+, 6%), 250 (81%), 214 (11%), 126 (96%), 41 (100%)
4	167	296 (M^+, 8%), 250 (100%), 214 (18%), 126 (88%)
5	172	310 (M^+, 3%), 264 (78%), 222 (16%), 126 (89%), 44 (100%)
6	197	310 (M^+, 9%), 264 (100%), 228 (26%), 223 (22%), 126 (100%)
7	146	324 (M^+, 2%), 278 (70%), 237 (16%), 126 (100%)
8	203	324 (M^+, 4%), 294 (11%), 278 (100%), 238 (16%), 223 (29%), 127 (17%)
9	190	324 (M^+, 2%), 278(37%), 234 (36%), 223 (17%), 157 (12%), 127 (100%)
10	221	324 (M^+, 17%), 295 (28%), 290 (28%), 278 (100%), 234 (83%), 223 (42%), 209 (69%), 157 (43%), 141 (37%), 127 (48%)
11	218	344(M^+, 2%), 298 (82%), 126 (51%), 104 (100%)
12	210	344 (M^+, 14%), 312 (41%), 233 (24%), 127 (77%), 118 (62%), 91 (100%)
13	205	284 (M^+, 2%), 238 (33%), 209 (15%), 155 (13%), 126 (100%)
14	207	299 ($M^+ + 1$, 48%), 268 (16%), 252 (76%), 222 (14%), 126 (86%)
15	202	360 ($M^+ + 1$, 8%), 314 (24%), 267 (12%), 220 (13%), 126 (100%), 108 (11%)
16	167	254 (M^+, 25%), 208 (70%), 172 (40%), 126 (100%)
17	195	282 (M^+, 16%), 267 (24%), 252 (17%), 236 (80%), 126 (92%), 55 (100%)
18	208	282 (M^+, 15%), 267 (15%), 252 (13%), 236 (89%), 126 (94%), 55 (100%)
19	219	282 (M^+, 3%), 236 (79%), 126 (88%), 55 (100%)

stituted six-membered ring compound (**1**) was more potent than either the five-membered ring homologue (**16**) or imidacloprid (**20**). Substitution with isopropyl (**6**) or *n*-butyl (**7**) at the 5-position of the diazacyclohexyl ring did not reduce activity. However, other straight or branched alkyl groups (**2**–**5**, **8**–**10**), as well as phenyl (**11**) and benzyl (**12**) groups decreased the activity substantially. An alcohol (**13**) or ethers (**14**, **15**) were less potent than unsubstituted compound (**1**) by a factor of 100. The seven-membered ring homologue (**19**) exhibited slightly lower potency than the unsubstituted compound (**1**). Also the activity of the imidazolidine compound (**16**) was decreased by geminally appending two methyl groups at the 4- (**17**) or 5-position (**18**), although compound **18** was three times more active than compound **17**. We could not find any definitive relationship between the insecticidal activity and the molecular hydrophobicity log *P*.

3.3 Binding activity

The binding activity of compound **1** was twice that of the five-membered ring homologue (**16**), and it was about 700-fold more active than imidacloprid (**20**). The activity was decreased by further enlarging to a seven-membered ring (**19**). The activity was also lowered drastically by putting alkyl, phenyl, hydroxyl and aralkyloxy groups at the 5-position of the diazacyclohexyl ring, with the exception of *sec*-butyl (**9**). Compound **9** showed the highest binding potency among the diazacyclohexyl derivatives. Another point to note is that the potencies were different by over 15 times in the regional isomers **17** and **18**. Here again no correlation between the binding activity and log *P* was observed.

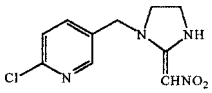
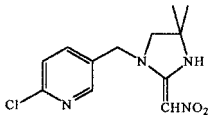
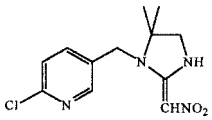
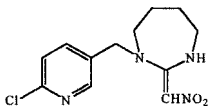
4 DISCUSSION

The CoMFA maps predicted the permissible steric field area in the area extending from the imidazolidine ring.[17,18] The slight enhancement of the binding activity by enlarging the ring from five-membered (**16**) to six-membered (**1**) is not inconsistent with this CoMFA result. However, introduction of substituents at the 5-position of diazacyclohexane as well as the introduction of methyl groups at either the 4- or 5-position of the imidazolidine ring were fairly unfavourable to activity, suggesting the existence of a sterically unfavourable field over this permissible field. CoMFA is in progress for the combined set of compounds.

As a whole, however, the relationships between substituents and biological potencies appear neither uniform nor simple. As for the insecticidal activity, all substituents tested excepting isopropyl (**6**) and *n*-butyl (**7**) decreased the potency of the parent molecule **1** by different scalars. It is puzzling why the *sec*-butyl derivative **9** exhibited 6–70 times higher potency than the other butyl isomers with respect to the binding activity. To uncover the structural features required for the enhancement of the biological activity a quantitative analysis is important.

Previously we reported that insecticidal activity was positively correlated with the binding activity for the substituted benzyl derivatives of chloronicotinyl in-

Table 3. Binding and insecticidal activities of chloronicotinyl compounds with modified imidazolidine ring

Compound no	R	Insecticidal PEC_{50} (M)[a]	Binding PK_i (M)[a]	Hydrophobicity log P
1	H	6.12 (±0.12) (2)	8.28 (±0.14) (2)	−0.30
2	CH_3	4.60 (±0.17) (3)	5.38 (±0.27) (7)	−0.10
3	di-CH_3	4.57 (±0.13) (2)	3.98 (±0.28) (5)	0.40
4	C_2H_5	5.34 (±0.13) (2)	4.98 (±0.06) (2)	0.50
5	*n*-C_3H_7	4.80 (±0.18) (2)	5.06 (±0.13) (2)	1.07
6	i-C_3H_7	6.01 (±0.28) (3)	5.43 (±0.02) (2)	0.91
7	*n*-C_4H_9	6.10 (±0.09) (2)	5.29 (±0.13) (2)	1.55
8	i-C_4H_9	4.84 (±0.07) (2)	5.04 (±0.17) (2)	1.46
9	*s*-C_4H_9	4.44 (±0.08) (2)	6.04 (±0.12) (2)	1.40
10	*t*-C_4H_9	4.72 (±0.18) (2)	4.19 (±0.16) (2)	1.25
11	C_6H_5	4.70 (±0.12) (2)	3.62 (±0.29) (2)	1.22
12	$C_6H_5CH_2$	4.19 (1)	5.01 (±0.07) (2)	1.44
13	OH	4.30 (±0.13) (2)	3.69 (±0.16) (2)	−0.80
14	CH_3O	3.99 (1)	3.62 (±0.23) (2)	−0.42
15	C_6H_5O	4.20 (±0.13) (2)	3.98 (±0.20) (2)	1.13
16		5.80[b]	7.93[c]	−0.19[d]
17		5.01 (±0.05) (2)	3.48 (±0.29) (3)	0.37
18		5.54 (±0.06) (2)	4.65 (±0.02) (3)	0.31
19		5.70 (±0.21) (2)	6.01 (±0.27) (2)	0.34
20	Imidacloprid	5.71[b]	5.43[c]	0.58[d]

[a] Mean values with standard deviation with the number of replications in parentheses.
[b] Cited from Reference 15.
[c] Cited from Reference 12.
[d] Cited from Reference 8.

secticides [eqn (1)].[15]

$$\mathrm{pEC_{50}} = 0.688\,(\pm 0.197)\ \mathrm{pK_i} + 0.395\,(\pm 1.071) \quad (1)$$
$$n = 16,\ r = 0.894,\ s = 0.439,\ F_{1,14} = 55.752$$

In eqn (1) and the following equations, n is the number of compounds, s is the standard deviation, r is the correlation coefficient, and F is the value of the ratio between regression and residual variances. The figures in parentheses following each coefficient are the 95% confidence intervals of the regression coefficient. We also found a high correlation between these two activities for *N*3-substituted imidacloprid derivatives after taking into account the structural difference of the nitroimino and nitromethylene moieties and a structural feature of the *N*3-substituents [eqn (2)].[14]

$$\mathrm{pEC_{50}} = 0.611(\pm 0.132)\ \mathrm{pK_i} - 0.923\,(\pm 0.303)\,I_{\mathrm{CHNO_2}} - 0.795\,(\pm 0.349)\,I_{\mathrm{branch/N3}} + 1.909\,(\pm 0.485) \quad (2)$$
$$n = 26,\ r = 0.929,\ s = 0.303,\ F_{3,22} = 46.101$$

In eqn (2), the $I_{\mathrm{CHNO_2}}$ term was set as 1 for compounds having nitromethylene group instead of nitroimino group in the imidazolidine moiety of imidacloprid. The $I_{\mathrm{branch/N3}}$ term (= 1) was given for the compounds to which a secondary alkyl or phenyl group was introduced. Since the coefficients of the $\mathrm{pK_i}$ terms were similar between eqns (1) and (2), these two sets

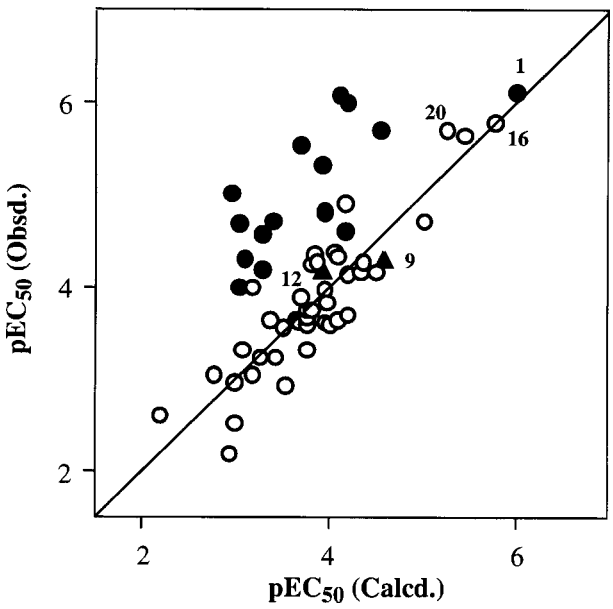


Figure 2. Plot of observed insecticidal activity vs calculated values from eqn (3). Newly measured compounds were shown by closed circles and triangles. Open circles are cited from our previous publications (References 13–15). Numbers in figure corresponds to the compound number in Fig 1 and Tables. Compounds **9** and **12** specified by closed triangles were not used to derive eqn (4).

of compounds were combined to formulate eqn (3):

$$pEC_{50} = 0.633\,(\pm 0.120)\ pK_i - 1.065\,(\pm 0.303)\,I_{CHNO_2} - 0.719\,(\pm 0.402)\,I_{branch/N3} + 1.824\,(\pm 0.460) \quad (3)$$

$$n = 41,\ r = 0.894,\ s = 0.369,\ F_{3,37} = 48.935$$

We calculated the insecticidal activity in terms of pEC_{50} of the present set of the compounds by eqn (3), and found that the observed pEC_{50} values were significantly higher than the calculated ones except for compounds **9** and **12** as shown in Fig 2. Thus, another indicator variable I_{mod} which takes 1 for the substituted diazacyclohexyl, dimethylimidazolidine and diazacycloheptyl compounds was considered to derive eqn (4) for all sets of compounds excluding compounds **1** (H), **9** (*sec*-Bu), and **12** ($CH_2C_6H_5$). For unsubstituted diazacyclohexyl compounds, I_{mod} is set as 0, because the activity is well predicted by eqn (3), as shown in Fig 2.

$$pEC_{50} = 0.626\,(\pm 0.104)\ pK_i - 1.049\,(\pm 0.309)\,I_{CHNO_2} - 0.726\,(\pm 0.427)\,I_{branch/N3} + 1.307\,(\pm 0.265)\,I_{mod} + 1.850\,(\pm 0.419) \quad (4)$$

$$n = 57,\ r = 0.912,\ s = 0.397,\ F_{4,52} = 64.131$$

Coefficients of pK_i, I_{CHNO_2}, and $I_{branch/N3}$, in eqn (4) are consistent with those in eqn (3). Equation (4) means that the insecticidal activity correlates linearly with the binding activity for all compounds. The new indicator variable I_{mod} term in eqn (4) shows that the insecticidal activity values of the substituted diazacyclohexyl, diazacycloheptyl and dimethylimidazolidine derivatives are about 20-fold higher than the predicted values from their binding activity. The reason why I_{mod} was insignificant for compound **1** is probably due to the bulkiness of 1,3-diazacyclohexyl ring being close to that of the imidazolidine ring, but not as large as that for alkylated 1,3-diazacyclohexyl or 1,3-diazacycloheptyl rings.

We have examined how hydrophobicity participates in the regression analysis for chloronicotinyl-related compounds in previous papers.[9,17,19,20] The correlation between the insecticidal and neurophysiological activities was improved by adding the negative $(\log P)^2$ term.[9,19,20] The relationship between the binding and neurophysiological activities was also improved by including the log *P* term with a positive sign.[17] Since the signs of the hydrophobicity parameter (log *P*) are opposite in these correlation equations (insecticidal–neurophysiological and neurophysiological–binding), the hydrophobicity parameter could be cancelled in the correlation between insecticidal and binding activities, as previously discussed.[14] In fact, the addition of log *P* in eqn (4) was insignificant.

To date we have discussed the QSAR based on the binding potencies using labelled α-BGTX. However, recently it has been suggested that the binding assay is more straightforward when imidacloprid is used instead of α-BGTX as radioligand.[11,29] There is also an interesting argument that neonicotinoids possibly bind to distinct sites from α-BGTX, although on the same receptor, or on different receptors.[30–33] We recently measured the activity of a set of compounds in a binding assay using [^{3}H]imidacloprid and obtained a similar structure–activity relationship for a set of compounds. However, further considerations are required for a structure–activity relationship study of the wide rage of structures as suggested by Wollweber and Tietjen.[34] We are continuing our research to elucidate the binding mode for neonicotinoids, including chloronicotinyl compounds, by comparing the correlation equations using both ligands.

ACKNOWLEDGEMENTS

We are grateful to Dr Everett Bandman of the University of California Davis for reviewing this manuscript. A part of this study was performed in the RI Centre of Kyoto University.

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