Binding activity of substituted benzyl derivatives of chloronicotinyl insecticides to housefly-head membranes, and its relationship to insecticidal activity against the housefly *Musca domestica*

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Abstract: Variously substituted benzyl derivatives of chloronicotinyl insecticides were synthesized with a wide range of substituents including halogens, NO_2, CN, CF_3 and small alkyl and alkoxy groups at the *ortho, meta* and *para* positions, as well as multiple-substituted benzyl analogues. Their binding activity to the α-bungarotoxin binding site in housefly (*Musca domestica*) head membrane preparations was measured. Among the compounds tested, the activity of the *meta*-CN derivative was the highest, being 20–100 times higher than those of imidacloprid, acetamiprid and nitenpyram. The synergized insecticidal activity against houseflies was also measured for selected compounds with the metabolic inhibitor, NIA16388 (propargyl propyl phenylphosphonate). For the nitromethylene analogues, including both benzyl and pyridylmethyl analogues, higher binding activity usually resulted in higher insecticidal activity.

Keywords: chloronicotinyl insecticides; housefly; *Musca domestica*; nicotinic acetylcholine receptor (nAChR); α-bungarotoxin

1 INTRODUCTION

A series of recently developed insecticides, referred to as chloronicotinyl or neonicotinoid insecticides, act at the acetylcholine binding site of the nicotinic acetylcholine receptor (nAChR) of insects.[1–9] To date, three compounds are available commercially; imidacloprid,[10] acetamiprid[11] and nitenpyram.[12] These insecticides are remarkably potent neurotoxic insecticides, and are characterized by their persistent effects, broad spectra, good systemic properties and low toxicity to mammals and aquatic life. We have recently shown that the activity of chloronicotinyl compounds, including these commercial insecticides, is very weak in the receptor binding assay using the rat membrane fraction.[13]

Two different binding modes of neonicotinoids have been proposed; by Yamamoto *et al*,[14] and by Kagabu.[15] According to Yamamoto *et al*, the nitrogen atoms of the pyridine and imidazolidine rings interact with the hydrogen-donating and electron-rich sites, respectively, of the receptor. According to Kagabu, the oxygen atom of the nitro group, but not the nitrogen atom of the pyridine ring, plays the role of hydrogen acceptor. Comparative molecular field analysis (CoMFA) is a method for the analysis of three-dimensional quantitative structure-activity relationships (3-D QSAR).[16] Using this method, we have found that the binding mode of a set of neonicotinoids is more likely to be that proposed by Yamamoto *et al.* [17] This CoMFA study also disclosed the occurrence of other important steric and electrostatic interactions encompassing the imidazolidine ring.[17,18] However, since the structural variation of the 6-chloropyridine moiety was limited in the set of compounds examined, the factors that affect the interaction of this moiety with the receptor site remained unknown.

In the present study, we have examined the structure-activity relationships of chloronicotinyl compounds to clarify the factors affecting the interaction of the pyridine ring moiety and the receptor site. Structural modification of a benzene ring allows for many more variations than that of a pyridine ring. We

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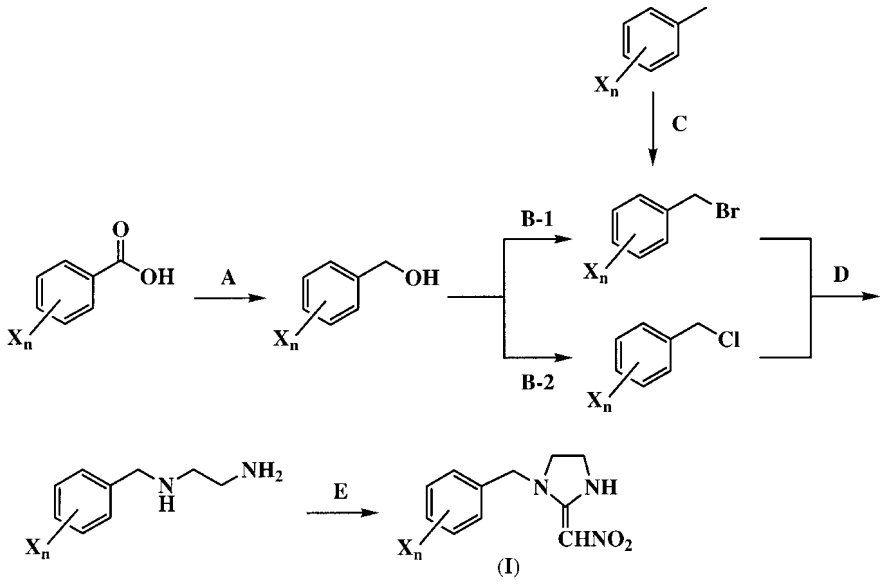


Figure 1. Synthetic scheme for benzyl derivatives of chloronicotinyl analogues.

therefore synthesized benzyl derivatives with various substituents at the *ortho, meta*, and *para* positions of the benzene ring in place of the 6-chloropyridine moiety of the representative chloronicotinyl compounds (**I**: Fig 1). In the imidazolidine moiety, a nitromethylene group was introduced instead of the nitroimino group of imidacloprid. This substitution was made because the binding activity of nitromethylene analogues having a pyridylmethyl group is higher than that of the corresponding nitroimine analogues.[4,8,17,18] We measured the binding activity of these compounds to α-bungarotoxin (α-BGTX) receptors of housefly-head membrane preparations, and also measured the insecticidal activity against houseflies for selected compounds and compared it with the binding activity.

2 EXPERIMENTAL METHODS

2.1 Chemicals

Compounds **1**–**41** listed in Table 1 were synthesized according to a published method.[19] Compounds **42**–**45** were kindly provided by Nihon Bayer Agrochem KK (Ibaraki, Japan). Compound **46** and compound **47** were from Nippon Soda Co, Ltd (Tokyo, Japan) and Takeda Chemical Industries, Ltd (Osaka, Japan), respectively. The synthetic scheme for the preparation of the benzyl compounds is shown in Fig 1. Reagents used for the syntheses were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan), Nacalai Tesque, Inc (Kyoto, Japan), Tokyo Chemical Industry Co, Ltd (Tokyo, Japan), and Aldrich Chemical Co (Milwaukee, WI, USA). α-BGTX was purchased from Sigma Chemical Co (St Louis, MI, USA), and [^{125}I]α-BGTX (*c* 74 TBq mmol^{-1}) was from Amersham Pharmacia Biotech (Buckinghamshire, UK). The metabolic inhibitor, NIA 16388 (NIA; propargyl propyl phenylphosphonate), was from our stock sample.[20] Intermediates and the final compounds were purified by column chromatography on Wakogel C-200 or by recrystallization if a solid. The structures of all compounds were confirmed by [^{1}H]NMR. Some intermediates were used without further purification. The authenticity of the final compounds was also confirmed by elemental analyses. The analytical values for C, H and N agreed with the calculated values within an error of ±0.3%. The [^{1}H]NMR analyses were performed using a Bruker AC-300 NMR spectrometer at 300 MHz in deuterochloroform ($CDCl_3$) with tetramethylsilane as the internal standard. Melting points of the compounds were measured with a Yanaco melting point apparatus (Kyoto, Japan) and were uncorrected. Melting points of the final compounds are listed in Table 1. Typical synthetic methods are described below.

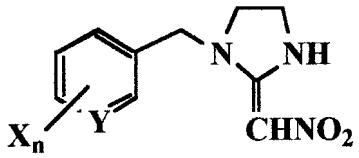
2.1.1 3,5-Di-tert-butylbenzyl alcohol (Step A)

3,5-Di-*tert*-butylbenzoic acid (3.00 g, 13 mmol) dissolved in anhydrous tetrahydrofuran (THF; 100 ml) was added dropwise to lithium aluminium hydride (0.97 g, 26 mmol) suspended in anhydrous THF (5 ml) while stirring in an ice-bath. The mixture was removed from the ice-bath and stirred overnight at room temperature. Water (2 ml) followed by aqueous sodium hydroxide solution (100 g litre^{-1}; 7 ml) were slowly added to the reaction mixture at 0 °C. After removing the precipitate by filtration, the filtrate was dried over anhydrous magnesium sulfate and evaporated to afford 3,5-di-*tert*-butylbenzyl alcohol as a colourless oil (2.61 g, 91% yield) for compound **38**. The necessary substituted benzyl alcohols to prepare compounds **6, 34**–**37** and **39** were synthesized from the corresponding benzoic acids using a similar procedure.

2.1.2 2,5-Dichlorobenzyl bromide (Step B-1)

Triphenylphosphine (3.02 g, 15 mmol) was added slowly to a solution of 2,5-dichlorobenzyl alcohol (1.77 g, 10 mmol) and carbon tetrabromide (3.32 g, 11 mmol) in anhydrous dichloromethane (10 ml), and the mixture was refluxed for 2 h. After evaporating the

Table 1. Binding and insecticidal activities of imidacloprid and its related compounds



Compound no	X_n	Y	Activity against houseflies: Binding pK_i (M) (±SEM) (n)	Activity against houseflies: Insecticidal pEC_{50} [a,b] (M)	mp (°C)
1	H	CH	4.65 (±0.18) (4)	3.61	145–149
2	2-F	CH	3.97 (±0.16) (2)	2.52	169–170
3	2-Cl	CH	3.43 (±0.18) (4)	2.09	197–199
4	2-CH_3	CH	3.53 (±0.15) (2)	<2.00 (13)	195–197
5	2-NO_2	CH	3.51 (±0.14) (2)	nd	209–211
6	2-CH_3O	CH	2.94 (±0.06) (2)	nd	147–150
7	2-CF_3	CH	3.01 (±0.05) (2)	nd	165–167
8	3-F	CH	5.66 (±0.13) (2)	3.60	149–152
9	3-Cl	CH	5.24 (±0.09) (2)	3.46	176–178
10	3-CH_3	CH	4.38 (±0.00) (2)	3.00	167–170
11	3-NO_2	CH	4.72 (±0.25) (3)	2.62	198–200
12	3-CH_3O	CH	4.04 (±0.15) (4)	nd	182–184
13	3-CF_3	CH	3.35 (±0.26) (4)	nd	164–168
14	3-CN	CH	6.73 (±0.28) (3)	3.98	182–185
15	3-C_6H_5O	CH	3.54 (±0.08) (2)	<2.55 (6)	154–155
16	4-F	CH	4.89 (±0.10) (2)	3.54	142–144
17	4-Cl	CH	5.40 (±0.30) (2)	4.18	169–172
18	4-Br	CH	4.90 (±0.01) (2)	nd	166–169
19	4-CH_3	CH	4.11 (±0.18) (2)	3.75	118–119
20	4-C_2H_5	CH	2.78 (±0.22) (2)	nd	122–125
21	4-*i*-C_3H_7	CH	2.87 (±0.09) (2)	nd	136–138
22	4-*t*-C_4H_9	CH	2.32 (1)	nd	210–213
23	4-NO_2	CH	3.54 (±0.22) (4)	nd	224–227
24	4-CH_3O	CH	2.99 (±0.15) (2)	nd	118–120
25	4-CF_3	CH	3.44 (±0.05) (2)	nd	150–152
26	4-CN	CH	3.18 (±0.16) (2)	nd	188–192
27	2,3,4,5,6-F_5	CH	2.83 (±0.08) (2)	nd	205–208
28	3,4-F_2	CH	5.42 (±0.11) (2)	3.39	167–170
29	3,5-F_2	CH	4.92 (±0.11) (2)	nd	161–164
30	2,3-Cl_2	CH	3.95 (±0.11) (2)	nd	198–200
31	2,5-Cl_2	CH	3.25 (±0.11) (2)	nd	208–211
32	2,6-Cl_2	CH	<3.48	nd	251–258
33	3,4-Cl_2	CH	5.92 (±0.17) (2)	3.23	181–184
34	3,5-Cl_2	CH	4.73 (±0.05) (2)	<2.73 (40)	184–186
35	2,3-$(CH_3)_2$	CH	3.52 (±0.11) (2)	nd	186–189
36	3,4-$(CH_3)_2$	CH	3.47 (±0.14) (2)	nd	120–124
37	3,5-$(CH_3)_2$	CH	4.39 (±0.15) (2)	nd	124–126
38	3,5-*t*-$(C_4H_9)_2$	CH	<3.57	nd	192–196
39	3,4-$(CH_3O)_2$	CH	3.21 (±0.13) (2)	nd	159–163
40	3-NO_2,4-CH_3	CH	4.80 (±0.09) (6)	2.69	191–195
41	3,4-OCH_2O-	CH	4.67 (±0.13) (2)	nd	174–176
42	H	N	7.43 (±0.13) (2)	5.50	—
43	6-Cl	N	7.93 (±0.11) (2)	5.64	—
44	6-CH_3	N	6.14 (1)	5.70	—
45	Imidacloprid		5.43 (±0.16) (2)	5.25	—
46	Acetamiprid		4.80 (±0.26) (2)	5.77	—
47	Nitenpyram		5.17[c]	5.63	—

[a] The values in parentheses are percentage mortality at the dose indicated.

[b] nd = not determined.

[c] From Reference 13.

solvent, the residue was suspended in diethyl ether (200 ml) and filtered to remove insoluble materials. The filtrate was evaporated and the residue was purified by silica-gel column chromatography with hexane as the mobile phase to afford 2,5-dichlorobenzyl bromide (2.22 g, 93% yield) for compound **31**. Other substituted benzyl bromides were synthesized from the corresponding alcohols to prepare compounds **7**, **30**, **36**, **37**, **39** and **41** using a similar procedure.

2.1.3 2,3-Dimethylbenzyl chloride (Step B-2)
After dissolving 2,3-dimethylbenzyl alcohol (1.25 g, 11 mmol) in thionyl chloride ($SOCl_2$; 2 ml), the solution was refluxed for 2 h. Excess $SOCl_2$ was evaporated and the residue was purified by silica-gel column chromatography to afford 2,3-dimethylbenzyl chloride as a colourless liquid (1.53 g, 90% yield). Other substituted benzyl chlorides were synthesized from the corresponding alcohols to prepare compounds **6**, **34**, **35** and **38** using a similar procedure.

2.1.4 3-Phenoxybenzyl bromide (Step C)
N-Bromosuccinimide (8.10 g, 50 mmol) and benzoyl peroxide (0.024 g, 0.1 mmol) were added to a solution of 3-phenoxytoluene (9.20 g, 50 mmol) in anhydrous carbon tetrabromide (30 ml) and the mixture refluxed for 2 h. After removing the precipitate by filtration, the filtrate was evaporated. The residue was purified by silica-gel column chromatography with hexane + ethyl acetate (9 + 1 by volume) as the mobile phase to afford 3-phenoxybenzyl bromide (1.76 g, 78% yield). The corresponding bromide for compound **14** was also synthesized using this method.

2.1.5 N-(2-Methylbenzyl)ethylenediamine (Step D)
2-Methylbenzyl chloride (1.40 g, 10 mmol) dissolved in acetonitrile (10 ml) was added dropwise to ethylenediamine (3.00 g, 50 mmol) while stirring in an ice-bath. After removing from the ice-bath and stirring overnight at room temperature, the solvent was evaporated. Aqueous sodium hydroxide (1 M; 50 ml) was added to the residue, which was then extracted with dichloromethane. The organic layer was dried over anhydrous magnesium sulfate and evaporated to afford *N*-(2-methylbenzyl)ethylenediamine (1.30 g, 79% yield). Diamines for other compounds were similarly prepared.

2.1.6 1-(4-Methylbenzyl)-2-nitromethylene-imidazolidine (Step E)
N-(4-Methylbenzyl)ethylenediamine (0.82 g, 5 mmol) dissolved in ethanol (20 ml) was added dropwise to 1,1-bis(methylthio)-2-nitroethylene (0.83 g, 5 mmol) dissolved in ethanol (10 ml). The mixture was refluxed overnight. After evaporating the solvent, the residue was recrystallized from ethanol to afford 1-(4-methylbenzyl)-2-nitromethylene-imidazolidine (0.66g, 57% yield). [^{1}H]NMR ($CDCl_3$/TMS) δ (ppm): 2.35 (3H, s), 3.56 (2H, t), 3.75 (2H, t), 4.26 (2H, s), 6.70 (1H, s), 7.11 (2H, d), 7.19 (2H, d), 8.71 (1H, br). Analysis: calculate, C (61.79), H (6.48), N (18.01); found, C (61.51), H (6.29), N (17.71). mp 118–119 °C. Compounds **1**–**18** and **20**–**41** were similarly prepared.

2.2 Bioassays

2.2.1 Insects
An insecticide-susceptible strain of the housefly (*Musca domestica* L, Takatsuki strain) was reared at 25 °C. Three- to six-day-old flies were used for the binding and insecticidal tests. Among the insects, females were selected for the insecticidal test.

2.2.2 [^{125}I] α-BGTX binding to membrane fraction
The procedures for the preparation of the housefly head membrane fraction and for the binding assay were fundamentally the same as those described previously.[17,18] Briefly, housefly heads (5 ml) were homogenized in sodium phosphate buffer (pH 7.4; 100 mM; 5 ml) containing sucrose (0.32 M) and EDTA (0.1 mM). The homogenate was filtered through gauze and centrifuged at 700*g* for 10 min, and the supernatant was further centrifuged at 125 000*g* for 60 min. The final precipitate was suspended in sodium phosphate buffer (pH 7.4; 10 mM; *c* 50 ml) containing sodium chloride (50 mM) and Triton X-100 (1 g litre^{-1}). This suspension was used for the binding assay. The protein concentration was measured by a bicichoninic acid protein-assay kit (Pierce Chemical Co, Rockford, IL, USA) with BSA as a standard. The final protein concentration of the membrane preparation was adjusted to 1.0 mg ml^{-1}.

The membrane preparation was pre-incubated with the test compounds (10^{-3} – 10^{-9} M) for at least 10 min, then [^{125}I]α-BGTX (0.2 nM) was added. After incubating 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 (1 g litre^{-1}). The filters were rinsed three times with sodium phosphate buffer (pH 7.4; 10 mM) containing sodium chloride (50 mM). After washing with methanol, drying and adding Microscinti-O (Packard Instrument Co, Meriden, CT, USA) as scintillation cocktail, the radioactivity was measured using a Topcount instrument (Packard Instrument Co, Meriden; CT, USA). The specific binding was determined as the difference of the radioactivity in the presence and absence of non-radioactive α-BGTX (10 μM). 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 value calculated by

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

where $[L]$ is the concentration of the [^{125}I]α-BGTX and K_d is the dissociation constant of α-BGTX. The K_d value was determined in each experiment by a non-

linear regression analysis from the saturation curve of [^{125}I]α-BGTX to nAChR using PRISM. The reciprocal logarithm of the K_i value, pK_i, was calculated for each compound listed in Table 1.

2.2.3 Insecticidal activity

The method for the determination of insecticidal activity was essentially the same as that reported by Yamamoto *et al*,[21] in which NIA16388 was topically applied 1 h before injection of the test compound. Female houseflies were anesthetized using carbon dioxide. A methanol solution (1 μl) containing NIA (2 g litre^{-1}) was topically applied to the abdomen. After 1 h at 25 °C, the flies were again anesthetized using carbon dioxide and solutions in ethanol + water (50 + 50 v/v; 0.24 μl) containing the test compounds at various concentrations were injected into the dorsal side of their thoraxes.

At high concentrations, the flies failed to regain consciousness. Early symptoms at lower, but effective, concentrations included hyperactivity and tremors of the body and legs. Some houseflies were unable to recover or right themselves upon awakening. All paralyzed insects were considered to be affected. In the preliminary test, we examined the time-response profiles for several compounds (data not shown). Since the most pronounced effect was seen 1 h after injection, we used 1 h for the determination of 50% effective concentration [EC_{50} (M)]. Control flies showed no adverse effects from carbon dioxide, the synergist alone or the injection procedure. Fifteen flies were used for each preparation. Six concentrations were generally used for each compound. The reciprocal logarithm of the EC_{50} value, pEC_{50}, was calculated for each compound using the probit method and defined as the insecticidal activity. The pEC_{50} values are listed in Table 1.

3 RESULTS AND DISCUSSION

3.1 Binding activity

The K_d value of α-BGTX was determined in each membrane preparation in order to calculate the K_i value for each compound. In this series of experiments, the K_d values varied from 0.24 to 3.58 nM. This large deviation seemed not to be unusual, because the K_d value has been found to vary over thirtyfold in other receptor binding assays using [^{3}H]apomorphine[22] and [^{3}H]spiperon.[23] In this study, the binding activity was expressed as pK_i as described above to minimize the deviation depending on the preparation. In addition, compound **40** was used as a reference compound for each membrane preparation and its standard deviation in the pK_i value was ± 0.09 for six determinations (Table 1).

Among the benzyl compounds, the 3-CN derivative (**14**) had the highest binding activity, being about 20 times greater than that of imidacloprid. It is interesting that the activity of compound **43**, which is the nitromethylene congener of imidaclopid, was 300 times greater than that of imidacloprid itself. Substituted pyridine compounds **42**–**44** had higher activities than the corresponding phenyl derivatives (**1** vs **42**; **17** vs **43**; **19** vs **44**). It is also interesting that these substituted-pyridyl compounds had much greater activities than the three standard compounds, imidacloprid (**45**), acetamiprid (**46**) and nitenpyram (**47**).

Introduction of any substituent at the *ortho* position of the benzene ring was unfavourable to the binding activity. Among *meta*-substituted derivatives, the single introduction of substituents such as F (**8**), Cl (**9**) and CN (**14**) significantly enhanced activity, whereas CH_3 (**10**), OCH_3 (**12**), CF_3 (**13**) and OC_6H_5 (**15**) groups decreased it. Compound **11** (3-NO_2) was equivalent to unsubstituted compound **1** in terms of pK_i. At the *para* position, introduction of Cl (**17**) was favourable to activity, although other halogens such as F (**16**) and Br (**18**) did not change the activity significantly. It was interesting that the activity of the 4-CN compound (**26**) was 1/30 of that of the unsubstituted compound **1**, whereas that of the 3-CN isomer (**14**) was 120 times higher than that of the unsubstituted compound **1**. Among the multiple-substituted derivatives, compounds **28** (3,4-F_2) and **33** (3,4-Cl_2) had much higher binding activities than compound **1**.

Previously, we analyzed the binding activity of imidacloprid and related compounds to the housefly nAChR using CoMFA[17,18] and agreed with the model proposed by Yamamoto *et al*,[14] in which the nitrogen atom of the pyridine ring possibly interacts with the positive site of the nAChR. In those analyses, two benzyl analogues, one of which was the same as compound **1** used in this study, were also included by superimposing the *meta*-C atom onto the N atom of the pyridine ring.[17,18] For this purpose, electrostatic charges were calculated using AM1, a semi-empirical molecular orbital method. The electronic charge of the C atom at the *meta* position of compound **1** was the most negative, being consistent with that observed for the corresponding pyridyl compound **42**, in which the nitrogen atom was the most negatively charged (Fig 2). Previously, we showed that the nitromethylene (or nitroimino) moiety and a portion of the imidazolidine ring were mainly surrounded by a sterically and electrostatically sensitive region of the receptor, but no electrostatic field appeared to surround the pyridine ring in that CoMFA.[17] To clarify the role of the benzyl and pyridyl moieties, classical and 3-D QSAR analyses are in progress in our laboratory using an expanded set of compounds.

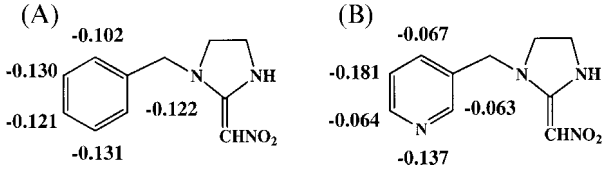


Figure 2. Charges of the carbon and nitrogen atoms on the aromatic rings calculated by the AM1 method: (A) compound **1** and (B) compound **42**.

Wollweber and Tietjen[24] suggested that imidacloprid and α-BGTX have different binding sites, because the binding activities of a large range of compounds evaluated using [^{3}H]imidacloprid and [^{125}I]α-BGTX as radioligands could not be correlated. Nevertheless, Liu *et al*[8] showed that the binding activities of chloronicotinyl compounds determined using [^{3}H]imidacloprid and [^{125}I]α-BGTX were correlated. Consequently, the binding activities evaluated with [^{125}I]α-BGTX have been used for a structure-activity study with the present set of compounds.

3.2 Insecticidal activity

The insecticidal activities of three standards, imidacloprid (**45**), acetamiprid (**46**) and nitenpyram (**47**), were very high, as expected. The activities of the substituted pyridyl compounds **42**–**44** were similar to those of the standards. Among the benzyl analogues tested, compound **17** (4-Cl) was the most potent, although it was 10 times less potent than imidacloprid (Table 1). The activity of compound **14** (3-CN), the binding activity of which was the highest among the benzyl analogues, was slightly less than that of compound **17**. Other substituted benzyl analogues were of similar or less potency than the unsubstituted compound **1**. Compounds **5**–**7**, **12**, **13**, **18**, **20**–**27**, **29**–**32**, **35**–**39** and **41** were not assayed, because their low binding activity indicated that their insecticidal activity would be less than that of the parent molecule (compound **1**).

The insecticidal activity against houseflies was plotted against binding activity (Fig 3). Except for imidacloprid (**45**), acetamiprid (**46**), nitenpyram (**47**) and compounds **19** and **44**, these two activity values were linearly correlated ($n = 15$, $r = 0.91$, $s = 0.43$). A similar relationship between insecticidal activity against houseflies and binding activity evaluated using [^{3}H]imidacloprid was reported by Tomizawa *et al.*[25] Imidacloprid (**45**) has the nitroimine substructure, and acetamiprid (**46**) and nitenpyram (**47**) do not carry the imidazolidine ring structure, and so differ from the mother structure of the present series of compounds. The replacement of the nitromethylene group with nitroimine on the imidazolidine ring and the opening of the cyclic imidazolidine moiety might be favourable to enhancing insecticidal activity. Structural similarities between two other outliers (compounds **19** and **44**) involve the position of the CH_3 group. Compounds **19** has a CH_3 group at the *para* position of the benzene ring and compound **44** has that at the corresponding position on the pyridyl moiety. The methyl group at these positions might have a role in increasing insecticidal activity compared with that expected from their binding activity.

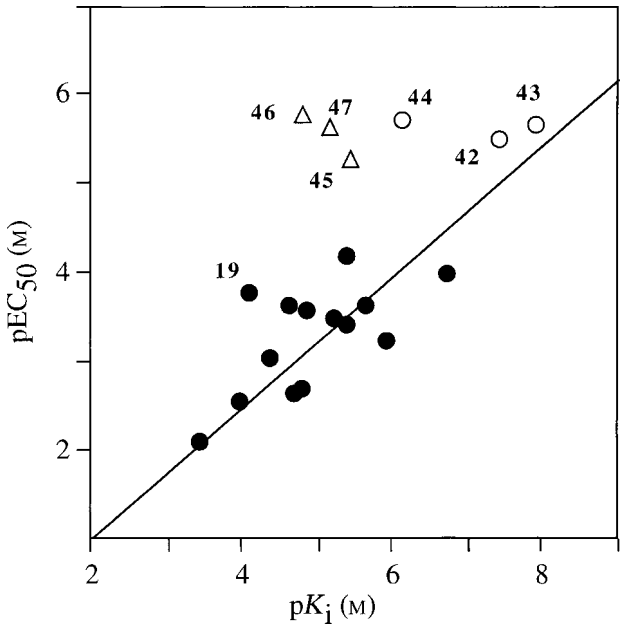


Figure 3. Relationship between the binding activity and the insecticidal activity against houseflies: (●) benzyl analogues, (○) pyridylmethyl analogues with a nitromethylene moiety, (△) imidacloprid, acetamiprid and nitenpyram. The regression line ($r = 0.91$ and $s = 0.43$) was derived for 15 compounds designated by open and closed circles except for compounds **19** and **44**.

When a compound arrives at the nAChR of insects, it interacts with the receptor to cause excitation and/or to block by competing with endogeneously released ACh. To evaluate the binding activity, radiolabelled nicotine[26,27] and imidacloprid[6–8,28] have also been used as radioligands. The binding activity evaluated with these and other ligands may reflect the behaviour of these compounds in the insect nervous system more directly than α-BGTX. We have only used the competitive binding activity evaluated with α-BGTX as an *in vitro* assay. Deflection of the insecticidal activity of compounds **19** and **44**–**47** from that expected from the binding activity (Fig 3) may be related to these factors.

In our earlier studies, insecticidal activity was found to increase with nerve excitatory activity for sets of imidacloprid-related compounds.[20,29] The insecticidal activity of compounds is governed by pharmaco-kinetic factors as well as pharmaco-dynamic ones. Since the compounds were injected in both the previous and the present experiments, the penetration phase in which chemicals move through the cuticle (a pharmaco-kinetic factor) was eliminated. Since the insecticidal activity of the previous sets of compounds against the American cockroach was enhanced by the use of NIA as well as piperonyl butoxide (PB),[20,29] another pharmaco-kinetic factor (detoxification) would be more-or-less eliminated under the conditions. It may be better to additionally use PB to determine the intrinsic insecticidal activity of the present set of compounds and to correlate with the binding activity.

In summary, the insecticidal activity of substituted benzyl and pyridyl derivatives of imidacloprid having a nitromethylene group at the imidazolidine ring generally increased with the binding activity. Three standard compounds showed a much higher insecticidal activity than the benzyl derivatives, but their binding activities were close to the average of the tested compounds. Of the benzyl compounds, the

3-CN derivative had the highest binding activity, but its insecticidal activity was only 1/13 that of imidacloprid.

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