Effect of substituents at the 5-position of the pyridine ring of imidacloprid on insecticidal activity against *Periplaneta americana*

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Abstract: The insecticidal activities of imidacloprid derivatives with a wide range of substituents at the 5-position on the pyridine ring against American cockroaches, *Periplaneta americana* (L.), were measured by injection with and without synergists propyl 2-propynyl phenylphosphonate and piperonyl butoxide. The log(1/MLD) value (MLD = minimal lethal dose in mol) without synergists was 7.96 for the methyl derivative, and the values were lower for other derivatives. Synergists enhanced the potencies of all the compounds tested. Considering these compounds and those with other substituents at this position, the region for maximum activity was predicted to be in the conjunction of the pyridyl 6-chlorine atom with a lipophilic small group in the 5-position.

Keywords: neonicotinoid insecticide; imidacloprid; American cockroach; *Periplaneta americana*; insecticidal activity; synergist; substituted pyridine

1 INTRODUCTION

Manifold structural variations of imidacloprid (**1a**; Fig. 1), the first neonicotinoid insecticide, have been contrived to find a new product with its own biological prominence.[1] Addition of a substituent to a site of the original structure or replacement with other groups is one of the most widely applied methodologies for drug design.[2] In the neonicotinoid field, introduction of a new substituent to the pyridine ring of imidacloprid or replacement of the chlorine atom at the 6-position with other groups has been studied.[3] However, structure–activity relationship studies showed that no variation of the original structure elevated the insecticidal potency of the original compound, regardless of the nature of the substituent and the site or the substituent number, except that the 6-fluoro derivative afforded comparable activity.[3,4] Similar insecticidal tendencies of single and double substitutions were reported in the case of the benzene derivative,[5,6] and the best fitness of the 6-chloro-3-pyridin-3-ylmethyl moiety for monosubstituted pyridin-3-ylmethyl molecules was verified by stereoelectronic considerations.[6,7] Against such a background, significant factors such as the relevant activities of 1-(5,6-dichloropyridin-3-ylmethyl)-2-nitromethylene or *N*-cyanoguanidine-imidazolidine[8,9] and the prediction of permissible steric regions in the vicinity of a chlorine atom at the 6-position on the pyridine ring of imidacloprid[6] may have gone unnoticed. Recently, Casida and co-workers[10–13] found that the introduction of an azido group at the 5-position of the pyridine ring (**1b**) retains or even improves the affinity to *Myzus* and *Drosophila* nAChRs. This new result has prompted an examination of the overall effect of added substituents at the 5-position on insecticidal activity as assessed by the authors' estimation method.[14] Firstly, the effect of halogen and pseudohalogen substituents such as azido and cyano on insecticidal activity was examined by injection into American cockroaches, *Periplaneta americana* (L.). Although the activity of azido compounds did not stand out against the insect tested, potency was observed to be strongly dependent on the substituents, and the fluoro derivative (**1c**) showed a high insecticidal potency comparable with that of imidacloprid.[14] The following experiments with 5-substituted alkoxy derivatives showed that all of the added substituents significantly lowered the potency of the parent compound.[15] In the present work, the effect of alkyl, trifluoromethyl, phenyl and ester derivatives of imidacloprid was examined, and the effect of substituents at this position was evaluated.

2 MATERIALS AND METHODS

2.1 Chemicals

All melting points (MP) are uncorrected. IR spectra were measured with a Perkin Elmer FTIR 1600 spectrometer. NMR spectra were obtained by a Varian Gemini 2000 C/H (400 MHz). The

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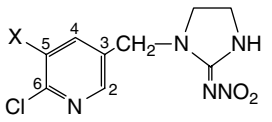


1a: imidacloprid (X=H)
1b: 5-azidoimidacloprid (X=N_3)
1c: 5-fluoroimidacloprid (X=F)

Figure 1. Imidacloprid and 5-substituted compounds.

chemical shifts δ were recorded in ppm and the coupling constants $\mathcal{J}$ in Hz. Mass spectra were recorded by a Jeol JMS-700. The following products were prepared according to procedures described previously:[16] 1-(6-chloro-5-methylpyridin-3-ylmethyl)-2-nitroiminoimidazolidine (**1o)**, 1-(6-chloro-5-trifluoromethylpyridin-3-ylmethyl)-2-nitroiminoimidazolidine (**1t)** and 1-(6-chloro-5-methoxycarbonylpyridin-3-ylmethyl)-2-nitroiminoimidazolidine (**1u**). Propyl 2-propynyl phenylphosphonate (NIA16388 or NIA) for insecticidal testing was obtained from the stock sample used in previous studies.[17–21] Piperonyl butoxide (PB), an inhibitor of mixed-function oxidases, was purchased from Nakalai Tesque (Kyoto, Japan).

2.2 Synthesis

2.2.1 N-*(6-Methoxypyridin-3-ylmethyl)morpholine (**3; Fig. 2**)*

Powdered potassium hydroxide (5.60 g, 100 mmol) was added rapidly to a stirred mixture of dibenzo-18-crown-6 (420 mg) in dry toluene (20 mL), with subsequent dropwise addition of a solution of *N*-(6-chloropyridin-3-ylmethyl)morpholine[10] (**2**; 4.42 g, 20.1 mmol) in dry methanol (4.2 mL). The mixture was warmed at 65 °C for 5 h. The toluene was evaporated, the residue was diluted 3 times with isopropyl ether (IPE; 3 × 15 mL) and the combined IPE-soluble fraction was dried. After evaporation of IPE, column chromatography of the residue on SiO_2, with IPE + hexane (1 + 1 by volume) as the eluate, afforded a colourless liquid. Yield: 2.73 g (62%). IR (liquid) (cm^{-1}): 1595 (m), 1510 (vs), 1455 (m), 1255 (vs), 1230 (m), 1130 (s), 740 (m), 670 (m). ^{1}H NMR (δ, $CDCl_3$): 2.42 (m, 2 × 2H, $NC\underline{H}_2CH_2O$), 3.42 (s, 2H, CH_2), 3.69 (s, 2 × 2H, $NCH_2C\underline{H}_2O$), 3.92 (s, 3H, CH_3), 6.71 (d, 1H, 3-Py-H, $\mathcal{J}$ = 8.6 Hz), 7.56 (dd, 1H, 4-Py-H, $\mathcal{J}$ = 8.6/2.3 Hz), 8.04 (d, 1H, 6-Py-H, $\mathcal{J}$ = 2.3 Hz); ^{13}C NMR: 53.4, 53.5, 60.0, 67.0, 110.7, 125.8, 139.8, 147.1, 163.7. MS *m/z* (%): 208 (M^+, 97), 207 (23), 177 (35), 137 (11), 136 (15), 135 (11), 123 (22), 122 (100), 86 (40). HRMS (EI) *m/z* (M^+): calculated for $C_{11}H_{16}N_2O_2$, 208.1211; found, 208.1236.

2.2.2 N-*(5-Ethyl-6-methoxypyridin-3-ylmethyl)morpholine (**4p**)*

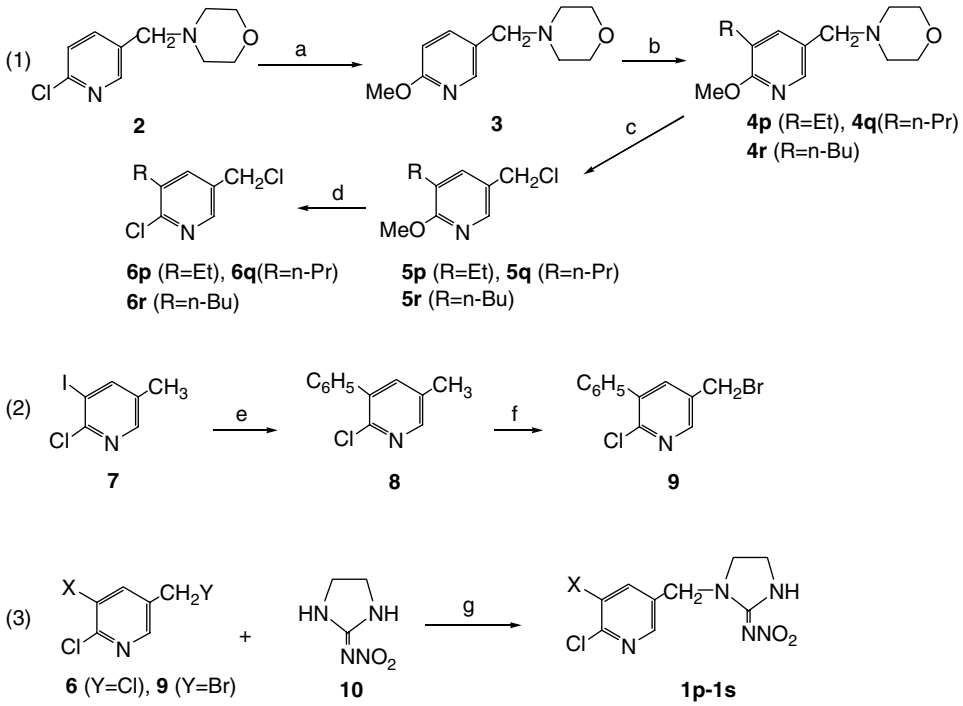
A solution of methyllithium (1.6 M in diethyl ether, 6.3 mL, 10 mmol) was added to tetrahydrofuran (THF; 8 mL) under an argon atmosphere at −45 °C. A solution of diisopropylamine (10 mg, 0.1 mmol) and **3** (416 mg, 2 mmol) in THF (0.5 mL) was added dropwise. The mixture was stirred at a temperature ranging from −10 to 0 °C for 3 h and then cooled again to −70 °C before dropwise introduction of a solution of iodoethane (1.56 g, 10 mmol) in THF (0.5 mL). The mixture was allowed to stand in a cooling bath overnight (the temperature rose to about 0 °C in this time). During ice cooling, a few drops of a mixture of concentrated hydrochloric acid and THF (2 mL) was added, and, after 5 min, aqueous sodium hydroxide (40 g L^{-1}, 2 mL) was added. After 30 min of stirring, the reaction mixture was poured onto water and extracted with IPE (3 × 10 mL). The solution was dried, the solvent was evaporated and the residue (280 mg), composed of products **4p** and **3** in a ratio of about 3:2 (NMR), was used in the next step. The following ^{1}H NMR shifts were assigned for compound **4p**. ^{1}H NMR (δ, $CDCl_3$): 1.21 (t, 3H, $C\underline{H}_3CH_2Py$, $\mathcal{J}$ = 7.3), 2.42 (m, 2 × 2H, $NC\underline{H}_2CH_2O$), 2.58 (q, 2H, $CH_3C\underline{H}_2Py$, $\mathcal{J}$ = 7.3), 3.40 (s, 2H, CH_2), 3.69 (m, 2 × 2H, $NCH_2C\underline{H}_2O$), 3.94 (s, 3H, CH_3), 7.39 (d, 1H, 4-Py-H, $\mathcal{J}$ = 2.4 Hz), 7.87 (d, 1H, 6-Py-H, $\mathcal{J}$ = 2.4 Hz).

*2.2.3 5-Ethyl-6-methoxypyridin-3-ylmethyl chloride (**5p**)*

The mixture of Section 2.2.2 in 20 mL of THF was treated with 280 mg of ethyl chloroformate and stirred at 60 °C for 2 h, and the volatile materials were evaporated. The residue was purified by chromatography on SiO_2, using IPE + hexane (1 + 4 by volume) as the eluate, to yield 135 mg of product. IR (liquid) (cm^{-1}): 2880 (m), 1610 (m), 1560 (m), 1510 (s), 1475 (vs), 1405 (m), 1250 (s), 1205 (s), 1025 (m), 835 (m), 760 (s), 690 (s). ^{1}H NMR (δ, $CDCl_3$): 1.20 (t, 3H, $CH_2C\underline{H}_3$, $\mathcal{J}$ = 7.4 Hz), 2.58 (q, 2H, $C\underline{H}_2CH_3$, $\mathcal{J}$ = 7.4 Hz), 3.95 (s, 3H, OCH_3), 4.53 (s, 2H, CH_2Cl), 7.43 (d, 1H, 4-Py-H, $\mathcal{J}$ = 2.6 Hz), 7.97 (d, 1H, 6-Py-H, $\mathcal{J}$ = 2.6 Hz); ^{13}C NMR: 13.0, 22.8, 43.7, 53.5, 126.1, 137.1, 138.9, 143.6, 162.2. MS *m/z* (%): 187 (12), 185 (M^+, 37), 171 (11), 170 (26), 151 (11), 150 (100), 136 (28), 107 (14). HRMS (EI) *m/z* (M^+): calculated for $C_9H_{12}ClNO$, 185.0608; found, 185.0621.

*2.2.4 6-Chloro-5-ethylpyridin-3-ylmethyl chloride (**6p**)*

$POCl_3$ (0.5 mL) was added to a solution of **5p** (130 mg) in *N*,*N*-dimethylformamide (DMF; 0.5 mL) with external ice cooling. The mixture was allowed to warm to room temperature and was then heated at 140 °C for 2 h. The cooled reaction mixture was poured onto cold water. IPE extracts were washed with water, dried and evaporated. Preparative chromatography of the residue on SiO_2 with hexane + IPE (4 + 1 by volume) afforded 100 mg (79%) of product. IR (liquid) (cm^{-1}): 2930 (m), 1595 (m), 1565 (m), 1430 (m), 1405 (vs), 1175 (m), 1080 (s), 925 (m), 740 (m), 690 (m). ^{1}H NMR (δ, $CDCl_3$): 1.28 (t, 3H, $CH_2C\underline{H}_3$, $\mathcal{J}$ = 7.5), 2.76 (q, 2H, $C\underline{H}_2CH_3$,



Reagents and conditions.
(a) KOH, MeOH, toluene, dibenzo-18-crown-6, 65°C, 5 h.
(b) (i) MeLi, diisopropylamine, THF, -45°C to 0°C, 3 h; (ii) RI, -70°C to 0°C, overnight.
(c) $ClCO_2Et$, THF, 60°C, 2 h.
(d) $POCl_3$ / DMF, room temp gradually to 140°C, 2 h.
(e) $C_6H_5B(OH)_2$, $[P(C_6H_5)_3]_4Pd(0)$, dimethoxyethane / H_2O, 85°C, 10 h.
(f) NBS, $(C_6H_5CO_2)_2$, CCl_4, reflux, 10 h.
(g) NaH / DMF, 0°C to room temperature, 2 h.

Figure 2. Route to 5-alkyl and phenyl substituted imidacloprid.

$\mathcal{J} = 7.5$ Hz), 4.56 (s, 2H, CH_2Cl), 7.60 (d, 1H, 4-Ph-H, $\mathcal{J} = 2.3$ Hz), 8.23 (d, 1H, 6-Py-H, $\mathcal{J} = 2.3$ Hz); ^{13}C NMR: 13.2, 26.2, 42.3, 123.5, 138.1, 139.5, 146.5, 151.2. MS *m/z* (%): 191 (26), 189 (M^+, 41), 176 (10), 175 (11), 174 (15), 156 (52), 155 (15), 154 (100), 142 (14), 140 (44), 118 (16), 117 (11), 91 (21). HRMS (EI) *m/z* (M^+): calculated for $C_8H_9Cl_2N$, 189.0112; found, 189.0123.

*2.2.5 1-(6-Chloro-5-ethylpyridin-3-ylmethyl)-2-nitroiminoimidazolidine (**1p**)*

A solution of 2-nitroimino-4-imidazoline (**10**; 130 mg, 1.00 mmol) in DMF (15 mL) was treated with sodium hydride (60% oil dispersion, 60 mg, 1.50 mmol) at 5 °C. The mixture was stirred at room temperature until no hydrogen was produced and then cooled again to 0 °C. A solution of **6p** (189 mg, 1.0 mmol) in DMF (5 mL) was added dropwise. The cooling bath was set aside and the solution was stirred at room temperature for 2 h. The reaction was quenched with one drop of acetic acid, and the DMF was evaporated under vacuum. Chromatography on SiO_2 with hexane + ethyl acetate (1 + 10 by volume) gave 122 mg (43% yield) of product, which was purified by recrystallization from methanol. MP: 146–147 °C. IR (KBr) (cm^{-1}): 3300 (m), 1600–1575 (s–vs), 1550 (vs), 1480 (s), 1450 (vs), 1405 (vs), 1280–1220 (s), 1080 (s), 1050 (s), 950 (s), 750 (m), 660 (m). ^1H NMR (δ, $CDCl_3$): 1.25 (t, 3H, $CH_2C\underline{H}_3$, $\mathcal{J} = 8.0$ Hz), 2.75 (q, 2H, $C\underline{H}_2CH_3$, $\mathcal{J} = 8.0$ Hz), 3.50 (m, 2H, NCH_2CH_2N), 3.80 (m, 2H, NCH_2CH_2N), 4.52 (s, 2H, Py-CH_2), 7.57 (d, 1H, 4-Py-H, $\mathcal{J} = 2.3$ Hz), 8.15 (d, 1H, 6-Py-H, $\mathcal{J} = 2.3$ Hz), 8.19 (bs, 1H, NH); ^{13}C NMR: 13.3, 26.3, 41.5, 45.2, 45.4, 130.0, 138.2, 138.5, 146.5, 151.4, 161.3. MS *m/z* (%): 283 (M^+, 3), 240 (41), 239 (100), 238 (100), 237 (100), 235 (54), 224 (27), 223 (54), 223 (36), 222 (73), 221 (42), 210 (31), 209 (77), 208 (61), 207 (56), 202 (100), 195 (33), 187 (72), 182 (40), 174 (23), 168 (30), 154 (90), 140 (24). Analysis. Found: C, 46.56; H, 4.82; N, 24.80. Calculated for $C_{11}H_{14}ClN_5O_2$: C, 46.57; H, 4.97; N, 24.69.

Compounds **1q** and **1r** were prepared according to the procedures above.

Pure 6-methoxy-5-*n*-propylpyridine-3-ylmethylmorpholine (**4q**) could not be isolated. A mixture (the ratio of **4q** to **3** was 2:5, based on ^1H NMR) was used to the next step. The following ^1H NMR shifts were assigned for compound **4q**. ^1H NMR (δ, $CDCl_3$): 0.94 (t, 3H, $\mathcal{J} = 7.4$ Hz), 1.62 (m, 2H), 2.42 (m, 4H), 2.53 (t, 2H, $\mathcal{J} = 7.4$ Hz), 3.69 (m, 4H), 3.93 (s, 3H), 7.37 (d, 1H, $\mathcal{J} = 2.3$ Hz), 7.87 (d, 1H, $\mathcal{J} = 2.3$ Hz).

*2.2.6 6-Methoxy-5-*n*-propylpyridin-3-ylmethyl chloride (**5q**)*

IR (liquid) (cm^{-1}): 2960 (m), 1610 (s), 1580 (m), 1475 (vs), 1405 (vs), 1315 (s), 1255 (vs), 1145 (m), 1025 (s), 765 (m), 695 (m). ^1H NMR (δ, $CDCl_3$): 0.95 (t, 3H, $CH_2CH_2C\underline{H}_3$, $J = 7.5$ Hz), 1.62 (m, 2H, $CH_2C\underline{H}_2CH_3$), 2.54 (t, 2H, $C\underline{H}_2CH_2CH_3$, $J = 7.4$ Hz), 3.95 (s, 3H, OCH_3), 4.52 (s, 2H, CH_2Cl), 7.43 (d, 1H, 4-Py-H, $J = 2.3$ Hz), 7.98 (d, 1H, 6-Py-H, $J = 2.3$ Hz); ^{13}C NMR: 13.90, 22.0, 31.8, 43.7, 53.5, 125.5, 126.0, 138.1, 143.7, 162.3. MS *m/z* (%): 201 (17), 199 (M^+, 51), 184 (58), 164 (100), 136 (38), 107 (22). HRMS (EI) *m/z* (M^+): calculated for $C_{10}H_{14}ClNO$, 199.0765; found, 199.0790.

*2.2.7 6-Chloro-5-*n*-propylpyridin-3-ylmethyl chloride (**6q**)*

IR (liquid) (cm^{-1}): 2950 (m), 1595 (m), 1565 (m), 1435 (m), 1405 (vs), 1350 (m), 1180 (m), 1080 (s), 730 (m), 690 (m). ^1H NMR (δ, $CDCl_3$): 0.99 (t, 3H, $CH_2CH_2C\underline{H}_3$, $J = 7.3$), 1.67 (m, 2H, $CH_2C\underline{H}_2CH_3$), 2.70 (t, 2H, $C\underline{H}_2CH_2CH_3$, $J = 7.3$ Hz), 4.56 (s, 2H, CH_2Cl), 7.60 (d, 1H, 4-Py-H, $J = 2.6$ Hz), 8.23 (d, 1H, 6-Py-H, $J = 2.6$ Hz); ^{13}C NMR: 13.7, 22.2, 34.9, 42.2, 132.3, 136.6, 138.8, 146.4, 151.2. MS *m/z* (%): 205 (38), 203 (M^+, 60), 176 (52), 175 (131), 174 (80), 170 (43), 168 (100), 140 (20), 104 (15). HRMS (EI) *m/z* (M^+): calculated for $C_9H_{11}Cl_2N$, 203.0269; found, 203.0298.

*2.2.8 1-(6-Chloro-5-*n*-propyl-pyridin-3-ylmethyl)-2-nitroiminoimidazolidine (**1q**)*

MP: 125–127 °C. IR (KBr) (cm^{-1}): 3400 (m), 2960 (m), 1600–1550 (s–vs), 1480 (m), 1450–1405 (s–vs), 1295 (vs), 1220 (vs), 1140 (s), 1090 (m), 1045 (s), 945 (s), 760 (m), 710 (m). ^1H NMR (δ, $CDCl_3$): 0.97 (t, 3H, $CH_2CH_2C\underline{H}_3$, $J = 7.3$ Hz), 1.66 (m, 2H, $CH_2C\underline{H}_2CH_3$), 2.69 (t, 2H, $C\underline{H}_2CH_2CH_3$, $J = 7.4$ Hz), 3.54 (m, 2H, NCH_2CH_2N), 3.83 (m, 2H, NCH_2CH_2N), 4.53 (s, 2H, CH_2Cl), 7.54 (d, 1H, 4-Py-H, $J = 2.5$ Hz), 8.16 (d, 1H, 6-Py-H, $J = 2.5$ Hz), 8.26 (bs, 1H, NH); ^{13}C NMR: 14.0, 22.6, 35.2, 41.8, 45.4, 45.5, 130.2, 137.2, 139.1, 146.7, 151.4, 161.4. MS *m/z* (%): 297 (M^+, 1), 253 (81), 251 (100), 223 (13), 222 (22), 221 (21), 213 (61), 209 (44), 187 (34), 168 (20), 118 (10), 104 (14). Analysis. Found: C, 48.52; H, 5.55; N, 23.59. Calculated for $C_{12}H_{16}ClN_5O_2$: C, 48.40; H, 5.42; N, 23.52.

2.2.9 N-*(5-*n*-Butyl-6-methoxypyridin-3-ylmethyl) morpholine (**4r**)*

A portion of a 1:1 mixture of the product and **3** obtained according to the above procedures was subjected to repeated separation by preparative thin-layer chromatography with IPE + hexane (1 + 4 by volume), and the purified product was obtained as colourless liquid. IR (liquid) (cm^{-1}): 2955 (m), 1610 (m), 1475 (s), 1405 (s), 1350 (m), 1250 (m), 1120 (vs), 1025 (s), 865 (m), 760 (m), 670 (m). ^1H NMR (δ, $CDCl_3$): 0.93 (t, 3H, $(CH_2)_2CH_2C\underline{H}_3$, $J = 7.3$ Hz), 1.36 (m, 2H, $(CH_2)_2C\underline{H}_2CH_3$), 1.55 (m, 2H, $CH_2C\underline{H}_2C_2H_5$), 2.42 (t, 2 × 2H, $NC\underline{H}_2CH_2O$, $J = 4.4$ Hz), 2.54 (t, 2H, $C\underline{H}_2CH_2C_2H_5$, $J = 7.7$ Hz), 3.40 (s, 2H, Py-CH_2), 3.69 (t, 2 × 2H, $NCH_2C\underline{H}_2O$, $J = 4.4$ Hz), 3.93 (s, 3H, OCH_3), 7.38 (d, 1H, 4-Py-H, $J = 2.6$ Hz), 7.87 (d, 1H, 6-Py-H, $J = 2.6$ Hz); ^{13}C NMR: 14.0, 22.6, 29.5, 31.2, 53.4, 53.5, 60.2, 67.0, 125.1, 125.5, 138.9, 144.2, 161.8. MS *m/z* (%): 264 (M^+, 46), 233 (11), 192 (25), 178 (100), 149 (54), 136 (14), 113 (12), 97 (14), 86 (25). HRMS (EI) *m/z* (M^+): calculated for $C_{15}H_{24}N_2O_2$, 264.1837; found, 264.1856.

*2.2.10 5-*n*-Butyl-6-methoxypyridin-3-ylmethyl chloride (**5r**)*

IR (liquid) (cm^{-1}): 2960 (m), 1560 (m), 1430 (s), 1405 (vs), 1265 (m), 1175 (m), 1095 (s), 1065 (vs), 930 (m), 735 (vs). ^1H NMR (δ, $CDCl_3$): 0.94 (t, 3H, $(CH_2)_2CH_2C\underline{H}_3$, $J = 7.3$ Hz), 1.36 (m, 2H, $CH_2CH_2C\underline{H}_2CH_3$), 1.62 (m, 2H, $CH_2C\underline{H}_2CH_2CH_3$), 2.54 (t, 2H, $C\underline{H}_2CH_2C_2H_5$, $J = 7.7$ Hz), 3.95 (s, 3H, OCH_3), 4.54 (s, 2H, Py-CH_2), 7.43 (d, 1H, 4-Py-H, $J = 2.5$ Hz), 7.98 (d, 1H, 6-Py-H, $J = 2.5$ Hz); ^{13}C NMR: 14.0, 22.6, 29.5, 31.1, 43.8, 53.6, 125.8, 126.1, 138.1, 143.7, 162.4. MS *m/z* (%): 215 (10), 213 (M^+, 30), 196 (40), 178 (100), 150 (20), 120 (15). HRMS (EI) *m/z* (M^+): calculated for $C_{11}H_{16}ClNO$, 213.0921; found, 213.0955.

*2.2.11 5-*n*-Butyl-6-chloropyridin-3-ylmethyl chloride (**6r**)*

IR (liquid) (cm^{-1}): 2560 (m), 1610 (m), 1580 (m), 1475 (vs), 1405 (vs), 1320 (s), 1255 (vs), 1145 (m), 1025 (vs), 780 (m), 695 (m). ^1H NMR (δ, $CDCl_3$): 0.97 (t, 3H, $CH_2CH_2CH_2C\underline{H}_3$, $J = 7.3$ Hz), 1.40 (m, 2H, $CH_2CH_2C\underline{H}_2CH_3$), 1.65 (m, 2H, $CH_2C\underline{H}_2CH_2CH_3$), 2.73 (t, 2H, $C\underline{H}_2CH_2C_2H_5$, $J = 7.6$ Hz), 4.55 (s, 2H, CH_2Cl), 7.58 (d, 1H, 4-Py-H, $J = 2.2$ Hz), 8.23 (d, 1H, 6-Py-H, $J = 2.2$ Hz); ^{13}C NMR: 14.1, 22.8, 31.5, 33.1, 42.6, 132.6, 137.2, 139.1, 146.8, 151.2. MS *m/z* (%): 217 (M^+, 65), 184 (36), 182 (100), 175 (81), 174 (71), 140 (60), 104 (36). HRMS (EI) *m/z* (M^+): calculated for $C_{10}H_{13}Cl_2N$, 217.0425; found, 217.0426.

*2.2.12 1-(5-*n*-Butyl-6-chloropyridin-3-ylmethyl)-2-nitroiminoimidazolidine (**1r**)*

MP: 115–118 °C. IR (KBr) (cm^{-1}): 3430 (m), 1575 (vs), 1480 (m), 1440 (vs), 1400 (s), 1325 (s), 1295 (vs), 1230 (s), 1140 (m), 1090 (m), 1045 (s), 940 (s), 780 (m), 710 (m). ^1H NMR (δ, $CDCl_3$): 0.96 (t, 3H, $(CH_2)_2CH_2C\underline{H}_3$, $J = 7.3$ Hz), 1.41 (m, 2H, $CH_2CH_2C\underline{H}_2CH_3$), 1.60 (m, 2H, $CH_2C\underline{H}_2CH_2CH_3$), 2.71 (t, 2H, $C\underline{H}_2CH_2C_2H_5$, $J = 7.7$ Hz), 3.50 (m, 2H, NCH_2CH_2N), 3.79 (m, 2H, NCH_2CH_2N), 4.52 (s, 2H, CH_2Cl), 7.54 (d,

Table 1. Insecticidal activities of substituted imidacloprid derivatives and (*S*)-nicotine[a]

Compound		log(1/MLD) (mol)			Compound		log(1/MLD) (mol)		
No.	X	Alone	+NIA	+(PB + NIA)	No.	X	Alone	+NIA	+(PB + NIA)
1a[b]	H	8.96	10.15	10.15	**1l**[c]	OPr(i)	6.99	7.89	8.10
1b[b]	N_3	7.37	8.18	8.18	**1m**[c]	OBu(n)	7.12	8.12	8.12
1c[b]	F	8.82	9.91	9.91	**1n**[c]	OPent(n)	5.86	6.46	6.46
1d[b]	Cl	8.35	9.05	9.05	**1o**	Me	7.96	9.26	9.26
1e[b]	Br	8.02	9.11	9.11	**1p**	Et	<6.97	7.58	7.88
1f[b]	I	7.54	8.64	8.64	**1q**	Pr(n)	<7.02	7.51	7.62
1g[b]	CN	<7.24	<7.24	6.30	**1r**	Bu(n)	<6.08	6.17	6.27
1h[b]	NO_2	<7.22	<7.22	5.77	**1s**	Ph	<7.22	7.52	7.92
1i[c]	OMe	7.43	8.74	8.74	**1t**	CF_3	6.69	7.99	8.20
1j[c]	OEt	7.07	7.87	8.08	**1u**	COOMe	<6.50	<6.50	<6.50
1k[c]	OPr(n)	<6.89	6.89	6.89	(*S*)-nicotine[d]			5.51	

[a] See Fig. 1 for the general structure.
[b] Ref. 14.
[c] Ref. 15.
[d] Ref. 21.

1H, 4-Py-H, $J = 2.5$ Hz), 8.14 (d, 1H, 6-Py-H, $J = 2.5$ Hz), 8.17 (bs, 1H, NH); ^{13}C NMR: 13.9, 22.5, 31.3, 32.9, 41.5, 45.2, 45.5, 129.9, 137.5, 139.0, 146.6, 151.6, 161.4. MS *m/z* (%): 313 (2), 311 (M^+, 5), 268 (43), 266 (100), 221 (55), 211 (45), 209 (99), 187 (81), 167 (10). Analysis. Found: C, 50.31; H, 5.65; N, 22.80. Calculated for $C_{13}H_{18}ClN_5O_2$: C, 50.08; H, 5.82; N, 22.47.

*2.2.13 2-Chloro-5-methyl-3-phenylpyridine (**8**)*

A mixture of **7**[14] (253 mg, 1 mmol), phenylboronic acid (195 mg, 1.5 mmol), tetrakis(triphenylphosphine) palladium(0) (20 mg) and potassium carbonate (202 mg, 1.5 mmol) in dimethoxyethane (4 mL) and water (1 mL) was stirred at 85 °C for 3.5 h. The mixture was diluted with IPE (20 mL) and insoluble materials were discarded. The separated organic phase was washed with brine and dried over magnesium sulfate. After evaporating the solvents, the residue was chromatographed on SiO_2 with hexane + IPE (4 + 1 by volume) as the eluate, and a colourless liquid product was obtained. Yield: 102 mg (50%). IR (liquid) (cm^{-1}): 3150 (m), 1565 (m), 1510 (vs), 1320 (m), 1220 (s), 1155 (m), 1140 (m), 1000 (s), 820 (s), 810 (s). ^{1}H NMR (δ, $CDCl_3$): 2.35 (s, 3H, CH_3), 7.38–7.48 (m, 5H, Ph), 7.48 (d, 1H, 4-Py-H, $J = 1.8$ Hz), 8.21 (d, 1H, 6-Py-H, $J = 1.8$ Hz); ^{13}C NMR: 17.6, 128.1, 128.2, 129.2, 132.3, 136.1, 137.5, 140.4, 146.7, 148.5. MS *m/z* (%): 205 (77), 204 (68), 203 (M^+, 100), 168 (71), 167 (61), 166 (46), 165 (18), 153 (19), 152 (18), 141 (36), 140 (32), 139 (25), 138 (21), 115 (32), 114 (31). Analysis. Found: C, 70.56; H, 4.79; N, 6.56. Calculated for $C_{12}H_{10}ClN$: C, 70.77; H, 4.95; N, 6.88.

*2.2.14 6-Chloro-5-phenylpyridin-3-ylmethyl bromide (**9**)*

A mixture of compound **8** (228 mg, 1.12 mmol) and *N*-bromosuccinimide (300 mg, 1.68 mmol) in carbon tetrachloride (15 mL) was stirred with a catalytic amount of benzoyl peroxide at reflux temperature for 10 h. The solids precipitated on cooling were filtered off, and the filtrate, after evaporation of the solvent, was subjected to column chromatography of the residual liquid on SiO_2 with hexane + IPE (10 + 1 by volume). Yield: 158 mg (50%). MP: 56–57 °C. IR (KBr) (cm^{-1}): 1440 (m), 1400 (vs), 1210 (s), 1105 (s), 1080 (m), 1045 (m), 1030 (m), 895 (m), 760 (s), 700 (vs). ^{1}H NMR (δ, $CDCl_3$): 4.49 (s, 2H, CH_2), 7.41–7.50 (m, 5H, Ph), 7.72 (d, 1H, 4-Py-H, $J = 2.2$ Hz), 8.40 (d, 1H, 6-Py-H, $J = 2.2$ Hz); ^{13}C NMR: 28.3, 128.4, 128.6, 129.2, 132.9, 136.8, 137.1, 140.2, 148.0, 149.5. MS *m/z* (%): 283 (18), 281 (M^+, 13), 204 (33), 202 (100), 167 (20), 166 (13), 140 (14), 139 (36). Analysis. Found: C, 49.98; H, 3.45; N, 4.79. Calculated for $C_{12}H_9BrClN$: C, 51.01; H, 3.21; N, 4.96.

*2.2.15 1-(6-Chloro-5-phenylpyridin-3-ylmethyl)-2-nitroiminoimidazolidine (**1s**)*

The compound was prepared analogously to compound **1p**. Yield: 36%. MP: 194–196 °C. IR (KBr) (cm^{-1}): 3400 (s), 2870 (m), 1580–1540 (s), 1440 (s), 1400 (vs), 1320 (s), 1290 (vs), 1220 (vs), 1100 (m), 1040 (m), 940 (m), 700 (m). ^{1}H NMR (δ, $CDCl_3$): 3.56 (m, 2H, NCH_2CH_2N), 3.80 (m, 2H, NCH_2CH_2N), 4.59 (s, 2H, CH_2), 7.40–7.49 (m, 5H, Ph), 7.66 (d, 1H, 4-Py-H, $J = 2.2$ Hz), 8.22 (bs, 1H, NH), 8.32 (d, 1H, 6-Py-H, $J = 2.2$ Hz); ^{13}C NMR: 41.5, 45.2, 45.3, 128.5, 128.6, 129.2, 130.1, 136.7, 137.3, 139.7, 147.7, 149.7, 161.2. MS *m/z* (%): 331 (M^+, 2), 287 (39), 286 (100), 249 (37), 202 (8), 167 (9), 139 (19). Analysis. Found: C, 54.63; H, 4.56; N, 20.86. Calculated for $C_{15}H_{14}ClN_5O_2$: C, 54.30; H, 4.25; N, 21.11.

2.3 Insecticidal tests

The insecticidal test against male adult *P. americana* was carried out as described previously.[17,21,22] Various volumes (1–10 μL) of solutions of each compound

in dimethyl sulfoxide (DMSO) containing some methanol were injected into the abdomen of a cockroach. Organic solvents alone in this range did not have any toxic effect. Details of the dosage were fundamentally the same as described previously.[22] The doses were varied by 0.1 in log units. In some experiments, a methanol solution (1 μL) containing NIA (50 μg) or a mixture of PB (50 μg) and NIA (50 μg) was injected 1 h before injection of the test compound. The metabolic inhibitors at these amounts did not have any toxic effect. Three insects were used to test each dose of each compound, and were kept at 24–26 °C for 24 h after injection. The minimum lethal doses (MLD; mol) for the test compounds were determined. The activity values of compounds **1o–1u** were expressed by log(1/MLD) and are listed in Table 1 together with previously reported data for **1a–1n** and (*S*)-nicotine. Each value is the mean of at least two experimental runs, with a deviation of ±0.2.

3 RESULTS AND DISCUSSION

3.1 Synthesis

The scheme in Fig. 2 represents the route to derivatives substituted in the 5-position of the pyridine ring of imidacloprid. The known 2-chloro-5-morpholinomethylpyridine (**2**)[10] was selected as the precursor for 5-alkyl-6-chloronicotinyl chloride. The first step of the electrophilic alkylation of position α to the 6-chlorine, using, as the base, the well-documented lithium diisopropyl amide (LDA), prepared *in situ* from diisopropylamine and butyllithium, failed under various conditions. Therefore, the precursor for the alkylation was changed to the 2-methoxy derivative (**3**) in expectation of its more powerful effect on chelating the lithium ion.[23] The chlorine atom was rather inert to nucleophilic attack by the methoxide ion in a conventional way. The substitution reaction proceeded at last with the aid of crown ether catalysis. However, alkylation using an equimolar amount of LDA again failed to give this methoxy derivative. Fortunately, a modifying procedure using methyllithium with a catalytic amount of diisopropylamine[24] afforded the alkylated products in 20–40% yields. The morpholinomethyl group could be converted to the chloromethyl group by an established procedure using ethyl chloroformate without isolating the alkylated products.[10] Another precursor, 6-chloro-5-phenyl-3-picoline (**8**), was accessible by the Suzuki coupling reaction of 6-chloro-5-iodo-3-picoline (**7**)[15] with phenylboronic acid using a palladium phosphine complex. The final imidacloprid derivatives substituted in the 5-position of the pyridine ring were obtained by the coupling reaction of 5-substituted 6-chloro-3-pyridylmethyl bromide or chloride with 2-nitroiminoimidazolidine (**10**) in DMF using sodium hydride as an acid acceptor.

The analytical data supported the structures of the new products (**1p–1s**). Substituted imidacloprid derivatives shared characteristic spectral features with imidacloprid.[25] Proton NMR spectra showed a typical A_2B_2 coupling pattern for the imidazolidine ring protons, and and AX pattern for 2,3,5-trisubstituted pyridine ring protons. The acidic imidazolidine NH appeared as a broad signal. The ^{13}C signal around 160 ppm is informative for confirming the existence of a guanidinyl central carbon. The molecular ion peaks of the present compounds appeared with very weak intensity or were often lacking in the electron impact mass method, like other imidacloprid-type compounds, because of the fragile $N–NO_2$ bond, while the ion with a relatively strong intensity due to the 'benzylic' fragment was a good mark for the structure.[25]

3.2 Insecticidal activity

The potencies of alkyl derivatives were considerably less than that of imidacloprid (**1a**). Of the set of alkyl compounds, the potency of the methyl compound (**1o**) was the highest, with log(1/MLD) of 7.96, which is 10 times weaker than imidacloprid (**1a**), and comparable with the bromo derivative (**1e**) at the molar level. Introduction of higher alkyl or phenyl substitutions in the 5-position drastically decreased the activity, so that the MLD values could not be determined. The methoxycarbonyl derivative (**1u**) afforded no determinable lethal value at 310 nmol dose.

NIA16388, which was originally reported as an inhibitor of the hydrolytic metabolism of tetramethrin,[26] a pyrethroid, has also been used to improve the activity of neonicotinoids. The previous experiments suggested that the synergistic effect of NIA could be due to the inhibition of oxidative metabolism at the carbon(s) in the α-position to the imidazolidine nitrogen atom(s).[17,21,27] Also, Liu *et al.*[28] observed a similar enhancement effect of NIA on insecticidal activity against houseflies. Recent experiments with houseflies have evidenced the retarded degradation of [^{3}H]imidacloprid in the presence of NIA.[29] The present results reflected well the synergistic effect of NIA, which increased the potencies by more than 1 log unit for compounds **1o** and **1t**, so that the potency of **1o** was raised to nanomolar level, and the log(1/MLD) values for the higher alkyl derivatives were elevated enough to allow their determination. The present authors have reported that the activity of neonicotinoid compounds could be further enhanced by adding PB, another oxidative metabolism inhibitor.[17,20] This showed a slight synergistic effect with the compounds tested here, except for the methyl derivative (**1o**), which was not affected. The MLD value of ester (**1u**) could not yet be determined even under these synergist-pretreated conditions. All of the newly prepared compounds except **1u** showed higher activity than nicotine.

The insecticidal potency under synergistic conditions should reflect the intrinsic activity more closely than that without the synergist. In order to compare their activities in the presence of combined synergists, the tested compounds were divided into four classes (1–4 below), and compounds were selected from each

class to compare the potencies among the classes (5 below).

1. H > F ≫ Br ~ Cl > I ≫ N_3.
2. Me ≫ CF_3 > Ph ~ Et > Pr (n) ≫ Bu (n).
3. OMe ≫ OBu(n) ~ OPr(i) ~ OEt ≫ OPr(n) > OPentyl(n).
4. CN, NO_2, CO_2Me.
5. (F ≫ Me > Cl > OMe > I ≫ CF_3 ~ N_3 ≫ CN).

It can be seen that (1) the activity is largely inversely proportional to the size of the atom or group in the same class; (2) the activity is not parallel to the electronic inductive effect of the group with the Hammet σ_m constants;[30] (3) the activity seems not to be determined primarily by lipophilicity; (4) CN, NO_2 and CO_2Me groups deactivate to an extraordinary degree.

Neonicotinoids act on the insect nicotinic acetylcholine receptors agonistically in the same way as nicotine.[31,32] The present authors proposed a binding model by emphasizing the important role of a nitro or cyano group conjugated to the amidine or guanidine part for the hydrogen bonding to the receptor (Fig. 3).[33] The distance (~4.5 Å) from the guanidinyl

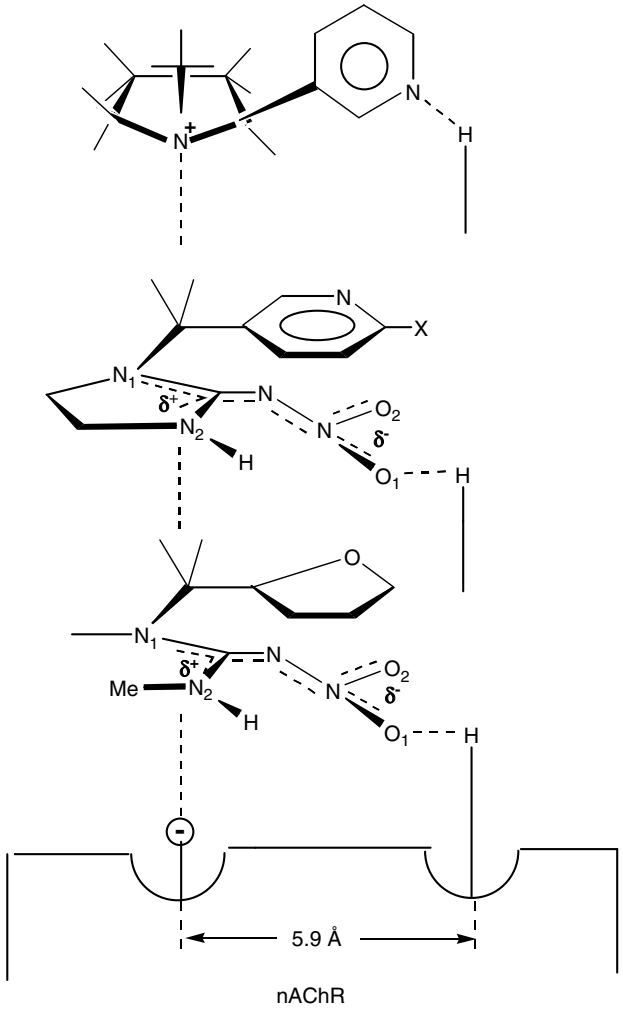


Figure 3. Binding model of agonists to nAChR: nicotine (upper), 6-substituted imidacloprid (middle) and dinotefuran (lower).

(amidinyl) nitrogen atom (N_1) to the electron-negative tip (O_1 or O_2) on one side and to the pyridyl nitrogen atom on the other side is also crucial for activity. As for the chloropyridyl part, it has been considered in the present model as a contributor to activity enhancement by participation in a certain bonding with the hydrophobic residues near the essential binding sites in the receptor peptide segment, instead of the primary role of the hydrogen bond of the pyridyl nitrogen that was advocated in the preceding model.[34] The prominent insecticidal activity of the later developed dinotefuran bearing a 3-tetrahydrofuranylmethyl moiety in place of the 3-pyridylmethyl group is in accord with the present model in that the ether oxygen in the former is apparently a weaker hydrogen bonding donator than the pyridyl nitrogen.[35]

The present study with an additional substituent at the neighbouring position to the 6-chlorine atom on the pyridine ring added another aspect to the binding model. The high activity of the fluoro derivative (**1c**), comparable with that of imidacloprid, means that there is a permissible region to accommodate a lipophilic group in proximity to the 6-chlorine, although it is limited in space from accepting larger groups, as seen in the significant activity decrease with increasing atom size or alkyl chain length of the substituent. Another activity tendency independent of the σ_m constant of the group suggests that the hydrogen bond strength related to the negative charge density of the pyridyl nitrogen does not primarily determine the level of activity, although its hydrogen bond accepting property seems necessary for neonicotinoids to exert activity.

The enormous degeneration of activity caused by NO_2, CN and CO_2Me is puzzling at first glance. It is suspected that this phenomenon arises as follows. The electronegative tips of the 5-substituents (nitro oxygen, carbonyl oxygen and cyano nitrogen) are separated from the pyridyl nitrogen by a similar distance to that between the nitro oxygen and the guanidinyl nitrogen atom in imidacloprid, so that these compounds possibly approach the imidacloprid-binding site on the receptor in a similar fashion to imidacloprid. However, there are two essential differences between the 'false' compounds and imidacloprid. Firstly, the pyridyl and guanidinyl nitrogen atoms are indeed of sp^2 configuration, but the former nitrogen possesses a lone electron pair extending in the horizontal direction, while the latter is inherently electron deficient and the lone pair is incorporated into the conjugation cascade. Secondly, as argued above, the substantial effect of the chloropyridyl moiety appending 4.5 Å from the nitroguanidinyl part (the main part hereafter) is needed for imidacloprid to exert its high activity. In molecules **1g, 1h** and **1u**, the guanidinyl part is located in turn at a similar distance from the pyridine part substituted with a nitro, cyano or methoxycarbonyl group at the 5-position (the main part in these molecules). However, the two main parts differ considerably

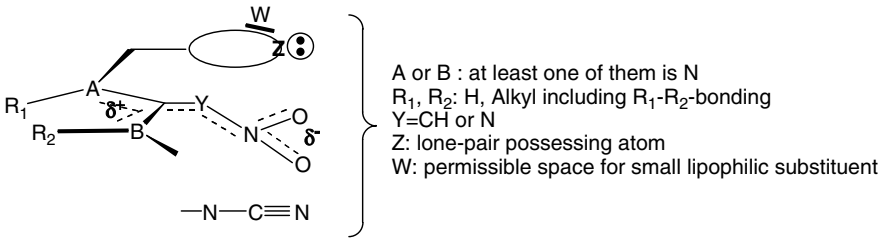


Figure 4. Hypothetical pharmacophore model for neonicotinoids.

from each other in their physicochemical properties such as hydrogen bond facility and lipophilicity. Such inadequate molecular frameworks combined would reduce the effective action at the receptor site.

Based on the present results, a schematic pharmacophore of neonicotinoid molecules is shown in Fig. 4. The pharmacophore is constructed with two parts lying at a certain distance; one is an amidine or guanidine conjugated to a powerful electronegative tip such as NO_2 or CN, and the other is an atom possessing a lone-pair electron in conjunction with a small round lipophilic moiety. A picture is being gained of the structural features around the nicotinoid-binding site on the putative insect nAChR.[36,37] The nitro or cyano group of neonicotinoids is predicted to interact electrostatically with a basic residue in the ligand-binding pocket, the amidine part would interact with the vicinal tryptophan residue and the heteroaromatic part such as chloropyridine will be oriented towards one of the tyrosine residues.[36,37] The positional aberration of each pharmacophore part or the deviation of the electronic nature causes the activity to degenerate significantly.

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