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Note

Preparation of Alkylene-Tethered Acyclic Divalent Neonicotinoids and Their Insecticidal and Neuroblocking Activities for American Cockroach (*Periplaneta americana* L.)

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C4-C8 and C12-alkylene- or 1,4-xylylene-tethered bis[1-(6-chloro-3-pyridylmethyl)]- or bis[1-(2-chloro-5-thiazolylmethyl)]-2-nitroimino-3-guanidines were prepared. The insecticidal potency against American cockroaches, *Periplaneta americana* L., determined by injection was weak as can be seen by their minimal lethal doses (MLD) over 110 nmol. Synergists piperonyl butoxide and NIA 16388, however, enhanced the potency of compounds for which the definitive values were determinable. The MLD values varied with the methylene-chain length, but the variation was not parallel to the tether length. The values abruptly decreased to 26.7 and 85.1 nmol respectively for the hexamethylene chloropyridylmethyl and chlorothiazolylmethyl derivatives. The neuroblocking potency in terms of BC (μM) using a nerve preparation containing the abdominal fifth and sixth ganglia of an American cockroach was mostly parallel to the insecticidal potency determined with the synergists. The above C6-compounds showed BC values of 26.3 and 14.8 μM, respectively, which were below one tenth of the values of the other tethered derivatives.

Keywords: neonicotinoid insecticide, alkylene-tethered divalent molecule, nicotinic acetylcholine receptor, neuroblocking activity, American cockroach.

INTRODUCTION

Nicotinic acetylcholine receptor (nAChR) is a target for current neonicotinoid insecticides.[1–4] The nAChR ligands are considered to bind at the α and non-α interface(s) of the subunits constituting the pentagonal assembly.[3,5,6] A wealth of structural variations of imidacloprid, the first product of this class, have been exploited to develop new commercial products or to elucidate the functional structures of insect nAChRs.[7] Previous studies seemed to concentrate on the monovalent structures (**I** and **II** in Fig. 1). Recently we presented a divalent neonicotinoid class like alkylene-tethered bis-imidacloprid derivatives (**III**; Het: 6-chloro-3-pyridyl) with highly insecticidal potency.[8] The profile of these divalent molecules displaying the tether length-dependent insecticidal activity will provide different strategic aspects for drug designs from those of monovalent ones whose optimization weighs much of substituent variation.[9] Further it will add a new dimension to the binding mode which has been argued exclusively on the basis of the monovalent ligand-receptor interaction. We examined in this study how the tether length relates to the biological activities if two acyclic potent pharmacophores (**II**) are linked to form another series of divalent neonicotinoids (**V**).

MATERIALS AND METHODS

1. *Preparation of Compounds*

The test compounds were prepared according to the scheme in Fig. 2. Preparation of 1,4-bis[1-(6-chloro-3-pyridylmethyl)-2-nitroguanidin-3-yl]butane (**1**) was conducted as follows. To a solution of 1-(6-chloro-3-pyridylmethyl)-2-methyl-3-nitroisothiourea (626 mg, 2.4 mmol) in ethanol (10 ml) was added 1,4-diaminobutane (71 mg, 0.8 mmol). The resulting mixture was heated under reflux for 36 hr. The solvent was distilled off under reduced pressure and the residual semi-solid was subjected to silica gel column chromatography with chloroform/ethanol (4 : 1, v/v) as eluent. Recrystallization from ethanol gave 82 mg (20% yield) of the product. Mp 224–227°C; ^{1}H-NMR (DMSO-d_6, δ, ppm) 1.51 (4H, bs), 3.24 (4H, bs), 4.45 (4H, s), 7.50 (2H, d, J=8.0 Hz), 7.77 (2H, dd, J=8.0/1.7 Hz), 8.36 (2H, d, J=1.7 Hz). Anal. Found: C, 41.80; H, 4.50; N, 27.50%. Calcd. for $C_{18}H_{22}Cl_2N_{10}O_4$: C, 42.11; H, 4.32; N, 27.39%.

The other compounds were prepared by similar procedures and gave the satisfactory elemental analysis data. **2**: mp 186–187°C; ^{1}H-NMR (DMSO-d_6, δ, ppm) 1.06 (2H, m), 1.52 (4H, m), 3.22 (4H, m), 4.49 (4H, s), 7.51 (2H, d, J=8.0 Hz), 7.84 (2H, dd, J=8.0/2.2 Hz), 8.36 (2H, d, J=2.2 Hz). **3**: 188–191 °C; ^{1}H-NMR (DMSO-d_6, δ, ppm) 1.23 (4H, m), 1.49 (4H, m), 3.21 (4H, m), 4.45 (4H, s), 7.52 (2H, d, J=8.4 Hz), 7.78 (2H, dd, J=8.4/2.5 Hz), 8.34 (2H, d, J=2.5 Hz). **4**: 175–177°C; ^{1}H-NMR (DMSO-d_6, δ, ppm) 1.25 (4H, bs), 1.49 (4H, bs), 3.19 (4H, bs), 4.51 (4H, s), 7.60 (2H, s). **5**: 58–71°C; ^{1}H-NMR (DMSO-d_6, δ, ppm) 1.25 (6H, m), 1.49 (4H, m), 3.21 (4H, m), 4.45 (4H, s), 7.51 (2H, d, J=8.0 Hz), 7.78 (2H, d, J=8.0 Hz), 8.34 (2H, bs). **6**: 138–140°C; ^{1}H-NMR (DMSO-d_6, δ, ppm) 1.22 (8H, m), 1.49 (4H, m), 3.22 (4H, m), 4.50 (4H, s), 7.51 (2H, d, J=8.6 Hz), 7.78 (2H, dd, J=8.6/2.3 Hz), 8.36 (2H, d, J=2.3 Hz). **7**: 116–118°C; ^{1}H-NMR (DMSO-d_6, δ, ppm) 1.22 (2H, bs), 1.49 (4H, m), 3.21 (4H, m), 4.45 (4H, s), 7.50 (2H, d, J=8.6 Hz), 7.72

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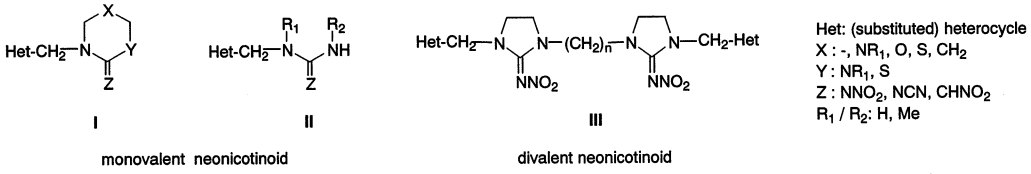

Fig. 1. Cyclic (**I**) and acyclic (**II**) monovalent neonicotinoids and cyclic divalent neonicotinoids (**III**).

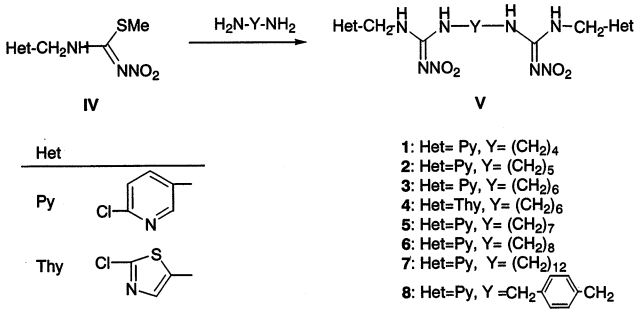

Fig. 2. Preparation of acyclic alkylene-tethered divalent neonicotinoids (**V**).

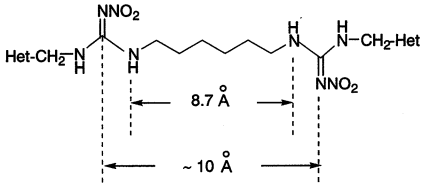

Fig. 3. Distances between the 3-guanidyl nitrogens and between the cationic centers of divalent hexamethylene molecules.

(2H, d, J=8.6 Hz), 8.32 (2H, bs). **8**: 135–137°C; ^{1}H-NMR (DMSO-d_6, δ, ppm) 4.47 (8H, bs), 7.23 (2H, bs), 7.70 (2H, bs), 8.32 (2H, bs).

2. Biological Tests

Insecticidal assays against male adult American cockroaches, *Periplaneta americana* L., were conducted with the test compounds with and without synergists piperonyl butoxide (PB) together with propargyl propyl benzenephosphonate (NIA 16388 or NIA).[8] Neurophysiological measurements using nerve preparations containing the abdominal fifth and sixth ganglia of male adult American cockroaches were conducted principally by the same procedures described previously.[8] The biological activities for the test compounds are listed in Table 1.

RESULTS AND DISCUSSION

The minimal lethal doses (MLD) of the test compounds for the cockroaches were over 110 nmol in the absence of synergists PB and NIA. These values were much larger than those of the corresponding cyclic tethered compounds (**III**),[8] and we did not try to get the MLD values at higher doses because of the poor solubility. The synergists potentiated the insecticidal magnitude of the tested compounds as far as we could determine the values. Such synergistic enhancement for the present divalent alkylenes is consistent with the tendency in monovalent neonicotinoids.[10–13] In principle the inherent insecticidal potency of a molecule appears when the enzymatic metabolism is eliminated by synergists. In the present case, we could discern the potency of several compounds in the presence of PB and NIA. The obtained potency varied significantly among the tethered molecules. However, we could not see any potency variation parallel to the tether length. The abruptly high potency of compound **3** was noticeable, as was the implicated activity level of thiazolyl

Table 1. Biological activities of tested compounds[a]

Compound No.	Insecticidal activity (MLD, nmol)[b]		Neuroblocking potency[c]
	Alone	+(PB+NIA)	BC (μM)
1	>142	141	173 (155–191)
2	>135	>135	355 (234–525)
3	>129	25.7	26.3 (17.5–39.8)
4	>134	85.1	14.8 (12.0–17.8)
5	>129	>129	173 (171–179)
6	>123	123	>204
7	>114	114	>171
8	>127	>127	>191
III[d]	2.2	0.29	0.9

[a] Chemical structures are shown in Fig. 2. The mark > means that the values are larger than those indicated.
[b] The value has a deviation of 0.64 to 1.6-fold.
[c] Values in parentheses are the deviation range estimated from a dose-response relationship where each point was determined from more than three runs.
[d] For compound **III** (Het: Py, n=6 in Fig. 1), the data were taken from Ref. 8.

analog **4**. This abrupt potency protrusion of the hexamethylene molecules is consistent with the previous results with the cyclic series.[8] The greater potencies of the C6-chain derivatives became more obvious in the straightforward estimation of the neuroblocking potencies involving the ligand-nAChR interactions. Compounds **3** and **4** blocked the nerve conduction respectively at 15 and 26 μM, 1/10 the concentration of the other congeners and xylylene derivative (**8**).

The N-$(CH_2)_6$-N length is about 8.7 Å (or ~10 Å between the amidinium ion centers) if the alkylene chain takes the extended conformation (Fig. 3). The finding that the highest potency was reached in the hexamethylene-tether of the present and the previous series[8] of divalent compounds would imply that this length is the best-fit for dual receptive sites of the nAChR where the ligands bind simultaneously. In monovalent neonicotinoids, the insecticidal potency generally decreases with the elongation of the alkyl substituents (R_2 in **II**).[11,12,14-16] This is not the case with the divalent tethered molecules.

The high affinity of polymethylene bis-quaternary ammonium ligands like hexamethonium and decamethonium to the isolated nAChR from a few animal species has been reported, and the involvement of more than a single negative subsite in the complexation with the cation or of their interaction within the channel pore in a folded conformation has been proposed.[17-22] Factually, some divalent molecules designed to accommodate to the dual binding sites are likely to find a pharmaceutical use.[23] For neonicotinoid insecticides the electron-deficient nitrogen atom conjugated with the nitroimine group has been assumed to play the corresponding role in the interaction with the anionic site on the nAChR. The study with divalent neonicotinoids may give a hint about the geometry and function of the binding sites of insect nAChR that has not been elucidated yet as well as the molecular design for new insecticides.

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