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Insecticidal and Neuroblocking Potencies of Variants of the Imidazolidine Moiety of Imidacloprid-Related Neonicotinoids and the Relationship to Partition Coefficient and Charge Density on the Pharmacophore

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The pharmacophore of the neonicotinoid insecticide imidacloprid, nitroiminoimidazolidine, was modified to heterocycles such as thiazolidine, pyrrolidine, dihydroimidazole, dihydrothiazole, and pyridone conjugated to nitroimine (=NNO_2) or nitromethylene (=$CHNO_2$). Their 6-chloro-3-pyridylmethyl or 5-chloro-3-thiazolylmethyl derivatives were examined for insecticidal activity against the American cockroach by injection and for neuroblocking activity using the cockroach ganglion. Most of the compounds having the neonicotinoidal pharmacophore exhibited insecticidal activity at the nanomolar level, which was enhanced in the presence of synergists, and high neuroblocking activity at the micromolar level. Quantitative analysis for the compounds showed that the neuroblocking potency is proportional both to the Mulliken charge on the nitro oxygen atom and to the partition coefficient log *P* value. The equation for the insecticidal versus neuroblocking potencies indicated that both potencies are related proportionally with each other when the other factors are the same.

KEYWORDS: Imidacloprid; neonicotinoid insecticide; pharmacophore; neuroblocking activity; Mulliken atomic charge; log *P*

INTRODUCTION

Neonicotinoids, synthetic insecticides acting on the insect nicotinic acetylcholine receptor (nAChR), have been increasingly used to control various insects during recent decades, since imidacloprid (**1**) was introduced to the market (*1*). The supreme biological profile of imidacloprid (*1*−*3*) is giving impulse to the development of a new product by modifying the structural features of the prototype (*4*). Now nitenpyram (*5*), acetamiprid (*6*), thiamethoxam (*7*), thiacloprid (*8*), clothianidin (*9*), and dinotefuran (*10*) are on the market with their own prominence (**Figure 1**).

The potent neonicotinoids share common structural features: a chlorine-substituted heteroaromatic ring bearing a nitrogen atom at the quasi meta position coupled through a methylene juncture to an amidinyl or guanidinyl moiety that is conjugated to *N*-nitroimine (=NNO_2), *N*-cyanoimine (=NCN), or nitromethylene (=$CHNO_2$). Exceptionally, dinotefuran bears a 3-tetrahydrofuran moiety in place of the chlorine-substituted 3-*N*-heteroaromatic ring.

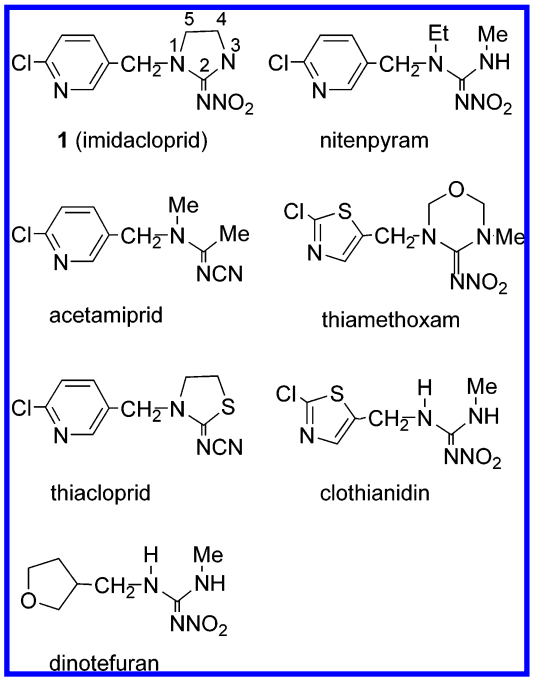

Figure 1. Commercialized neonicotinoid insecticides.

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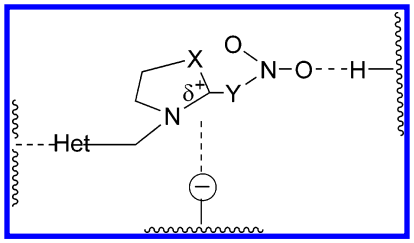


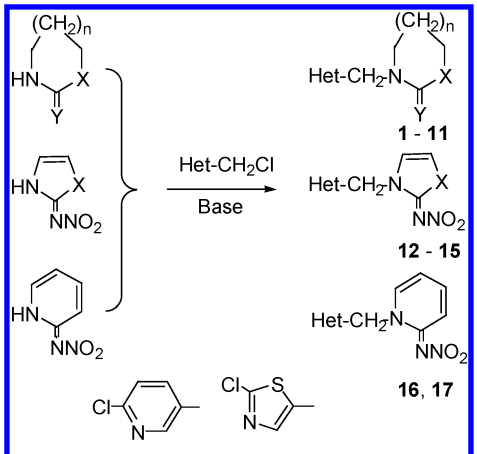
Figure 2. Schematic model for binding of neonicotinoid to nAChR (Y = N, CH; X = NH, S, CH_2). The nitro oxygen atom interacts through H-bonding, the electron-deficient moiety (δ^+) of the central ring through the electrostatic force, and the heteroaromatic nucleus (depicted as Het) in an auxiliary way with the recognition site on the nAChR.

On the basis of the structural features of neonicotinoid isotypes, we have proposed a binding model: the ligand molecule interacts with the group on the receptor by a Coulomb interaction through an electron-deficient (e-deficient) nitrogen atom on the guanidine (or amidine) and by a hydrogen bond (H-bond) through the H-accepting tip at the electron-withdrawing group and the binding of the appending heterocyclic group to the receptor in an auxiliary way (*11*) (**Figure 2**). Matsuda et al. rectified this model on the PM3 calculations so that the electronic flow cascade from the electron-donating nitrogen atom to the electron-negative tip can be fortified by the H-bond (*12*). Tomizawa et al. evolved our model so that the guanidine (or amidine) part would function as a π-acid toward the tryptophan residue on the putative insect receptor (*13*). Nakayama and Sukekawa (*14*) and Okazawa et al. (*15*) rationalized the necessity of a partially positive electrostatic amidine/guanidine site and the electronegative NO_2 or CN group for the binding to the receptor on the basis of similarity indices and comparative molecular field analysis, respectively. All of these pharmacophore models commonly underscore the importance of the negatively charged (n-charged) H-accepting tip conjugated to the amidine moiety.

Not only the pharmacophore but also the pharmacokinetics related to the physicochemical properties of the whole molecule including the auxophore such as the water solubility or the molecular size are other indispensable aspects for the drug action (*16*, *17*). In the neonicotinoid field, questions regarding how the lipophilicity difference among the functional groups (=NNO_2, =$CHNO_2$, =NCN) or the cyclic/acyclic bioisosteres or the size difference among the alkyl substituents affects the biological response have been addressed (*4*, *18*).

We examined in this paper the influence of the modification of the imidazolidine ring of imidacloprid on the physicochemical properties and the resultant biological trend. The modified ring includes thiazolidine, pyrrolidine, unsaturated 1,3-dihydroimidazole and -thiazole, or a fully conjugated pyridone ring (hereafter these are referred to as the central ring), and the central rings of the tested molecules are conjugated to nitroimine or nitromethylene. As the appending heteroaromatic ring were chosen 2-chloro-5-pyridyl (ClPy) and 2-chloro-5-thiazolyl (ClTh) moieties. Different atom alignments in the amidine (guanidine) moiety would produce the electronic distribution and the molecular lipophilicity differently, and the constructed molecules will exhibit the biological behavior in the reflection upon the newly attained physical properties. We further studied the quantitative relationships between the biological potency and the quantum chemical index, the Mulliken charge, and a lipophilicity parameter, log *P*, the octanol/water partition coefficient.

We have thus far pursued the relationship of the insecticidal activity of the neonicotinoids to the following structural parts



1 n=0, Het=Pyr, X=NH, Y=NNO_2(imidacloprid)
2 n=1, Het=Pyr, X=NH, Y=NNO_2
3 n=0, Het=Pyr, X=NH, Y=$CHNO_2$
4 n=0, Het=Pyr, X=CH_2, Y=$CHNO_2$
5 n=0, Het=Pyr, X=S, Y=$CHNO_2$
6 n=0, Het=Thy, X=NH, Y=NNO_2
7 n=0, Het=Thy, X=NH, Y=$CHNO_2$
8 n=0, Het=Thy, X=S, Y=NNO_2
9 n=0, Het=Thy, X=CH_2, Y=$CHNO_2$
10 n=0, Het=Pyr, X=NMe, Y=NNO_2
11 n=0, Het=Pyr, X=NMe, Y=$CHNO_2$
12 n=0, Het=Pyr, X=NH
13 n=0, Het=Pyr, X=S
14 n=0, Het=Thy, X=NH
15 n=0, Het=Thy, X=S
16 n=0, Het=Pyr
17 n=0, Het=Thy

Figure 3. Tested compounds and general preparation scheme.

on the basis of toxicity estimation by injection and nerve impulse measurements using the American cockroach (AmC): the substituents on the pyridine ring (*19*, *20*), imidazolidine N^3-alkyl substituents (*21*, *22*), acyclic compounds (*23*−*25*), and the enantiomers at the juncture (*26*). We expect in the present work that the approach based on the quantitative consideration for the biological response using the quantum chemical and the physiochemical parameters will deepen our insight into the binding mode for neonicotinoid insecticides.

MATERIALS AND METHODS

Preparation of Compounds. Compounds **2** (*27*), **3**, **10**, and **11** (*28*), **6** (*29*), and **12** and **13** (*30*) were prepared according to the described procedures. The other compounds disclosed on patents (*8*, *31*−*35*) without the specific preparative procedures were obtained according to the scheme in **Figure 3**, and the structures were confirmed by the analytical data (see Supporting Information).

Biological Tests. *Chemicals.* Biological data for compounds **1** and **11** (*22*), and **12** (*36*) were taken from our previous literature. Reagent-grade piperonyl butoxide (PB) purchased from Tokyo Kasei Kogyo Ltd. (Tokyo, Japan) was used as an inhibitor of oxidative metabolism. Propargyl propyl benzenephosphonate (NIA 16388) was the same sample used in our previous studies (*27*). NIA originally was an inhibitor of the hydrolytic metabolism of pyrethroids (*37*), and recently Nishiwaki et al. evidenced the interference of the enzymatic hydroxylation at the imidazolidine ring of imidacloprid by NIA (*38*).

Insecticidal Test against AmC. The insecticidal assay against male adult AmC, *Periplaneta americana* L., was conducted as described previously (*19*−*22*, *27*). Various volumes (1−10 μL) of each compound dissolved in methanol containing some amount of dimethyl sulfoxide

Table 1. Biological Potencies of Tested and Reference Compounds

	insecticidal potency[b]		neuroblocking potency[c]	
compd[a]	alone/ratio[d]	+(PB/NIA)/ratio[e]	obsd[f]	calcd[g]
1[h]	8.96/1	10.15(10.07)/15	5.64 (5.60–5.69)	5.38
2	8.22/5.5	10.12(9.77)/80	5.35 (5.26–5.44)	5.56
3	9.19/0.59	10.19(9.87)/10	5.02 (4.95–5.09)	5.17
4	9.84/0.13	10.44 (10.85)/3.9	6.43 (6.32–6.59)	6.13
5	9.76/0.15	10.37(10.62)/4.0	6.19 (6.12–6.27)	6.20
6	8.42/3.5	9.62(9.71)/16	5.97 (5.89–6.02)	5.54
7	9.34/0.42	10.24(9.99)/8.1	5.73 (5.58–5.87)	5.58
8	7.42/35	8.91(9.39)/32	6.00 (5.92–6.09)	5.69
9	8.96/1.0	10.06(9.95)/13	6.31 (6.17–6.46)	6.50
10	8.53/2.7	8.62(8.87)/1.3	3.81[i]	4.22
11[j]	7.77/15	8.07(7.99)/2.0	3.84 (3.74–3.97)	3.76
12[k]	7.72/17	8.72(9.16)/10	4.31 (4.26–4.39)	5.57
13	8.85/1.3	10.25(10.11)/25	5.68 (5.54–5.89)	5.54
14	8.12/6.9	8.82(8.94)/5.1	4.61 (4.56–4.66)	5.45
15	7.85/13	8.85(8.84)/10	5.79 (5.75–5.84)	6.39
16	8.88/1.2	9.99(9.63)/13	5.09 (5.02–5.14)	5.31
17	8.92/1.1	9.74(9.42)/6.7	5.75 (5.61–5.88)	5.63

[a] Structure, see **Figure 3**. [b] log(1/MLD) (mol). [c] log(1/BC) (M). [d] MLD (in mol) ratio to that of imidacloprid (**1**). [e] Values in parenthesss are calculated from eq 6; ratio of MLD(PB+NIA) to MLD(alone) in mol. [f] Values in parentheses indicate the range of error estimated from standard deviation. [g] Calculated from eq 4. [h] Imidacloprid. Biological data taken from ref *22*. [i] The value was predicted from the datum in ref *21*. [j] Biological data taken from ref *22*. [k] Biological data taken from ref *36*.

(DMSO) were injected into the abdomen of an AmC. Organic solvents alone in this range did not have a toxic effect. Details of the dosage were fundamentally the same as described previously (*19*, *20*, *27*). The doses were varied by every 1.25 times in moles. In some experiments, a methanol solution (1 μL) containing PB (50 μg) and NIA (50 μg) was injected 1 h before injection of the test compound. The metabolic inhibitors in these amounts did not have a toxic effect. Three insects were used to test each dose of each compound and were kept at 22−25°C for 24 h after injection. The minimum dose at which two of three insects were considered killed was taken as the minimum lethal dose (MLD in moles). Paralyzed insects were also counted as having died. The MLD values for the test compounds are listed as log(1/MLD)-(mol) in **Table 1**. Each value is the mean of at least two experiments with a deviation of ±0.2 in log units.

Neurophysiological Assay. The neurophysiological test of the compounds was conducted as described previously (*21*−*27*). In brief, a nerve preparation containing the abdominal fifth and sixth ganglia of a male adult AmC was excised and placed in a saline solution. One of two bundles of the nerve cord was taken up from the thoracic side with saline into a glass tube, in which a silver wire was set as an electrode. As the reference electrode, another wire was set outside the cut end of the tube. The silver wires were thinly coated with silver chloride. The number of spontaneous discharges that were larger than approximately 15 μV was consecutively counted with a pulse counter (MET-1100, Nihon Kohden, Tokyo, Japan) for every 30-s period. The frequency was usually quite high for a few minutes after setting and then normally subsided. When the frequency decreased at around a range of 30−400 counts per 30 s for about 2 min, the saline solution was substituted to one with a test compound dissolved in methanol containing some amount of DMSO. The final concentration of the organic solvents was lower than 1% (v/v), which did not essentially affect the nerve activity. Measurements were conducted at 22−25°C. The neuroblocking concentration in terms of log(1/BC) (M) defined below are listed in **Table 1** along with their deviation ranges.

Hydrophobicity Parameter. Log *P*, where *P* is the partition coefficient of compounds in the 1-octanol/water partitioning system, was determined by the shaking-flask method (*21*). The concentration of compounds in the water phase was measured by HPLC using an ODS column (LiChrosorb RP-18, Merck, Darmstadt, Germany) with a mixture of acetonitrile and water (3:7 to 1:1 v/v) as the mobile phase. The log *P* values are listed in **Table 2**.

Quantum Chemistry Calculations. The geometry optimization was carried out at the B3LYP/6-31G(d) level using the Gaussian 98 program (*39*). The optimized geometries are confirmed with no imaginary frequencies by the vibrational analyses. We used the Mulliken charges to evaluate the charges at C, X, Y, O1, and O2 in compounds **I**−**XIV** (**Figure 5**), which are summarized in **Table 2**.

Correlation Analysis. Variations in the neuroblocking activity were analyzed by using free energy related physicochemical parameters of compounds according to eq 1 (*40*, *41*)

$$\log(1/\mathrm{BC}) = aQ + b(\log P) - c(\log P)^2 + dS - eS^2 + \text{constant} \quad (1)$$

where *Q* represents the Mulliken charges of compounds represented in **Figure 5**. Log *P* is the hydrophobicity parameter, the values of which are listed in **Table 2**. *S* is the steric parameter of the compounds. To determine the existence of an optimum in the hydrophobicity and steric dimensions, the squared parameter terms were added so that c and $e \geq 0$.

We examined the relationship between the insecticidal activity against AmC, which was measured with the synergists, and the nerve-blocking activity with the AmC nerve cord using eq 2 (*42*).

$$\log(1/\mathrm{MLD}) = a(\log 1/\mathrm{BC}) + b(\log \mathrm{P}) - c(\log \mathrm{P})^2 + \text{constant} \quad (2)$$

The squared log *P* term was added to identify the optimum hydrophobic effect, so that $c \geq 0$. The coefficients a, b, c, d, and e, and the constants in the equations were determined by the least-squares method. Unless otherwise noted, statistical significance levels of the correlation equations and the independent terms in each equation were above 95% as examined by the t test.

RESULTS AND DISCUSSION

The biological potencies are listed in **Table 1**. In the insecticidal estimation without synergist (alone), nitromethylene compounds (**3**−**5**, **7**, and **9**) surpassed significantly the related nitroimines (**1**, **2**, **6**, and **8**) irrespective of the kind of central or heteroaromatic ring. Of them the activities of compounds **3**−**5** and **7** stood out. These compounds were 2−8 times as potent as imidacloprid (**1**). The influence of the central ring on the activity was not uniform; the imidazolidine, pyrrolidine, and thiazolidine rings made no appreciable difference among nitromethylene derivatives irrespectively substituted with the ClPy (**3**−**5**) or the ClTh group (**7** and **9**). The effect of the introduction of a C═C bond to the central five-membered rings was not unified either; the ClPy-1,3-dihydroimidazole derivative (**12**), apparently, and ClTh derivative (**14**) were somewhat lower in activity than, respectively, imidacloprid (**1**) and compound **6**, whereas ChTh thiazole derivatives **8** and **15** showed almost equal potency. On the other hand, interestingly, the fully conjugated pyridone systems afforded high activity (**16** and **17**), which should be contrasted with the enlarged saturated ring showing an appreciable drop (**1** vs **2**). Methylation at the N^3-position on the imidazolidine ring resulted in compounds of low activity (**10** and **11** vs **1** and **3**). As for the appending heteroaromatic nucleus, the activity of the ClPy residue was great over the ClTh one.

In principle the insecticidal potency improves when synergists eliminate or minimize the enzymatic metabolism. We have thus far observed the activity enhancement of neonicotinoid compounds by the presence of PB and NIA (*19*−*27*). Also in the present case, the potency improvement was observed in all compounds (**Table 1**). The high synergistic effect for compounds **2**, **8**, and **13** was noticeable; as a result their potencies were as high as or even higher than that of imidacloprid (**1**) under synergistic conditions. The metabolic pathways for neonicotinoids include the oxidation at several molecular sites

Table 2. Calculated Mulliken Charges and Partition Coefficients of Selected Nitro Compounds

	structural features		Mulliken charges[a]				log P^b	
type	W/$(CH_2)_n$/X,Y,Z	X–Me	C	Y	O1	O2	Pyr	Thy
I	0/N,NH,N	–0.468	0.834	–0.253	–0.470	–0.398	**1** (0.53)	**6** (0.61)
II	0/N,NMe,N	–0.461	0.810	–0.449	–0.429	–0.399	**10** (–0.07)	
III	0/N,NH,CH	–0.486	0.630	–0.268	–0.490	–0.435	**3** (–0.18)	**7** (0.03)
IV	0/N,NMe,CH	–0.472	0.638	–0.471	–0.463	–0.432	**11** (–0.84)	
V	1/N,NH,N	–0.471	0.832	–0.258	–0.484	–0.401		**2** (0.57)
VI	0/N,CH_2,CH	–0.441	0.416	0.043	–0.453	–0.426	**4** (0.45)	**9**
VII	0/N,S,N	–0.401	0.385	0.263	–0.416	–0.391		**8** (0.80)
VIII	0/N,S,CH	–0.402	0.143	0.264	–0.434	–0.427	**5** (0.47)	
IX	CH═CH/N,NH,N	–0.472	0.823	–0.243	–0.493	–0.416	**12** (0.33)	**14** (0.27)
X	CH═CH/N,S,N	–0.414	0.365	0.360	–0.432	–0.403	**13** (0.53)	**15** (0.96)
XI	pyridone/N,CH,N	–0.467	0.581	0.035	–0.441	–0.397	**16** (0.51)	**17** (0.67)
XII	0/CH,NH,CH	–0.007	0.423	–0.249	–0.479	–0.424	**24**	
XIII	0/CH,O,CH	–0.007	0.455	–0.454	–0.410	–0.415	**25**	
XIV	0/CH,S,CH	0.054	–0.070	0.263	–0.411	–0.409	**26**	
XV	0/CH,CH_2,CH	0.019	0.186	0.049	–0.416	–0.396	**27**	

[a] Hydrogens are summed into the heavy atoms. [b] Log *P* in parentheses denotes the log of the partition between octanol and water; the bold figure stands for the number of the corresponding compound; log *P* was not measured for compounds **24**–**27**.

and the reduction at the nitro group (*43*). The present synergists, PB and NIA, were proved to interfere with the former enzymatic action (*38*). Inherently highly active compounds do not show a drastic activity drop until a substantial amount of the active structure is decomposed (*43*). This is the case of nitromethylene derivatives of **3**−**5** and **9** displaying the modest synergistic effect, judging from the higher binding affinity to insect nAChR of the nitromethylene analogues than the corresponding nitroimines (*44*). The lower sensitivity of the nitromethylene moiety to reductive enzymes (*43*) will be an additional contributor to the smaller synergistic effect. The high synergistic effect for compounds **8**, **13**, and **15** is possibly due to the sulfide moiety sensitive to the enzymatic oxidation in the absence of synergists as observed in other neonicotinoidal sulfide derivatives (*27*). The synergistic ratio at 80 for the diazacyclohexane compound (**2**) compared to 15 for the five-membered homologue (**1**) is conspicuous. The extra methylene at the sixth position is assumed to be responsible for it, considering that the methylene at the more remote position from the e-deficient nitroguanidine part should be more oxidation sensitive than the conjunct methylenes.

The primary target of the neonicotinoids is the nAChR. The cation-permeable ion channels of the nAChR desensitize in response to full occupation of the ligand binding sites with acetylcholine. Thus, prolonged exposure of the nAChR to nonhydrolyzable agonists such as imidacloprid results in a block of cholinergic neurotransmission. The excitation duration time (the time until the blocking begins) and the frequency of nerve impulses is in principle proportional to the binding capacity to the receptor and the quantity of the ligand on it. One can estimate the neuroexcitation or neuroblocking capacity of a ligand by comparing the minimum concentration to induce the two-phase electric episode (*18*, *22*, *24*).

Figure 4 exemplifies the time courses for compound **13**, in which each symbol indicates cumulative counts for every 30 s in each nerve preparation at three concentrations. The two-phase characteristics, first increasing and then decreasing, were clearly observed, and generally the dose for the excitation of the nerve is smaller than that for the following blockade. Between these two effects, the nerve-blocking measurement seems to be related to the insecticidal tendency more significantly than the nerve excitation (*22*, *24*), so we evaluated only the BC in the present study. To evaluate the neuroblocking activity, the time when the number of firings decreased to a level lower than 10 counts per 30 s, which was defined as *t* in minutes, was determined. **Figure 4** shows how the effect on the frequency and the time for subsidence depended on the concentration of the compound. Similar measurements were conducted at least at two concentrations for each compound. More than three nerve preparations were used for each concentration. From a concentration−response relationship for each compound (data not shown), the concentration required to reach 1 in terms of log *t* was determined and was defined as BC (in moles). **Table 1** lists the log(1/BC) values of tested compounds.

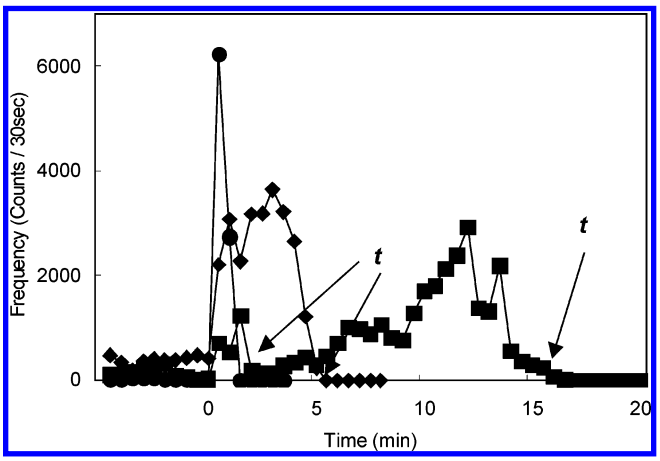


Figure 4. Time courses of the effect of compound **13** on spontaneous discharges in the excised central nerve cords of American cockroaches. After counting for 5 min, the nerve preparations were started to treat with the compound (●, 3.1×10^{-5} M; □, 2.4×10^{-6} M; ■, 1.2×10^{-6} M).

The blocking potencies of nitromethylene compounds (**4**, **5**, and **9**) surpassed that of imidacloprid (**1**). Of them the activities of pyrrolidine derivatives **4** and **9** were outstanding. Extendedly conjugated systems, 1,3-dihydrothiazole derivatives (**13** and **15**) with BC values of, respectively, 2.1 and 1.6 μM, and pyridone (**17**) with a BC value 1.8 μM showed excellent neuroblocking activity. Most of the other molecules elicited a neuroblocking effect at the micromolar level, and such high activity is understandable from their common structure, that is, a conjugated system composed of an electron-donating amidine or guanidine part and a powerful electron-withdrawing group. On the other hand, however, N^3-methyl imidazolidine derivatives (**10** and **11**) showed extraordinarily low neuroblocking potencies,

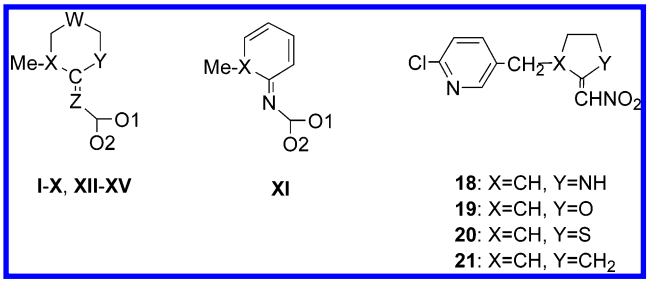


Figure 5. Molecular structures for calculation of the atomic charge in **Table 2** and reference compounds **18**–**21**.

and those of dihydroimidazole compounds (**12** and **14**) were only modest despite their sharing the common pharmacophore, which suggests that some other factors should be also involved in the activity exhibition.

To investigate the structural and physicochemical parameters associated with the biological potencies, we calculated the Mulliken charges of the model compounds (**I**−**XV**; **Figure 5**), where the heteroarylmethyl residue is simplified as a methyl group (**Table 2**). **Table 2** not only outlines the tendency of the charge distribution among the composed atoms in a structure but also demonstrates that the charge of the individual atom varies among the constituted structures. We have emphasized in the proposed model the importance of the presence of the nitro oxygen atom as the H-acceptor. First, we examined the relationship of the nitro oxygen atom O2 to the neuroblocking potency. The O2 atom of all of the structures is strongly n-charged, and compounds of high neuroblocking potencies such as compounds **4**, **5**, **8**, and **9** have factually larger negative charges on it. To certify the relationship of the charge to the activity, we took up compounds **18**−**21** for referral (**Figure 5**). The reported binding strengths (k_i) of compounds **18**, **19**, and **20** to the *Drosophila* nAChR are 1.2 ± 0.3, 180 ± 15, and 36000 ± 2750 nM (*45*), respectively. The calculated charge for **XII**−**XV** (**Table 2**) is actually highly related with the reported binding strength order as well as the nil insecticidal activity of **21** (*46*). As above, we could grasp the parallel relationship between the neuroblocking activity and the nitro oxygen charge. However, the log(1/BC) values of some compounds such as **10** and **11** were apparently lower than predicted from the charge magnitudes. Actually, we could not derive an equation of significant quality relating solely with the charge on the O2 for the listed compounds, even excepting **10** and **11**.

The activity strength is determined not only by the pharmacodynamic but also by the pharmacokinetic factors related to the bioavailability. It is well proved that hydrophilic or ionized molecules elicit only a mediocre effect because of the poor permeability through the ion barriers in the neurons despite fulfilling the pharmacophoric requirements (*16*). To examine how the hydrophobicity affects concurrently the biological trend of the compounds, we quantitatively analyzed the relationship between neuroblocking potency and the Mulliken charge on the O2 (Q_{O2}) and the log *P* term using eq 1 and found eq 3 as the most fitting one.

$$\log(1/\mathrm{BC}) = -7.651(\pm 8.609) - 29.883(\pm 20.499)(Q_{O2}) + 1.976(\pm 0.737)(\log P) \quad (3)$$

$$n = 17,\ s = 0.477,\ r = 0.839,\ F_{2,14} = 16.57$$

In this 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 the intercept and the regression coefficients are their 95% confidence intervals.

From careful examinations of the results, the experimentally measured log(1/BC) values for compounds **12** and **14** seemed to be much lower than the values calculated by eq 3 (data not shown). By excluding these compounds, eq 3 was much improved to eq 4.

$$\log(1/\mathrm{BC}) = -8.527(\pm 5.786) - 32.303(\pm 13.791)(Q_{O2}) + 1.983(\pm 0.495)(\log P) \quad (4)$$

$$n = 15,\ s = 0.315,\ r = 0.930,\ F_{2,12} = 38.16$$

Addition of the $(\log P)^2$ term did not improve the correlation. Moreover, addition of the steric terms for compounds such as Vw and/or $(\mathrm{Vw})^2$, where Vw is the van der Waals volume (*47*), did not improve these equations either. Because the steric dimension of the tested compounds was not much varied as a whole, steric parameters seemed to be inexpedient to improve the correlations. Equation 4 indicates that the higher the O2 charge of the nitro group and the greater the hydrophobicity of the molecule, the higher the neuroblocking activity. The activities calculated by eq 4 are listed in **Table 1**. From the equation, we can understand, for example, that the larger log *P* value is contributing to the superior neuroblocking ability of imidacloprid (**1**) to the nitromethylene compound (**3**), and in contrast the detrimental effect by N^3-methyl substitution for compounds **10** and **11** must be caused by the smaller log *P* values [the unusual water solubility of **10** and **11** has been ascribed to the distortion of the π-conjugation coplanarity by N-methylation (*28*)]. For the deviation of compounds **12** and **14** from eq 4, we suspect that a different factor is needed to determine the ligand−receptor interaction because of the strongly acidic NH proton (*36*, *48*).

We could not derive any significant quantitative equation by using the Mulliken charge on O1. This atom is taken to be involved in a six-membered intramolecular H-bonding with the N^3-H on the imidazolidine ring (*11*), and the resultant geometrical or electronic situations may complicate the relationship for the interaction with the amino acid residue on the receptor.

As for the charge on the amidinyl nitrogen atom (X in **Figure 5**), it has been controversial if the N atom is positive or negative (*1*, *3*, *12*, *13*). In our calculation at B3LYP/6-31G*, the nitrogen atom in question was evidently negative. Even the nitrogen atom of tetramethylammonium was as negative as −0.359, and considering the atomic charges of 0.334 and 0.225, respectively, for C and H, we can recognize that the nitrogen atom withdraws electrons from the surrounding four methyl groups, and in turn the hydrogen atoms are positively charged (p-charged) to a great extent. We can figure the situation in a neonicotinoid molecule in a similar fashion as the n-charged nitrogen atom is tacked with the p-charged hydrogen atoms on the conjunct methylenes.

The pivotal carbon atom on the amidinyl part in almost all of the tested molecules is p-charged (**Table 2**), in contrast with the n-charged methyl carbon in the ammonium ion. This difference will provide a picture of a neonicotinoid molecule delineating an electronic flow from the amidinyl part to the nitro oxygen group by π-conjugation. The amidine part has been assumed to interact with the aromatic π-nucleus of tryptophan on the receptor (*13*). We attempted to derive an equation including the Mulliken charge (**Table 2**) or the LUMO

coefficients (data not shown) on the C for the neuroblocking potency, but we could not attain one with a sufficiently reliable coefficient.

Although the log(1/MLD) measured with the synergists did not show any clear relationship with the Mulliken charges, we could derive the following equation between the insecticidal and the neuroblocking potencies by referring eq 2.

$$\log(1/\mathrm{MLD}) = 6.057(\pm 1.414) + 0.748(\pm 0.266)(\log 1/\mathrm{BC}) - 1.568(\pm 0.845)(\log P)^2 \quad (5)$$

$$n = 17,\ s = 0.397,\ r = 0.871,\ F_{2,14} = 22.09$$

Because the experimentally determined insecticidal activities of ClTh compounds **6**−**9**, **14**, **15**, and **17** tended lower than those calculated by eq 5, an indicator variable term *I* was added to improve the equation, giving eq 6, where the *I* value for the seven compounds was unity.

$$\log(1/\mathrm{MLD}) = 5.634(\pm 1.178) + 0.851(\pm 0.226)(\log 1/\mathrm{BC}) - 1.296(\pm 0.708)(\log P)^2 - 0.524(\pm 0.378)I \quad (6)$$

$$n = 17,\ s = 0.317,\ r = 0.926,\ F_{2,14} = 26.08$$

Insecticidal activities calculated by eq 6 are listed in **Table 1**. The coefficient of the *I* term means that the insecticidal activity of the ClPy compounds was about 3 times higher than the ClTh compounds when other factors were the same. Furthermore, eq 6 indicates that the high neuroblocking activity gives the potent insecticidal activity. Addition of the log *P* term to eq 6 or replacement of the $(\log P)^2$ term by the log *P* term did not improve the quality of the equation. In each set of the ClPy and the ClTh compounds with the same neuroblocking activity, the insecticidal activity was parabolically related to the log *P* value with an optimum of around zero. Similar parabolic relations were observed in such cases as the insecticidal activity against the AmC for other series of neonicotinoids with the neuroblocking (*22*, *24*) and with the neuroexcitatory (*19*, *21*) activities.

We have above discussed the neuroblocking potency using the Mulliken charges of the ligands. However, consideration involving the atoms of the ligand placing a protonated basic residue on the receptor segment should be necessary for the precise evaluation of the ligand receptor. Further investigation on this point will be performed in the future.

In conclusion, most of the variations of the pharmacophore structure in neonicotinoid afforded insecticidal activity against the AmC at 0.1−30 nM concentrations under synergistic conditions. The potency was dependent on the heterocyclic ring conjugated with NNO_2 or $CHNO_2$. The neuroblocking potency was proportional both to log *P* and the Mulliken charge on the nitro oxygen atom. For the insecticidal potency, the high neuroblocking effect gave the potent insecticidal effect when the hydrophobicity and structure in the ClPy or ClTh moiety were separately considered.

ABBREVIATIONS USED

nAChR, nicotinic acetylcholine receptor; H-bond, hydrogen bond; n-charged, negatively charged; p-charged, positively charged; e-dificient, electron-deficient; ClPy, 2-chloro-5-pyridyl; ClTh, 2-chloro-5-thiazolyl; AmC, American cockroach; PB, piperonyl butoxide; (PB), NIA 16388, propargyl propyl benzenephosphonate; MLD, minimum lethal dose; BC, neuroblocking concentration.

Supporting Information Available: Preparation of compounds **4**, **5**, **8**, **9**, and **14**−**17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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