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Bioorganic & Medicinal Chemistry 13 (2005) 1333–1340
Bioorganic & Medicinal Chemistry

Classical and three-dimensional QSAR for the inhibition of [^{3}H]ponasterone A binding by diacylhydrazine-type ecdysone agonists to insect Sf-9 cells

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Received 15 October 2004; revised 4 November 2004; accepted 5 November 2004
Available online 23 November 2004

Abstract—The activity of 52 diacylhydrazine congeners was evaluated by measuring the inhibition of the incorporation of [^{3}H]ponasterone A into intact Sf-9 cells. Eleven compounds were newly synthesized in this study. Results showed that the substitution of the 2-CH_3 or 3-OCH_3 moiety of methoxyfenozide with other groups or the removal of either group was unfavorable to the activity. The activity was quantitatively analyzed using both classical QSAR (Hansch-Fujita) and three-dimensional QSAR methods (comparative molecular field analysis, CoMFA). Sterically favorable fields were observed at the 3- and 4-positions of the benzene ring opposite from the *t*-butyl group (B-ring), and a sterically unfavorable field was evidenced at the 2-position. Another sterically unfavorable field developed surrounding the favorable field observed at the 4-position of the B-ring. Electrostatically negative fields were observed near the CO moiety, above the benzene ring, and at the 4-position of the B-ring. The optimum hydrophobicity of compounds in terms of their log*P* values was calculated to be ≈4.1. Results of the three dimensional structure–activity relationship analyses were consistent with those obtained from the previously reported classical QSAR for 2-chlorobenzoyl analogs containing various *para*-substituents. The high activity of potent insecticides such as tebufenozide and chromafenozide were rationalized by CoMFA. Thus, this CoMFA result will be useful in the design of new compounds and in understanding the molecular mechanism of the ligand–receptor interactions.

1. Introduction

Diacylhydrazines are non-steroidal molting hormone agonists that have insecticidal activity.[1–7] To date four compounds, tebufenozide,[8] methoxyfenozide,[9] chromafenozide,[10] and halofenozide[11] are currently used as insecticides. Although a steroid compound, 20-hydroxyecdysone (20E), is commonly utilized in insects as the molting hormone, selective toxicity is observed among different insect orders for these diacylhydrazine-type compounds. Tebufenozide, methoxyfenozide, and chromafenozide are very potent against Lepidoptera, but weak or inactive against other insect orders such as Diptera and Coleoptera; while halofenozide is effective on Coleoptera.[12] Among the Lepidoptera-selective diacylhydrazines, methoxyfenozide,[9] and chromafenozide[13] have more potent activity, compared to tebufenozide. The former two dibenzoylhydrazines commonly have multiple substitution patterns on the benzene ring opposite from the *t*-butyl group (B-ring), resulting in physicochemical properties that are considered to be essential to compound potency.

The hormonal activity of diacylhydrazines is due to their interaction with the ecdysone receptor.[14–16] The binding affinity of methoxyfenozide to receptor proteins was reported to be higher than that of tebufenozide against *Plodia interpunctella* (Pyralidae), which was consistent with the higher insecticidal activity of methoxyfenozide.[9] However, for the *Chilo suppressalis* (Pyralidae) ecdysone receptor, the binding affinity of methoxyfenozide and chromafenozide to the receptor proteins was at the same level as that of tebufenozide, and no significant difference was observed.[16] The binding affinity of methoxyfenozide to the ecdysone receptor proteins of the Sf-9 cell line, which was established from *Spodoptera frugiperda* (Noctuidae), was three times less potent than

Keywords: Ecdysone agonists; Structure–activity relationships; Diacylhydrazines; QSAR.
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doi:10.1016/j.bmc.2004.11.004

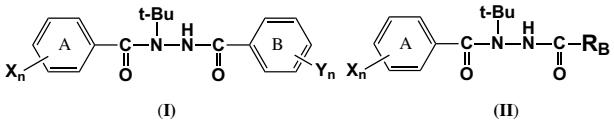


Figure 1. Structures of diacylhydrazines.

tebufenozide.[14] Thus, it is evident that the effects of the physicochemical properties of dibenzoylhydrazines on the receptor binding depend on the origin of the receptor, even among Lepidoptera.

Since the interaction of a compound with the ecdysone receptor is the most important key for the expression of hormonal activity, structural factors that affect the ligand–receptor interaction are of great concern. Previously, we measured the activity of a set of dibenzoylhydrazines with various substituents at the *para*-position of the B-ring using intact Sf-9 cells, and quantitatively analyzed the substituent effects on the receptor binding in Sf-9 cells.[17] In this study, an expanded set of compounds including methoxyfenozide, chromafenozide, and alkanoyl analogs (II, Fig. 1) were prepared to extensively examine the structure–activity relationship for their receptor binding in Sf-9 cells. Particular attention was directed to the tentatively unfavorable effects of 2,3-disubstitution in the B-ring of ligands on the Sf-9 ecdysone receptor. For this purpose, several methoxyfenozide congeners were newly synthesized to increase the variety of compounds with this type of substitution. The activity was evaluated by measuring the inhibition of [^{3}H]ponasterone A ([^{3}H]PoA) binding to intact cells,[14,18,19] and the activity was quantitatively analyzed using the Hansch-Fujita classical QSAR[20] and a three dimensional QSAR method, a comparative molecular field analysis (CoMFA).[21]

2. Experimental

2.1. Compounds

Compounds **2**, **7**–**11**, **15**–**17**, **41**, and **52** listed in Table 1 were newly synthesized according to the conventional method as shown below.[2,3,22] The chemical structures were confirmed by NMR and elemental analyses. The analytical values for C, H, and N in the elemental analysis agreed with the calculated values within ±0.3% as shown in Table 2. The melting points of newly synthesized compounds are listed in Table 2. Chromafenozide

Table 1. Inhibition of [^{3}H]ponasterone A binding by diacylhydrazine analogs in Sf-9 cells

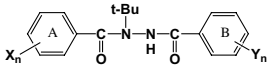
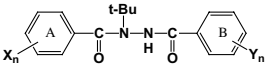
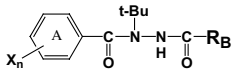
No	Compounds		Binding activity [pIC_{50} (M)] Eq. 2			
						
	X_n	Y_n	Obsd[a]	Calcd[b]	Δ[c]	Log P[d]
1	H	H[e]	6.44[f]	6.10	0.34	2.45[g,h]
2	H	4-Cl (RH-0345)[i]	6.48 ± 0.18 (2)	6.73	−0.25	3.37
3	H	4-Et	7.64[j]	7.58	0.06	3.51[k]
4	2-OCH_3	H	6.57 ± 0.09 (2)	6.62	−0.05	2.04[g,h]
5	3-Cl	H	6.56 ± 0.14 (2)	6.49	0.07	3.28[g,h]
6	3,5-$(CH_3)_2$	H	7.27 ± 0.23 (2)	6.80	0.47	3.39[g,h]
7	3,5-$(CH_3)_2$	2-CH_3	6.86 ± 0.11 (2)	7.00	−0.14	3.98
8	3,5-$(CH_3)_2$	3-CH_3	7.36 ± 0.02 (2)	7.47	−0.11	3.98
9	3,5-$(CH_3)_2$	3-OH	6.80 ± 0.05 (2)	6.95	−0.15	3.34
10	3,5-$(CH_3)_2$	3-OCH_3	7.09 ± 0.04 (2)	6.83	0.26	3.75
11	3,5-$(CH_3)_2$	3-OEt	6.60 ± 0.04 (2)	6.49	0.11	4.28
12	3,5-$(CH_3)_2$	4-Et[l]	8.81[f]	8.63	0.18	4.39[k]
13	3,5-$(CH_3)_2$	4-*n*-Bu	7.08[j]	7.17	−0.09	5.39[k]
14	3,5-$(CH_3)_2$	2-CH_3–3-OCH_3[m]	8.46[f]	8.44	0.02	3.93[k]
15	3,5-$(CH_3)_2$	2-CH_3–3-OEt	7.25 ± 0.19 (2)	7.17	0.08	4.78
16	3,5-$(CH_3)_2$	2-CH_3–3-OH	7.01 ± 0.04 (2)	7.30	−0.29	3.84
17	3,5-$(CH_3)_2$	2,3-$(CH_3)_2$	7.92 ± 0.12 (2)	7.79	0.13	4.43
18	3,5-$(CH_3)_2$	2-CH_3–3,4-$(CH_2CH_2CH_2O)$[n]	8.78 ± 0.11 (3)	8.66	0.12	4.80
19	2-Cl	H	7.08[k]	6.87	0.21	2.59[g,h]
20	2-Cl	2-NO_2	6.04[j]	6.25	−0.21	1.99[o]
21	2-Cl	2-CH_3	7.10 ± 0.08 (2)	7.15	−0.05	2.91[g,o]
22	2-Cl	3-CH_3	6.88 ± 0.03 (2)	7.12	−0.24	3.11[g,o]
23	2-Cl	3-OCH_3	6.95 ± 0.00 (2)	7.10	−0.15	2.81[o]
24	2-Cl	4-F	7.29[p]	7.28	0.01	2.87[g,o]
25	2-Cl	4-Cl	6.89[p]	6.99	−0.10	3.51[g,o]
26	2-Cl	4-Br	7.69[p]	7.31	0.38	3.73[o]
27	2-Cl	4-I	7.30[p]	7.58	−0.28	3.96[o]
28	2-Cl	4-CF_3	6.96[p]	7.05	−0.09	3.68[o]
29	2-Cl	4-NO_2	6.42[p]	6.32	0.10	2.78[o]
30	2-Cl	4-CN	5.74[p]	5.99	−0.25	2.44[o]
31	2-Cl	4-CH_3	7.28[p]	7.45	−0.17	3.15[g,o]
32	2-Cl	4-Et	7.89[p]	7.87	0.02	3.59[o]

Table 1 (*continued*)

No	Compounds		Binding activity [pIC_{50} (M)] Eq. 2			
						
	X_n	Y_n	Obsd[a]	Calcd[b]	Δ[c]	Log P[d]
33	2-Cl	4-*n*Pr	7.64[p]	7.86	−0.22	4.06[o]
34	2-Cl	4-*i*Pr	7.82[p]	7.79	0.03	4.11[o]
35	2-Cl	4-*n*-Bu	8.27[p]	8.05	0.22	4.60[p]
36	2-Cl	4-*t*Bu	7.66[j]	7.95	−0.29	4.48[o]
37	2-Cl	4-Ph	5.17[p]	7.28	−2.11	4.49[o]
38	2-Cl	4-OCH_3	7.21[p]	6.72	0.49	2.82[o]
39	2-Cl	4-SO_2CH_3	5.54[p]	5.67	−0.13	1.46[q]
40	2-Cl	4-$COCH_3$	6.37[p]	6.21	0.16	2.42[q]
41	2-Cl	2-CH_3, 3-OCH_3	8.48 ± 0.01 (2)	8.52	−0.04	3.46
42	2-Cl	2,3-CH_3	8.24 ± 0.08 (2)	8.30	−0.06	3.40[o]
43	2-Cl	2,6-F_2	7.52[j]	7.53	−0.01	2.35[o]
						
	X_n	R_B				
44	3,5-$(CH_3)_2$	*n*-Pr	5.92 ± 0.06 (2)	6.19	−0.27	2.60[k]
45	3,5-$(CH_3)_2$	*n*-Bu	6.79 ± 0.07 (2)	6.67	0.12	3.13[k]
46	3,5-$(CH_3)_2$	*n*-Pentyl	7.44 ± 0.17 (2)	7.41	0.03	3.66[k]
47	3,5-$(CH_3)_2$	*i*-Pentyl	7.49 ± 0.08 (2)	7.19	0.30	3.53[k]
48	3,5-$(CH_3)_2$	*n*-Hexyl	7.62 ± 0.03 (2)	7.48	0.14	4.19[k]
49	3,5-$(CH_3)_2$	*i*-Hexyl	7.88 ± 0.10 (3)	8.03	−0.15	4.06[k]
50	3,5-$(CH_3)_2$	*n*-Heptyl	6.70 ± 0.01 (2)	6.94	−0.24	4.72[k]
51	3,5-$(CH_3)_2$	*n*-Nonyl	6.07 ± 0.08 (2)	6.11	−0.04	5.78[k]
52	2-Cl	*n*-Hexyl	5.75 ± 0.16 (3)	7.94	−2.19	3.50

[a] Unless noted measured in this study. With the mean standard deviation for the number of replications indicated in parentheses.
[b] Calculated by Eq. 2.
[c] Difference between observed and calculated values.
[d] Unless noted evaluated in this study.
[e] RH-5849.
[f] Ref. 27.
[g] Experimentally measured.
[h] Ref. 3.
[i] Halofenozide.
[j] Ref. 19.
[k] Ref. 14.
[l] Tebufenozide (RH-5992).
[m] Methoxyfenozide (RH-2485).
[n] Chromafenozide (ANS-118).
[o] Ref. 2.
[p] Ref. 17.
[q] Ref. 5.

(**18**) was a generous gift from Sankyo Agro Co., Ltd and Nippon Kayaku Co., Ltd, and other compounds were from our stock and are described in previous laboratory publications. The experimental procedures for the synthesis of *N*-3,5-dimethylbenzoyl-*N*-*t*-butyl-*N*′-2-methyl-3-hydroxybenzoylhydrazine are shown below.

2.2. *N*-*t*-Butyl-*N*-3,5-dimethylbenzoyl-*N*′-2-methyl-3-hydroxybenzoylhydrazine

3-Acetoxy-2-methylbenzoic acid 0.40 g (2.1 mmol) in thionyl chloride 0.4 mL (5.5 mmol) was refluxed at 80 °C for 5 h. Excess thionyl chloride was removed azeotropically with toluene to afford crude 3-acetoxy-2-methylbenzoyl chloride which was dissolved in anhydrous dichloromethane (3.6 mL). This benzoyl chloride in dichloromethane containing triethylamine 1.2 mL (8.6 mmol) was added dropwise to *N*-*t*-butyl-*N*-(3,5-dimethylbenzoyl)hydrazine 0.48 g (2.2 mmol) dissolved in anhydrous dichloromethane (3.6 mL) under the ice-cold condition, and stirred overnight at room temperature. The reaction mixture was washed with aqueous 1 M HCl and saline, which was submitted to the following reaction without purification. To this dichloromethane solution containing *N*-*t*-butyl-*N*-(3,5-dimethylbenzoyl)-*N*′-(3-acetoxy-2-methylbenzoyl)hydrazine was added 1 M NaOH (34 mL) and stirred overnight at room temperature. After adding 3 M HCl (15 mL) to the aqueous layer, the mixture was extracted with ethyl acetate. The organic layer was washed with saline and dried over

Table 2. Melting points and elemental analysis values of newly synthesized compounds

No[a]	Mp (°C)	Elemental analysis values
2	153–155	Found: C-65.27, H-5.89, N-8.33 Calcd: C-65.35, H-5.79, N-8.47
7	163–164	Found: C-74.57, H-7.75, N-8.26 Calcd: C-74.52, H-7.74, N-8.28
8	162–165	Found: C-74.49, H-7.73, N-8.35 Calcd: C-74.52, H-7.74, N-8.28
9	218–219	Found: C-70.38, H-7.11, N-8.17 Calcd: C-70.56, H-7.11, N-8.23
10	159–162	Found: C-70.98, H-7.24, N-7.90 Calcd: C-71.16, H-7.39, N-7.90
11	133–134	Found: C-71.86, H-7.68, N-7.87 Calcd: C-71.71, H-7.66, N-7.60
15	167–169	Found: C-72.29, H-7.98, N-7.36 Calcd: C-72.22, H-7.91, N-7.32
16	214–216	Found: C-71.19, H-7.30, N-7.93 Calcd: C-71.16, H-7.39, N-7.90
17	175–176	Found: C-75.02, H-8.26, N-7.99 Calcd: C-74.97, H-8.01, N-7.95
41	171–172	Found: C-63.80, H-6.13, N-7.49 Calcd: C-64.08, H-6.18, N-7.47
52	117–118	Found: C-63.71, H-7.84, N-8.21 Calcd: C-63.80, H-8.03, N-8.27

[a] Corresponds to the compound number given in Table 1.

anhydrous $MgSO_4$, then the solvent was evaporated. The residue was recrystallized from ethyl acetate to afford colorless plate 0.50 g (1.4 mmol). Yield 66%. NMR δ_H ($CDCl_3$): 1.59 (9H, s), 1.76 (3H, s), 2.29 (6H, s), 6.11 (1H, dd, $J = 7.5$, 1.0 Hz), 6.75 (1H, dd, $J = 8.0$, 1.1 Hz), 6.88 (1H, dd, $J = 7.7$ Hz, 7.9 Hz), 7.08 (3H, s). NMR δ_C ($CDCl_3$): 12.1, 21.3, 28.2, 62.4, 117.4, 118.6, 123.8, 125.5, 127.1, 132.1, 136.4, 138.8, 138.9, 157.0, 171.3, 176. Other new dibenzoylhydrazine analogs were synthesized similarly.

2.3. Bioassay

The inhibition of the binding of [^{3}H]PoA (ARC Inc, Carlsbad, CA, USA) to Sf-9 cells was examined according to previously reported methods.[14,18] In brief, 400 μL of cell suspension (4×10^6 cells/mL) was incubated with 1 μL of DMSO solution of the test compound and 2 μL of the 70% ethanol solution of [^{3}H]PoA (0.5 μM, ca. 60,000 dpm) for 30 min at 25 °C. The reaction mixture was immediately filtered through a glass filter (GF/F) and washed three times with water (1 mL). The radioactivity collected in the filter was counted with a liquid scintillation counter (LSC) in 3 mL of Aquasol-2 (Packard Instrument Co., Meriden, CT, USA). The concentration-response curve for the inhibition of the [^{3}H]PoA binding was drawn for each compound. The concentration required to give 50% inhibition (IC_{50}) was determined by probit analysis,[23,24] and its reciprocal logarithm of IC_{50}, pIC_{50}, was used as the index of the activity.

2.4. QSAR analyses

The Hansch-Fujita QSAR analysis was performed using QREG system ver.2.05.[25] Log P (P: partition coefficient in 1-octanol/water system) values of newly synthesized compounds were calculated by CLOG P program MacLogP Ver.4.0 (BioByte Corp., Claremont, CA, USA).[26] Clog P values of representative compounds **1**, **6**, and **19** were 2.48, 3.48, and 2.76, which are close to the respective measured values. Other log P values were cited from our previous reports.[2,3,17,22,27] In all equations n is the number of compounds used for the regression analyses, s is the standard deviation, r is the correlation coefficient, and the values in parentheses are 95% confidence intervals of the regression coefficient and intercept.

All computations for CoMFA were performed with the molecular modeling software package SYBYL ver 6.91 (Tripos Co., St. Louis, MO, USA). Structures were generated by modifying the structure of RH-5849 (**1**), whose X-ray structure has been previously reported.[28] The geometry of all structures are fully optimized with PM3 and charges were calculated by AM1. All diacylhydrazines are automatically aligned using the SYBYL module (Align Database), in which common skeletal chain C–C–N–N–C–C was used as a template. The molecules were superposed in the lattice space of 23.78 Å × 21.36 Å × 25.84 Å ($X = -8.72$ to 15.06, $Y = -9.85$ to 11.51, $Z = -7.40$ to 8.44). The superposition of all 52 compounds is shown in Figure 2. CoMFA was executed using the SYBYL QSAR module. The lattice spacing was 2 Å, and $a + 1$ charge and a SP^3 carbon was used as probes to estimate the electrostatic and steric fields, respectively. The electrostatic and steric potential energies at each lattice point were calculated using Coulombic and Lennard–Jones potential functions, respectively. The hydrophobic effect was evaluated using log P and $(\log P)^2$ as the lattice independent external descriptors. The correlation analyses were analyzed with the partial least squares (PLS) method.[29,30] We initially selected the number of latent variables in the set from the leave-one-out method setting the column filtering at 2 kcal/mol, and then performed the conventional analysis using optimum component number, m. CoMFA results were represented by the conventional equations with statistic values of the leave-one-out cross-validated correlation coefficient, q^2, the cross-validated standard error, S_{cv}, the conventional correlation coefficient, r, and the standard deviation, s. The relative contribution (%) of descriptors to the correlation equations was also shown. The results were visualized by contour maps using connected lattice points having an equivalent coefficient level for each molecular field.

3. Results

3.1. Inhibition of the binding of [^{3}H]PonA to Sf-9 cells

The activity values of 52 diacylhydrazine congeners in terms of pIC_{50} (M) are listed in Table 1. Chromafenozide

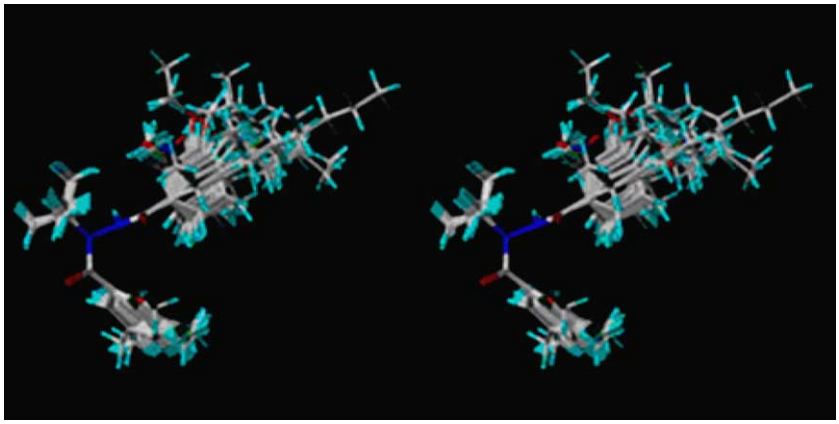


Figure 2. Stereoview of the superpositions of all 52 compounds.

(**18**) was equipotent to tebufenozide (**12**) with the highest activity, whereas methoxyfenozide (**14**) was half as potent as tebufenozide. By replacing the 3-OCH_3 group on the B-ring of methoxyfenozide with H (**7**), OH (**16**), or OC_2H_5 (**15**) the activity decreased 16–30-fold, but this decrease was only 3.5-fold for compound **17** in which the 3-OCH_3 was substituted with 3-CH_3. Thus, the effects of *meta*-substituents on the enhancement of the activity were in the following order, OCH_3 (**14**) > CH_3 (**17**) > OEt (**15**) > OH (**16**) ⩾ H (**7**). This sequence was greatly different from that observed for mono *meta*-substituted compounds, being decreased in the order of CH_3 (**8**) > H (**6**) > OCH_3 (**10**) > OH (**9**) > OEt (**11**).

Interestingly, the effects of structural changes in the B-ring moiety on the activity are different between the compound groups having 2-Cl-type A ring and 3,5-dimethyl-type A ring, as shown in Figure 3. Typically, the introduction of a *n*-Bu group on the B-ring is 10 times more effective in compounds containing a 2-Cl on the A-ring (**19** vs **35**) than in compounds containing 3,5-dimethyl groups (**6** vs **13**). This observation holds

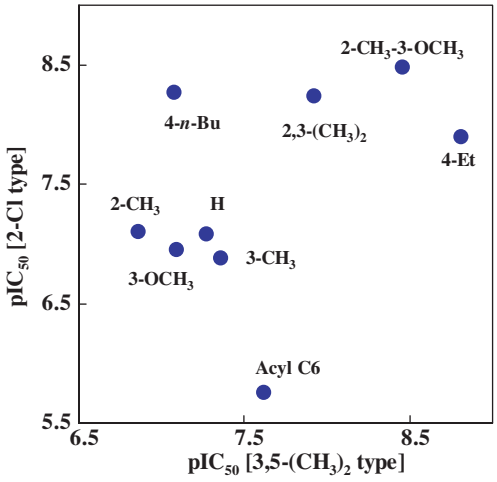


Figure 3. Comparison of the activity values between 3,5-dimethyl and 2-Cl A-ring series of compounds.

true even though the compound with 3,5-dimethyl-substituents on the A-ring and 4-Et on the B-ring (tebufenozide, **12**) was still 10 times more potent than that with 2-Cl on the A-ring and 4-Et on B-ring (**32**). With respect to the compounds with heptanoyl (R_B = *n*-hexyl) instead of benzoyl as the B-ring moiety, the activity of 2-Cl-A-ring type compound **52** was about 100 times less potent than the corresponding 3,5-dimethyl-A-ring type compound **48**. The effects of other substituents (H, 2-CH_3, 3-CH_3, 3-OCH_3, 2-CH_3–3-OCH_3, 2,3-diCH_3) were similar between 3,5-dimethyl- and 2-Cl-A-ring type compounds.

3.2. QSAR analyses

Previous classical QSAR analyses for the activity of diacylhydrazines indicated that the molecular hydrophobicity in terms of log *P* is essential for obtaining significant correlation equations.[17] Therefore, we began the analysis using log *P* to formulate the initial significant correlation (Eq. 1), in which compounds **37** and **52** are omitted. Compound **37** was omitted in our previous QSAR analysis,[17] and compound **52** was exceptionally weak as show in Table 1 and Figure 3.

$$\begin{aligned} \mathrm{pIC}_{50} &= 2.557(\pm 1.132)\log P - 0.302(\pm 0.156)(\log P)^2 \\ &\quad + 2.151(\pm 1.999), \\ &\quad n = 50, \quad s = 0.588, \quad r^2 = 0.410, \\ &\quad F_{2,47} = 16.318, \quad \log P_{\mathrm{opt},} = 4.23. \end{aligned} \tag{1}$$

As shown in Eq. 1, 40% of the total variation of activity of 50 diacylhydrazines was explained by log *P* and its squared term. The optimum log *P* value, $\log P_{\mathrm{opt}}$, was evaluated to be 4.23 from Eq. 1. Since steric and electronic effects participate to vary the activity in addition to the hydrophobicity, the addition of other physicochemical parameters in Eq. 1 should improve the correlation. However, since compounds **44–51** contain alkanoyl instead of benzoyl moieties, it is not easy to apply the classical QSAR method to the combined set of these compounds. Therefore, CoMFA was used to explore electronic and steric effects of diacylhydrazines to derive Eq. 2.

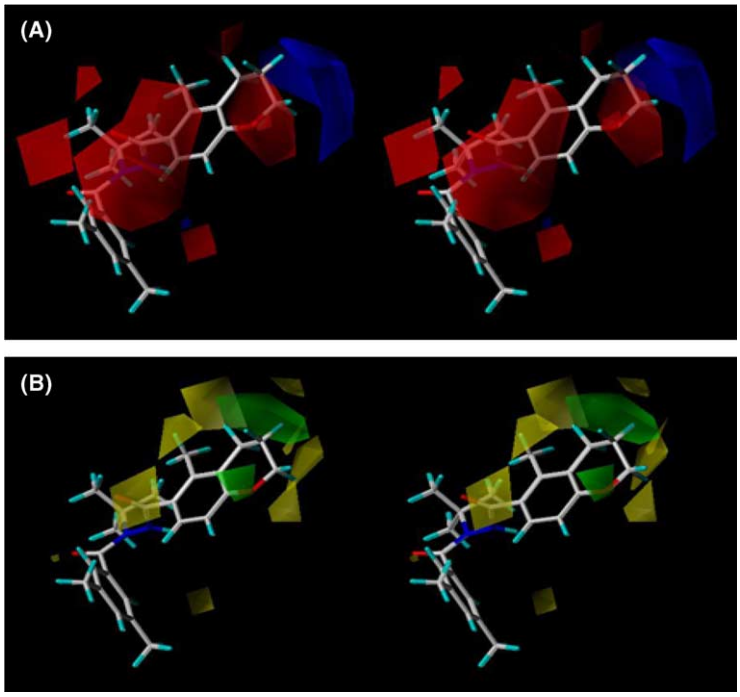


Figure 4. Stereoviews of the CoMFA models with chromafenozide. (A) The contours are shown to surround regions where a higher steric bulk increases (green) or decreases (yellow) the binding. (B) The contours are shown to surround regions where a positive (blue) and a negative (red) electrostatic potential increases the binding.

$$\begin{aligned} \mathrm{pIC}_{50} &= 1.620\log P - 0.197(\log P)^2 \\ &\quad + [\mathrm{CoMFA}] + 2.891 \\ n &= 50, \quad s = 0.214, \quad r^2 = 0.928 \qquad (2) \\ (q^2 &= 0.593,\ S_{\mathrm{cv}} = 0.510,\ F_{6,43} = 92.755,\ m = 6) \\ \mathrm{Steric} &= 35.3\%, \\ \mathrm{Electrostatic} &= 18.4\%,\ \log P = 24.6\%, \\ (\log P)^2 &= 21.7\%,\ \log P_{\mathrm{opt}} = 4.11. \end{aligned}$$

The $\log P_{\mathrm{opt}}$ value (=4.11) estimated in Eq. 2 was close to that in Eq. 1. Views of the steric and electrostatic CoMFA were shown as STDEV * COEFF contour maps in Figure 4. Sterically favorable fields (green) appeared above the 3- and 4-positions of the B-ring, and sterically unfavorable fields (yellow) appeared covering 2-position as shown in Figure 4A. Another sterically unfavorable field developed surrounding the favorable field observed at the 4-position of B-ring. A large positive electrostatic-potential network (blue) appeared surrounding the 3,4-positions of the B-ring, and negative potential networks (red) appeared near the C=O moiety and above the B-ring as shown in Figure 4-B. The relationship between observed and calculated values is shown in Figure 5.

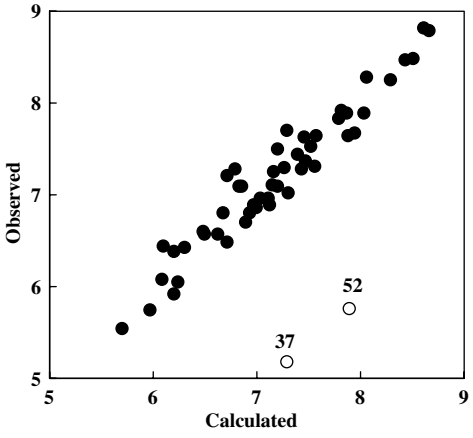


Figure 5. Graphical representation of observed pIC_{50} values versus pIC_{50} values calculated with Eq. 2 for 50 compounds (closed circles). Open circles are omitted compounds (**37** and **52**) in the correlation analysis.

4. Discussion

Previously we reported the classical-QSAR for the activity of a set of dibenzoylhydrazine congeners with various *para*-substituents at the B-ring against Sf-9 cells.[17] The activity of 17 *para*-substituted compounds (I: X_n = 2-Cl in Fig. 1) was quantitatively analyzed with electronic, steric, and hydrophobic substituent parameters to formulate Eq. 3.

$$\begin{aligned} \mathrm{pIC}_{50} &= 0.607\log P - 0.822\sigma - 0.367\Delta B_1 + 5.502 \\ n &= 17, \quad s = 0.243, \quad r^2 = 0.917, \quad F_{3,13} = 46.026. \end{aligned} \qquad (3)$$

In Eq. 3, σ is the regular Hammett's value representing the electron withdrawal property.[31] B_1 is the STERIMOL parameter which represents the minimum width of substituents in the projection on the plane perpendicular to the axis connecting the α-atom of the substituents with the rest of the molecule, and Δ means that

the reference of the B_1 value was shifted to that of H.[32] The equation indicated that the electron-donating *para*-substituents at the B-ring moiety were favorable for activity. The electron donating effect from the B-ring is likely to increase the electron density of the carbonyl oxygen, and therefore, we propose that one of the driving forces of the ligand binding could the electron transfer from the carbonyl oxygen to the acidic site of the receptor.

The result of the 3D-QSAR analysis in the present study using an expanded set of compounds was consistent with previously reported results. As shown in Figure 3, the electrostatically positive (blue) fields appear surrounding the 3- and 4-positions of the B-ring, showing that the presence of electron-donating substituents on these positions is favorable for activity. Furthermore, the appearance of the electrostatically negative field near the C=O group and above the B-ring strongly supports our proposed mechanism of ligand–receptor interaction. According to this CoMFA study, 50 compounds are fairly well predicted as shown in Table 1 and Figure 5, whereas omitted compounds (**37** and **52**) were both predicted to be 100 times more potent. The receptor binding modes of these compounds may not be same as that of ordinary dibenzoylhydrazine-type compounds.

In Eq. 2 the squared $\log P$ term became significant, although it was insignificant in Eq. 3. This observation is most likely due to the fact that a number of compounds with supra-optimum molecular hydrophobicity were included in the 50 compounds used to formulate Eq. 2. In fact, the squared $\log P$ term was significant in the QSAR of the hormonal activity against *C. suppressalis* as indicated in Eq. 4,[27] although the biological activity is different from the binding activity used in Eqs. (1)–(3). Log P values of diacylhydrazine congeners used in Eq. 4 vary from 2.60 to 5.78.

$$\begin{aligned} \mathrm{pEC}_{50} &= 7.779\log P - 0.775(\log P)^2 - 0.893D_{\mathrm{A}} \\ &\quad - 0.609D_{\mathrm{B}} - 3.227 \\ n &= 23, \quad s = 0.557, \quad r^2 = 0.863, \\ F_{4,18} &= 28.291, \quad \log P_{\mathrm{opt}} = 5.15. \end{aligned} \tag{4}$$

The $\log P_{\mathrm{opt}}$ value (5.15) estimated in Eq. 4 was higher than that (4.11) evaluated for the activity to Sf-9 cells. In Eq. 4, D_{A} and D_{B} express the length of the A-ring and B-ring moieties of the diacylhydrazine congeners, respectively.

The existence of the optimum molecular hydrophobicity in the ligand binding is likely to account for different substituent effects at the B-ring between 3,5-dimethyl and 2-Cl type compounds. Since the hydrophobicity values of these two types of A-ring moieties are considerably different, the introduction of the same substituent into the B-ring may have a non-linear effect on the activity as a result of the change in the total molecular hydrophobicity. For example, the $\log P$ value of 3,5-dimethyl-type compound **13** containing a *n*-Bu group on the B-ring was 5.39, whereas that of the corresponding 2-Cl type compound **35** was 4.60. The former is 10 times more hydrophobic than the optimum $\log P$, whereas the latter was closer to the optimum $\log P$ value. In fact, the binding activity of compound **13** was about 10 times lower than that of compound **35**. By contrast, 3,5-dimethyl-type compound **12** containing an Et group on the B-ring has a $\log P$ value of 4.39, very close to the optimum value. In the case of the corresponding 2-Cl type compound **32**, the $\log P$ value (3.59) was fairly low, contributing to the reduction in the activity.

The 3D-QSAR result demonstrated that the presence of a substituent at the 2-position on the B-ring is essentially unfavorable for receptor binding due to steric effects, although one of the commonly used insecticides, methoxyfenozide, contains a CH_3 group at this position. In fact, methoxyfenozide has reduced receptor binding activity, compared to tebufenozide, which contains no substituent at the corresponding position. Chromafenozide, however, is equipotent to tebufenozide, in spite of the presence of 2-CH_3 group on the B-ring, which is located in a sterically unfavorable region near the *ortho*-position of the B-ring. This observation is probably due to counterbalancing by the oxygen atom in the chromane ring located in the electrostatically negative region.

Previously we reported that the binding affinity of 10 ecdysone agonists to Sf-9 cells is linearly correlated with the hormonal activity in cultured integument of *C. suppressalis*, in which both steroidal and non-steroidal compounds were considered.[14,15] Here we examined the relationship between these two activities for an expanded set of ecdysone agonists (n = 25) including ecdysteroids as shown in Figure 6, and still obtained a strong linear correlation. Some scatter is certainly recognized in this correlation, which is probably due to the small difference of the binding pockets of receptors between *S. frugiperda* and *C. suppressalis*. However, the overall similarity in QSAR provides useful findings for designing new potent compounds against various Lepidoptera insects.

In conclusion, the binding affinity of various diacylhydrazines was correlated with CoMFA steric and

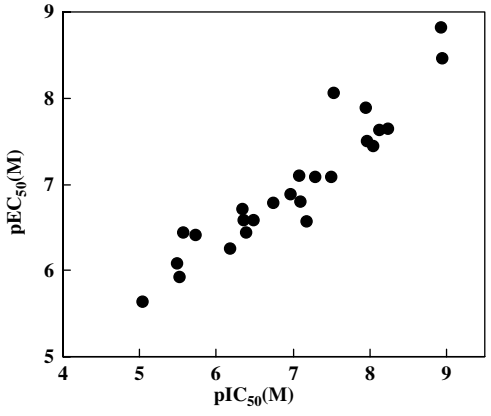


Figure 6. Relationship between the binding (pIC_{50}) of ecdysone agonists to Sf-9 cells and their molting hormonal activity (pEC_{50}) measured in the cultured integument of *C. suppressalis*.

electrostatic parameters as well as the molecular hydrophobicity parameters. This CoMFA result was consistent with the previous classical QSAR result[17] and reasonably predicted the potent analogs such as tebufenozide, chromafenozide, and methoxyfenozide. We also demonstrated that QSARs will be useful to verify the ligand–receptor binding mode.

Acknowledgements

We are thankful to Dr. Craig Wheelock for carefully reviewing this manuscript. We express our sincere thanks to Sankyo Agro, Co. Ltd and Nippon Kayaku Co., Ltd for the gift of chromafenozide and Mr. Masahiro Kawakatsu for his skillful cooperation in synthetic experiments. We also thank to Mr. Ryoichi Takami for his advice in the setting of the computer system. A part of this study was performed in the RI center of Kyoto University. This investigation was supported, in part by a Grant-in-Aid for Scientific Research from Ministry of Education, Culture, Sports, Science, and Technology (formally Ministry of Education, Science, and Culture) of Japan (09660117, 10161207).

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