

Manuscript prepared for publication in *Bioorganic Medicinal Chemistry Letters*

Structural requirement and stereospecificity of tetrahydroquinolines as potent
ecdysone agonists

Seiya Kitamura^a, Toshiyuki Harada^a, Hajime Hiramatsu^b, Ryo Shimizu^b, Hisashi
Miyagawa^a, Yoshiaki Nakagawa^{a*}

^a Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

^b Mitsubishi Tanabe Pharma Corporation, Kashima 3-16-89, Yodogawa, Osaka 532-8505,
Japan

Corresponding author

Dr. Yoshiaki Nakagawa

Graduate School of Agriculture

Kyoto University

Kyoto 606-8502, Japan

Tel: +81-75-753-6117

Fax: +81-75-753-6128

E-mail: naka@kais.kyoto-u.ac.jp

Abstract

Tetrahydroquinoline (THQ)-type compounds are a class of potential larvicides against mosquitoes. The structure-activity relationships (SAR) of these compounds were previously investigated (Sumith et al., *Bioorg. Med. Chem. Lett.*, 2003, 13, 1943-1946), and one of the *cis*-forms (with respect to the configurations of 2-methyl and 4-anilino substitutions on the THQ basic structure) was stereoselectively synthesized. However, the absolute configurations of C2 and C4 were not determined. In this study, four THQ-type compounds with *cis* configurations were synthesized, and two were submitted for X-ray crystal structure analysis. This analysis demonstrated that two enantiomers are packed into the crystal form. We synthesized the *cis*-form of the fluorinated THQ compound, according to the published method, and the enantiomers were separated via chiral HPLC. The absolute configurations of the enantiomers were determined by X-ray crystallography. Each of the enantiomers was tested for activity against mosquito larvae *in vivo* and competitive binding to the ecdysone receptor *in vitro*. Compared to the (2*S*, 4*R*) enantiomer, the (2*R*, 4*S*) enantiomer showed 55 times higher activity in the mosquito larvicidal assay, and 36 times higher activity in the competitive receptor binding assay.

Keywords: tetrahydroquinoline; ecdysone; stereospecificity; mosquito; larvicide

Two major classes of peripheral insect hormones, juvenile hormones (JHs) and molting hormones regulate insect growth. The principal molting hormone of insects is 20-hydroxyecdysone (20E).¹ 20E and its agonist bind to ecdysone receptors (EcRs) in collaboration with the heterodimeric partner, ultraspiracle (USP), and transactivate molting-related genes.² In a few arthropods, other steroid compounds such as ponasterone A (PonA), makisterone A, and ecdysone act as molting hormones.³ To date, the primary sequences of EcRs and USPs have been identified in various insects.^{3,4} Three-dimensional structures of the ligand binding domains of EcRs with ponasterone A as the ligand molecule were solved by X-ray crystal analysis in three insects: *Heliothis virescens*,⁵ *Bemisia tabacii*,⁶ and *Tolilolium castaneum*.⁷ The ligand-binding characteristics of 20E, which are similar to that of PonA, were also solved in *H. virescens*.⁸ The binding sites of non-steroidal ecdysone agonists for diacylhydrazine (DAH; BYI06830)⁵ and two imidazole type compounds, BYI08346 and PDB 3IXP,⁹ were also solved by X-ray analysis. However, these structures differ from those of PonA and 20E.

Compounds that regulate insect molting and metamorphosis are known as insect growth regulators (IGRs) or more recently as insect growth disruptors (IGDs), and some of these compounds are used as insecticides in the agricultural field.¹⁰ IGDs can be classified into three major categories, juvenile hormone agonists, chitin synthesis inhibitors and molting hormone agonists. Among molting hormone agonists, five diacylhydrazine (DAH)-type compounds (tebufenozide, methoxyfenozide, chlomafenozide, fufenozide, and halofenozide) are used currently. Most of DAH-type compounds are selectively toxic to Lepidoptera, except for halofenozide, which is registered to control both Lepidoptera and

Coleoptera. However, the binding affinity of halofenozide to coleopteran receptors is not stronger than that of other lepidopteran-specific DAHs.¹¹

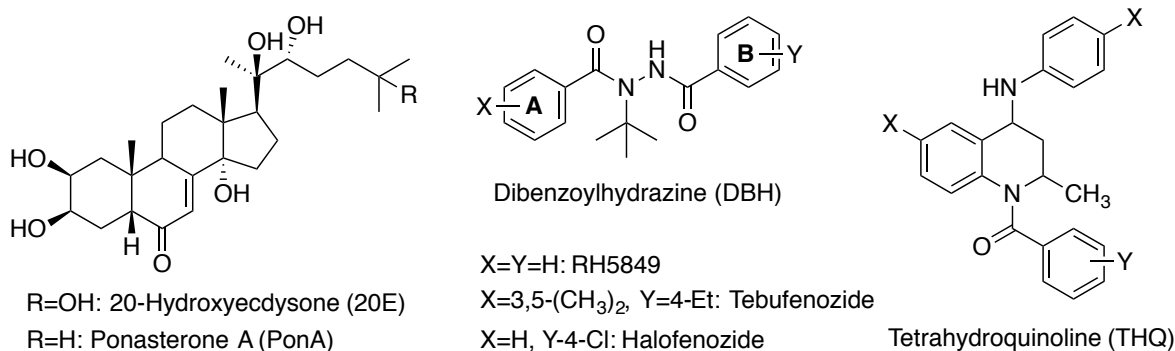


Fig. 1. Structures of ecdysone agonists

The high degree of specificity of DAHs against Lepidoptera triggered a search for new ecdysone agonists, in both random and rational manners. Although novel non-steroidal ecdysone agonists have been reported, none of these has been used commercially.¹² Among new ecdysone agonists, tetrahydroquinoline (THQ)-type compounds are reported to be dipteran-specific,¹³ particularly to the mosquito EcR.¹⁴ These compounds have a unique specificity toward mosquitos, which may offer a more selective and environmentally friendly pest-management option. Therefore, THQs are promising leads to develop into larvicides for mosquito control. A previous structure-activity relationship (SAR) study of THQs focused on the optimization of the substituents X and Y, and showed that a fluorinated THQ (X=F, Y=4-Cl: THQ in Fig. 1) had the highest ecdysone agonistic activity for the *Aedes aegypti* EcR among the 35 compounds tested.¹³ We initially synthesized a

few THQ analogs according to the published method,^{13, 15} and compared the binding and insecticidal activity of the analogs with the reported activities as shown in Table 1. We also reported that compounds lacking the benzene ring of the quinoline structure and the aniline moiety, as well as the *trans* isomer of the 2,4-positions of quinoline moiety were inactive.¹⁵

According to Smith and co-workers¹³, one of two possible *cis*-stereoisomers (2*R*, 4*S*) was obtained as a final product by the Doebner-von Miller reaction. However, convincing data regarding the stereochemistry of this compound was not reported. In the present study, we observed that Smith's synthesis method did not yield a stereochemically pure intermediate. Instead, it yields a racemic mixture via Doebner-von Miller reaction. We therefore hypothesized that the THQ reaction mixture prepared by this method also contained the (2*S*, 4*R*) *cis* enantiomer. In our preliminary study, we analyzed the X-ray crystal structure to determine the absolute configuration of the *cis* isomers of compounds **1** (CCDC 984528: Supplement Table 1S) and **2** (CCDC 984529: Supplement Table 1S), in which the two enantiomers are packed, respectively. Smith and co-workers reported the stereochemistry of THQs based on H-NMR analysis, which is difficult^{13, 16} without using special chiral analysis techniques such as chiral NMR solvent.

We then asked which enantiomer was biologically active. To determine this, the *cis* product of compound **4** (Y=4-Br) containing two enantiomers [**4a** (2*S*, 4*R*) and **4b** (2*R*, 4*S*)] was prepared according to the reported procedure²¹, and analyzed by chiral HPLC.²² A baseline separation of the two enantiomers was achieved with an amylose-based chiral HPLC column,¹⁷ packed with a silica gel bound to tris (3,5-dimethylphenylcarbamate) derivatives of amylose and eluted with hexane/ethanol (90/10, v/v). The enantiomer excess

was determined by chiral HPLC and found to be >99%. Each fraction was recrystallized from hexane-ethyl acetate and the absolute configurations were determined by X-ray crystallography as shown in Fig. 2 with their chemical structures.²³

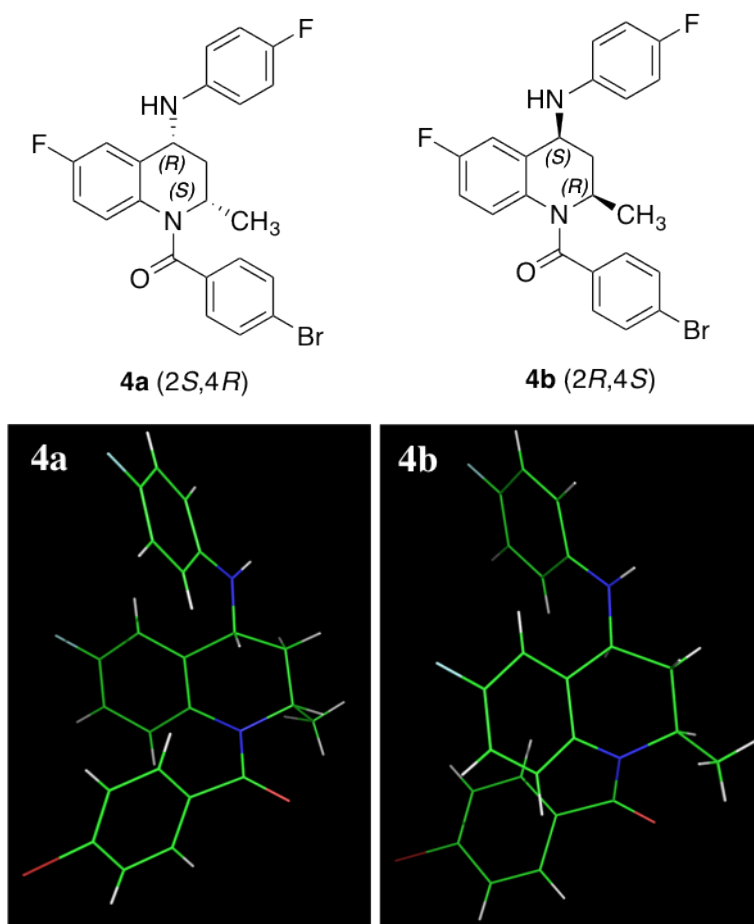


Fig. 2. X-ray structures of enantiomer **4a** and **4b**.

Crystal data and the data collection parameters for compounds **4a** (CCDC 984530) and **4b** (CCDC 984531) are listed in Table 1.

Table 1. X-ray crystallographic data for enantiomers **4a** and **4b**

	Retention time (HPLC)	
	4.3 min 4a	5.8 min 4b
Empirical formula	C ₂₃ H ₁₉ BrF ₂ N ₂ O	C ₂₃ H ₁₉ BrF ₂ N ₂ O
Formula weight	457.32	457.32
Crystal system	orthorhombic	orthorhombic
Space group	P2 ₁ 2 ₁ 2 ₁ (#19)	P2 ₁ 2 ₁ 2 ₁ (#19)
a (Å)	7.726 (1)	7.7240 (2)
b (Å)	11.425 (1)	11.423 (3)
c (Å)	23.284 (2)	23.288 (5)
V (Å ³)	2055.4 (3)	2054.8 (8)
Z, D _{calc} (g/cm ³)	4, 1.478	4, 1.478
F (000)	928.00	928.00
μ (Cu Kα) (mm ⁻¹)	3.024	3.025
T (K)	243	243
no. obsd	3506	3338
(I > 2.00 σ (I))		
no. parameters	265	265
R ₁ , wR ₂	0.040, 0.156	0.043, 0.145
Flack parameter	0.01(2)	0.01(2)
GOF	1.300	1.031

The structure-activity relationships for compounds **1–3** were consistent with the previously reported structure-activity relationships (SAR).¹³ The newly synthesized compound **4** is three times more toxic against mosquitoes than compound **3**, as shown in Table 2. The *cis*-(2*R*, 4*S*) enantiomer (**4b**) showed 55 times higher larvicidal activity against mosquito *Culex pipiens pallens* than the *cis*-(2*S*, 4*R*) enantiomer (**4a**).²⁴ The concentration-response relationships for the larvicidal activity of these enantiomers are shown in Fig. 3. Consistently, the active compound **4b** had approximately two times higher larvicidal activity than the racemic compound **4**.

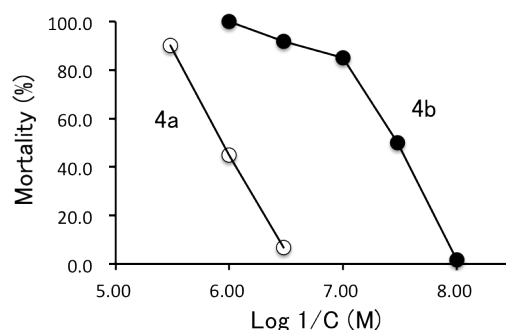


Fig. 3. Concentration-response relationships for **4a** (○) and **4b** (●) against the survival of mosquito larvae.

The EcR-THQ interaction was then investigated to understand the specific larvicidal activity of these enantiomers *in vitro*. We performed a competitive binding assay using [³H]PonA as a radioactive ligand for *Aedes albopictus* (AeAl) cell (NIAS-AeAl-2) binding.^{18,19,25} The results revealed that **4b** has a binding affinity for EcR that is 36 times more potent than **4a** (Table 2). These data indicate that differences in binding affinity toward EcR account for the observed larvicidal activity.

Table 2. *In vivo* and *in vitro* biological activities of ecdysone agonists^a

No.	Compounds (THQ) ^b		Larvicidal activity pLC ₅₀ (M)	Binding activity pIC ₅₀ (M)
	X	Y		
1	CH ₃	4-CH ₃	5.33	n.d. ^c
2	CH ₃	4-Cl	5.38	5.93
3	F	4-Cl	6.52	n.d.
4	F	4-Br	6.92 ^d	6.65 ± 0.13 (n=3)
4a	F	4-Br	5.65 ± 0.42 (n=2)	5.70 ± 0.16 (n=2)
4b	F	4-Br	7.40 ± 0.04 (n=2)	7.26 ± 0.04 (n=2)
	Ponasterone A		n.d.	9.01 ± 0.01 (n=2)
	Tebufenozide		n.d.	7.12 ± 0.03 (n=2)

^a Mean values with standard deviation. Number of experiments.

^b Basic structure is shown in Fig. 1.

^c n.d. not determined.

^d The trans-type compound ($2S,4S + 2S,4S$) is inactive (<4.52).

Figure 4 shows a summary of the SARs of THQs. The methyl group in position 2 is required to be in the $2R$ conformation, and the methyl substituent can be replaced with hydrogen. Conversely, the $2S$ methyl substituent leads to lower activity. This implies that the $2S$ methyl group has a steric collision with the receptor, whereas the $2R$ methyl group and the hydrogen do not. The aniline moiety in position 4 is essential for the larvicidal activity as reported previously¹⁵ and the $4S$ chiral conformation is also critical for activity. The phenyl group on tetrahydroquinoline ring is also required for activity, but the substituent R_3 is not essential.¹³ The larvicidal potency was improved approximately 3 fold by changing R_4 from 4-chloro to 4-bromo, which can be further optimized in future studies. This SAR summary provides valuable information for the molecular design of novel ecdysone agonists.

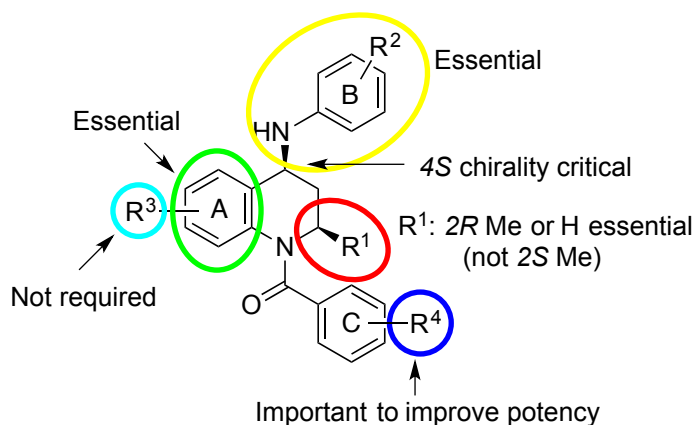


Fig. 4. Structural requirements of THQs for ecdysone agonist activity

Acknowledgements

We thank Dr. Qing X. Li of the University of Hawaii for reviewing the manuscript. The *Culex pipiens* eggs were kindly provided by Sumitomo Chemical Co. *Aedes albopictus* cells (NIAS-AeAl-2) were obtained from the GeneBank of National Institute of Agrobiological Sciences (NIAS). This study was supported in part by the Ministry of Education, Culture, Sports, Science, and Technology of Japan (No. 25450070) and the 21st century COE program for Innovative Food and Environmental Studies Pioneered by Entomomimetic Sciences. Dr. Toshiyuki Harada was a recipient of a Research Fellowship of the Japan Society for the Promotion of Science for Young Scientist.

References and Notes

1. Truman, J. W. *Vitam Horm* 2005, **73**, 1.
2. Palli, S. R., Hormann R. E., Schlattner U., Lezzi M. *Vitam Horm* 2005, **73**, 59.
3. Nakagawa, Y., Henrich V. C. *The FEBS journal* 2009, **276**, 6128.
4. Morishita, C., Minakuchi C., Yokoi T., Takimoto S., Hosoda A., Akamatsu M., Tamura H., Nakagawa Y. *J Pestic Sci* in press
5. Billas, I. M., Iwema T., Garnier J. M., Mitschler A., Rochel N., Moras D. *Nature* 2003, **426**, 91.
6. Carmichael, J. A., Lawrence M. C., Graham L. D., Pilling P. A., Epa V. C., Noyce L., Lovrecz G., Winkler D. A., Pawlak-Skrzecz A., Eaton R. E. *et al J Biol Chem* 2005, **280**, 22258.
7. Iwema, T., Billas I. M., Beck Y., Bonneton F., Nierengarten H., Chaumot A., Richards G., Laudet V., Moras D. *EMBO J* 2007, **26**, 3770.

8. Browning, C., Martin E., Loch C., Wurtz J. M., Moras D., Stote R. H., Dejaegere A. P., Billas I. M. *J Biol Chem* 2007, **282**, 32924.
9. HomePage <http://www.ncbi.nlm.nih.gov/Structure/mmdb/mmdbsrv.cgi?unid=3ixp> (accessed on Nov 15, 2013)
10. Pener, M. P., Dhadialla T. S. In: *Advances in Insect Physiology*. Edited by Dhadialla TS. Oxford: Elsevier Ltd.; 2012: 1.
11. Ogura, T., Minakuchi C., Nakagawa Y., Smagghe G., Miyagawa H. *FEBS J* 2005, **272**, 4114.
12. Dinan, L., Nakagawa Y., Hormann R. E. *Adv Insect Physiol* 2012, **43**, 251.
13. Smith, H. C., Cavanaugh C. K., Friz J. L., Thompson C. S., Sagers J. A., Michelotti E. L., Garcia J., Tice C. M. *Bioorg Med Chem Lett* 2003, **13**, 1943.
14. Palli, S. R., Tice C. M., Margam V. M., Clark A. M. *Arch Insect Biochem Biophys* 2005, **58**, 234.
15. Soin, T., Swevers L., Kotzia G., Iatrou K., Janssen C. R., Rouge P., Harada T., Nakagawa Y., Smagghe G. *Pest Manag Sci* 2010, **66**, 1215.
16. Funabashi, M., Iwakawa M., Yoshimur. *J B Chem Soc Jpn* 1969, **42**, 2885.
17. Enomoto, N., Furukawa S., Ogasawara Y., Akano H., Kawamura Y., Yashima E., Okamoto Y. *Anal Chem* 1996, **68**, 2798.
18. Nakagawa, Y., Minakuchi C., Takahashi K., Ueno T. *Insect Biochem Mol Biol* 2002, **32**, 175.
19. Nakagawa, Y., Minakuchi C., Ueno T. *Steroids* 2000, **65**, 537.
20. Sheldrick, G. M. *Acta Crystallogr A* 2008, **64**, 112.
21. Compounds **1–4** were synthesized according to the reported methods.¹³ Briefly, ice-cold acetaldehyde (5 ml, 157 mmol) was added drop-wise to a mixture of *p*-toluidine (9.57 g, 89.3 mmol) and benzotriazole (2.13 g, 17.9 mmol) in 90 ml of EtOH, and the mixture was stirred at room temperature for 4 days. After evaporating the solvent, the residue was purified by column chromatography (hexane: ethyl acetate=4:1) to give 2,6-dimethyl-4-(4-methylphenylamino)-1,2,3,4-tetrahydroquinoline (3.49 g, 13.1 mmol, 29 %) as a *cis/trans* mixture. 2,6-dimethyl-4-(4-methylphenylamino)-1,2,3,4-tetrahydroquinoline in THF and anhydrous pyridine were added drop-wise to a solution of 4-methylbenzoyl chloride

(1.14 ml, 7.39 mmol) in anhydrous THF at 0°C. The reaction mixture was warmed up to room temperature and stirred overnight. After adding 240 ml of THF, the solution was washed with a saturated NaHCO₃ aqueous solution and brine. The organic layer was dried over MgSO₄ and concentrated. The residue was purified by column chromatography (hexane: ethyl acetate = 4:1) to give *cis* isomer compound **1** (270 mg (0.702 mmol), 19%). mp: 185°C. Anal. Calcd for C₂₆H₂₈N₂O: C, 81.21; H, 7.34. Found: C, 81.32; H, 7.56. Compound **2**. mp: 183°C. Anal. Calcd for C₂₅H₂₅ClN₂O: C, 74.15; H, 6.22. Found C, 74.27; H, 6.36. Compound **3** mp: 178°C. Anal. Calcd for C₂₃H₁₉ClF₂N₂O: C, 66.91; N, 6.79; H, 4.64. Found: C, 66.80; N, 6.82; H, 4.76. Compound **4**: ¹H-NMR (CDCl₃, 400 MHz); δ (ppm) 1.26 (3H, d, *J* = 6.4 Hz), 1.35 (1H, m), 2.81 (1H, m), 3.72 (1H, br), 4.33 (1H, dd, *J* = 4.6, 12.0 Hz), 4.88 (1H, m), 6.48 (1H, br), 6.61 (2H, m), 6.68 (1H, m), 6.96 (2H, m), 7.06 (1H, m), 7.12 (2H, d, *J* = 6.6 Hz), 7.41 (2H, d, *J* = 6.8 Hz). mp: 187°C. Anal. Calcd for C₂₃H₁₉BrF₂N₂O: C, 60.41; N, 6.13; H, 4.19. Found: C, 60.21; N, 6.17; H, 4.23.

22. Separation of enantiomers of compound **4** by chiral HPLC: The enantiomers were separated on a chiral HPLC column containing a silica gel bound to tris (3, 5-dimethylphenylcarbamate) derivatives of amylose (ADMPC) at a 2.0 ml/min flow rate at 40°C using hexane/ethanol (90/10). The analytes were detected by UV absorption at 254 nm. An ADMPC-bound silica gel column was prepared according to the published method (Method-II in the report by Enomoto and coworkers¹⁷). The particle size of the column material was 5 μm and was packed in a stainless steel tube (250 mm × 4.6 mm). The retention times for each fraction are **4a** (4.3 min) and **4b** (5.8 min), respectively. This HPLC fractionation was repeated 200 times, and all fractions were combined. Enantiomer excess (*ee*) was determined by the same method and found to be >99%. After evaporating the solvent, each compound was recrystallized from hexane-ethyl acetate to obtain **4a** and **4b** crystals. Melting points of these compounds were both 201°C. The HRMS were 456.0647, 458.0631 (**4a**) and 456.0655, 458.0636 (**4b**), (Calcd. 456.0649, 458.0628). Optical rotations were $[\alpha]_D^{29} + 443$ (*c* = 0.040, ethanol) for **4a**, and $[\alpha]_D^{29} - 441$ (*c* = 0.037, ethanol) for **4b**.

23. The crystals were mounted on a glass fiber, and measurements were made on a Rigaku RINT RAPID/R with graphite monochromated Cu K α radiation ($\lambda = 1.5418 \text{ \AA}$) at 243_K. All data were processed and corrected for Lorentz and polarization effects. Intensity data within $2\theta \leq 136.4^\circ$ were measured using an imaging plate area detector. The structure was solved by direct method using the SHELXS-97 program²⁰. Positional parameters of non-H atoms were refined by full-matrix least squares using the SHELXL-97 program²⁰. All non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were placed in geometrically idealized positions and standard riding atoms. Crystal data, data collection parameters, and results of the analyses for compound **1** and **2** are listed in Supplement Table S2 and deposited under the designations CCDC 984530 (**4a**) and 984531 (**4b**). Crystal data for compounds **1** and **2** were also deposited under designations 984528 and 984529, respectively.
24. The eggs of the mosquito *Culex pipiens pallens* were kindly provided by Sumitomo Chemical Company (Hyogo, Japan), and reared to the 2nd instar in the laboratory using a 300 cm² (approx. 5 cm water depth) tank. Three tablets of Ebios (Asahi Food and Health) were added under conditions of a long-day photo period (16 h light: 8 h dark). Twenty larvae grown for two days after hatching and were transferred to a paper cup containing 30 ml of water and a small amount of Ebios. To this was added 10 μ l of DMSO solution with varying concentrations of the test compounds. After rearing for three additional days, the mortality of the mosquitos was evaluated. In each experiment, DMSO was used as a negative control, and tebufenozide treatment (33 μ M) was used as a positive control (100% mortality). The median lethal concentration (LC₅₀) was evaluated from the concentration-response curves, and the reciprocal logarithm of LC₅₀ (pLC₅₀) was used as the index of larvicidal activity.
25. The binding assay procedure is the same as that reported previously^{18, 19}. Insect cells of the forest day mosquito *Aedes albopictus* (NIAS-AeAl-2) were obtained from the

National Institute of Agrobiological Sciences (NIAS) GeneBank (http://www.gene.affrc.go.jp/index_en.php). AeAI cells were cultured at 25°C in 25-cm³ tissue culture flasks containing approximately 5 ml of the culture medium EX-Cell 401 (SAFC Biosciences) supplemented with 10% fetal bovine serum (FBS). Cell suspensions (400 µl) were added to disposable glass tubes (12 mm × 75 mm) containing 1 µl of the test compound in a DMSO solution at the bottom of the glass tube. Two microliters (ca. 60000 dpm) of [³H] PonA (140 Ci/mmol; American Radiolabeled Chemicals, Inc., St Louis, MO, USA) solution diluted with 70% ethanol was then added at 1-min intervals and incubated at 25°C. After 30 min of incubation, 3 ml of water was added to the tubes. The contents were immediately filtered through glass filters (GF-75, φ25 mm; ADVANTEC, Tokyo, Japan) and washed two times with 3 ml of water. The filters were dried under an infrared lamp, and placed into LSC vials. The amount of radioactivity collected on the filters was measured in 3 ml of Aquasol-2 (Packard Instrument Co., Meriden, CT, USA) using an Aloka LSC-5000 counter (Aloka Co., Ltd, Tokyo, Japan). The concentration required to give 50% inhibition of the binding of [³H]PonA (IC₅₀, M) was determined from the concentration-response curve, and the reciprocal logarithm of IC₅₀ (pIC₅₀) was used as the index of binding activity.

Table S1. X-ray crystallographic data for enantiomers **1** (*cis*) and **2** (*cis*)

	1 (<i>cis</i>)	2 (<i>cis</i>)
Empirical formula	C ₂₆ H ₂₈ N ₂ O	C ₂₅ H ₂₅ N ₂ OCl
Formula weight	384.52	404.94
Crystal system	Triclinic	Triclinic
Space group	$\bar{1}$ P1 (#2)	$\bar{1}$ P1 (#2)
a (Å)	9.614 (2)	9.602 (2)
b (Å)	14.574 (3)	14.447 (3)
c (Å)	16.551 (3)	16.622 (3)
α (deg)	93.57 (1)	93.47 (1)
β (deg)	93.72 (1)	95.23 (1)
γ (deg)	107.20 (1)	108.37 (1)
V (Å ³)	2202.7 (7)	2169.5 (7)
Z , D_{calc} (g/cm ³)	4, 1.159	4, 1.240
F (000)	824.00	856.00
μ (Cu K α) (mm ⁻¹)	0.546	1.688
T (K)	253	253
No. obsd ($I > 2.00 \sigma(I)$)	6216	9700
No. parameters	639	588
R_1 , wR_2	0.039, 0.104	0.048, 0.123
GOF	0.862	1.005

Table S2. X-ray crystallographic data for enantiomers **9a** and **9b**

	4.3 min	5.8 min
	9a	9b
Empirical formula	C ₂₃ H ₁₉ BrF ₂ N ₂ O	C ₂₃ H ₁₉ BrF ₂ N ₂ O
Formula weight	457.32	457.32
Crystal system	orthorhombic	orthorhombic
Space group	P2 ₁ 2 ₁ 2 ₁ (#19)	P2 ₁ 2 ₁ 2 ₁ (#19)
a (Å)	7.726 (1)	7.7240 (2)
b (Å)	11.425 (1)	11.423 (3)
c (Å)	23.284 (2)	23.288 (5)
V (Å ³)	2055.4 (3)	2054.8 (8)
Z, D _{calc} (g/cm ³)	4, 1.478	4, 1.478
F (000)	928.00	928.00
μ (Cu Kα) (mm ⁻¹)	3.024	3.025
T (K)	243	243
no. obsd	3506	3338
(I > 2.00 σ (I))		
no. parameters	265	265
R ₁ , wR ₂	0.040, 0.156	0.043, 0.145
Flack parameter	0.01(2)	0.01(2)
GOF	1.300	1.031