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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

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ABSTRACT

Tetrahydroquinoline (THQ)-type compounds are a class of potential larvicides against mosquitoes. The structure–activity relationships (SAR) of these compounds were previously investigated (Smith et al., *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1943–1946), and one of *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.

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] *Bemicia tabacii*,[6] and *Tolibolium 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 an imidazole type compound (BYI08346; 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 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]

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.

* Corresponding author. Tel.: +81 75 753 6117; fax: +81 75 753 6128.
E-mail address: naka@kais.kyoto-u.ac.jp (Y. Nakagawa).

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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-workers[13], 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 S1) and **2** (CCDC 984529: Supplement Table S1), in which the two enantiomers are packed, respectively. Smith et al. 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 procedure[21], 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 Figure 2 with their chemical structures.[23]

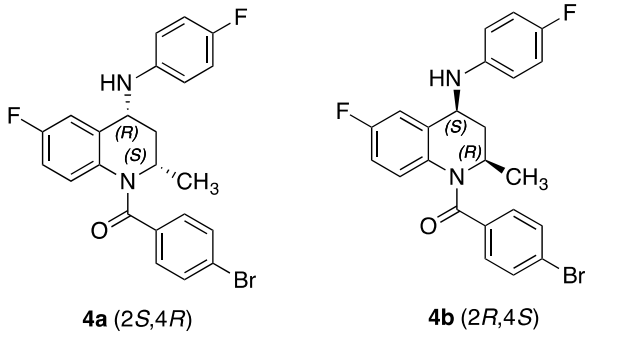
Crystal data and the data collection parameters for compounds **4a** (CCDC 984530) and **4b** (CCDC 984531) are listed in Table 1.

The SARs for compounds **1**–**3** were consistent with the previously reported SARs.[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 Figure 3. Consistently, the active compound **4b** had approximately two times higher larvicidal activity than the racemic compound **4**.

The EcR–THQ interaction was then investigated to understand the specific larvicidal activity of these enantiomers in vitro. We

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

	Retention time (HPLC)	
	4.3 min **4a**	5.8 min **4b**
Empirical formula	$C_{23}H_{19}BrF_2N_2O$	$C_{23}H_{19}BrF_2N_2O$
Formula weight	457.32	457.32
Crystal system	Orthorhombic	Orthorhombic
Space group	$P2_12_12_1$ (#19)	$P2_12_12_1$ (#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 ($I > 2.00\sigma(I)$)	3506	3338
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



4a (2*S*,4*R*) **4b** (2*R*,4*S*)

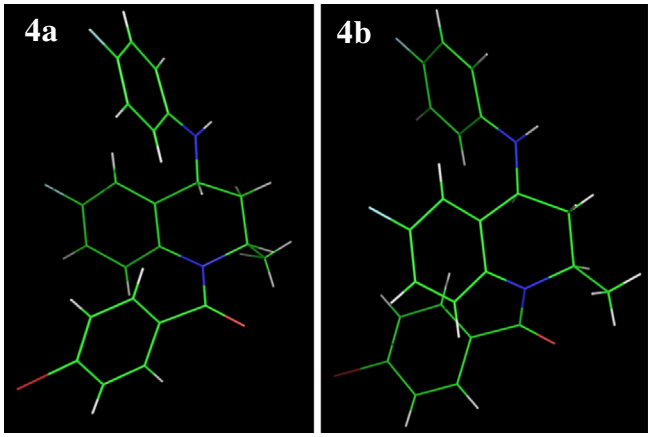
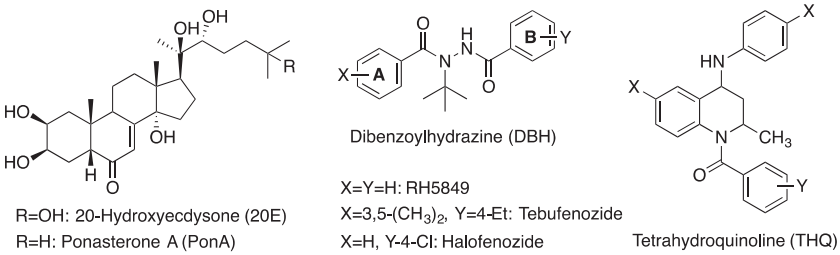


Figure 2. X-ray structures of enantiomer **4a** and **4b**.



R=OH: 20-Hydroxyecdysone (20E)
R=H: Ponasterone A (PonA)

Dibenzoylhydrazine (DBH)

X=Y=H: RH5849
X=3,5-$(CH_3)_2$, Y=4-Et: Tebufenozide
X=H, Y-4-Cl: Halofenozide

Tetrahydroquinoline (THQ)

Figure 1. Structures of ecdysone agonists.