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# Structure-activity relationship of imidazothiadiazole analogs for the binding to the ecdysone receptor of insect cells

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**Abstract**

Diacylhydrazines are the first non-steroidal ecdysone agonists, and five compounds are used as insecticides in agriculture. After the discovery of diacylhydrazine-type compounds, numerous non-steroidal structures were reported as ecdysone agonists. Among various ecdysone agonists, imidazothiadiazoles are reported to be very potent *in vitro;* however the experimental detail for the structure identification and bioassays are not stated in the paper (Holmwood and Schindler, *Bioorg. Med. Chem.*, **17**, 4064-4070, 2009). In our present study, we synthesized 18 imidazothiadiazole-type compounds and confirmed the chemical structures by spectrometric analyses. The binding activity of the synthesized compounds to the ecdysone receptor was evaluated in terms of the concentration required for 50% inhibition of [$^3$H]ponasterone A incorporation [$IC_{50}$ (M)] into lepidopteran (Sf-9), coleopteran (BCRL-Lepd-SL1), and dipteran (NIAS-AeAl2) cells. 6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]-thiadiazolyl-5-yl)acrylamide analogs with –CONHR (secondary amide) were very potent against Sf-9 cells, but further alkylation (tertiary amide: $–CONR_2$) decreased the activity dramatically. Additionally, a primary amide analog ($–CONH_2$) was inactive. The activity also decreased 150-fold by the saturation of olefin region of the acrylamide moiety. In addition, various substituents were introduced at the 2-position of the imidazothiadiazole ring to disclose the physicochemical properties of the substituents which are important for receptor binding. The activity increased by 7500-fold with the introduction of the $CF_2CF_2CF_3$ group compared to the unsubstituted compound against Sf-9 cells. Quantitative structure-activity relationship analysis for these substituents indicated that hydrophobic and electron-withdrawing groups were favorable for binding. Some of the compounds with strong receptor binding activity showed good larvicidal activity against *Spodoptera litura*. In contrast, the binding affinity of imidazothiadiazole analogs was low or not observed against dipteran and coleopteran cells.

**Keywords:**

ecdysone agonists, molting inhibitor, imidazothiadiazole, Sf-9, ecdysone receptor

## 1. Introduction

Arthropods, including insects, grow by repeated molting, which is regulated by molting hormones such as 20-hydroxyecdysone (20E; Fig. 1). Steroidal compounds with 20E-like activity are categorized as ecdysteroids, and have been identified in plants, animals, and microorganisms. To date, more than 400 ecdysteroids have been characterized (http://ecdybase.org), but no ecdysteroids have been launched as insecticides. Using steroids as insecticides may not be practical because of their high cost and synthesis difficulty. In addition, steroids do not easily penetrate the integument and are rapidly excreted from insects.

The discovery of diacylhydrazine (DAH)-type compounds (Fig. 1) enabled the development of novel ecdysone agonist insecticides [1, 2]. Currently, five DAHs, namely, tebufenozide, methoxyfenozide, chromafenozide, fufenozide, and halofenozide, are available on the market. These DAH-type compounds are generally used in agriculture against Lepidoptera, but halofenozide also shows control of Coloeoptera.

Fig. 1

Because the insecticidal spectrum of DAHs is narrow, other chemical structures have been screened as ecdysone agonists [3]. Among them, tetrahydroquinoline (THQ) [4], *N*-alkyl-3,5-di-*tert*-butyl-4-hydroxy-benzamide [5], α-acylaminoketone [6], oxadiazoline [7], and γ-methylene-γ-lactam [8] have been described over the past two decades. In 2009, Holmwood and Schindler reported that imidazole (IMD) and imidazothiadiazole (ITD)-type compounds are ecdysone agonists (Fig. 2) [9]. Although the biological activity was evaluated quantitatively in terms of $pInd_{50}$ (EcR induction assay), experimental procedures and target insect species have not been described. Analytical data for the synthesized chemicals were not reported.

The binding mode of IMD-type compounds was reported to be similar to that of DAHs based on crystal structure analysis. The binding mode of ITD-type compounds is,

however, thought to differ from those of DAHs and steroidal agonists such as ponasterone A (PonA). The ITD substructure is very interesting, because some ITD-type compounds are reported to show anti-inflamatory [10], anticancer [11] and antitubercular activity [12].

The aim of this study was to quantitatively measure the ligand-receptor binding activity of ITD analogs and discuss the structure-activity relationship (SAR). For the SAR study, various ITD analogs were chemically synthesized. The substituents X at 2-position of imidazothiadiazole ring (Fig. 2) were substituted with H, $CH_3$, $CF_3$, $CF_2CF_3$, $CF_2CF_2CF_3$, $SCH_3$, $S(=O)CH_3$, $SO_2CH_3$, and the amide moiety to vary primary, secondary, and tertiary structure (Fig. 2). The linker between the imidazole ring and amide moiety was fixed as either *trans* -CH=CH- or -$CH_2CH_2$- (Fig. 2). Thioamide and sulfonamide analogs were also synthesized (Fig. 2). The binding affinity of these compounds was measured to the ecdysone receptors of three insect cells. The effect of substituents X on ligand-receptor binding against Sf-9 was quantitatively analyzed using classical quantitative structure-activity relationship (QSAR) analysis (Hansch-Fujita method) [13]. Docking simulation was also performed to predict the ligand-receptor interaction of ITDs.

Fig. 2

## 2. Materials and Methods

### 2.1. Synthesis

#### 2.1.1. Chemicals

Chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Nacalai Tesque Inc. (Kyoto, Japan). Oven-dried glassware and positive argon pressure were used to maintain anhydrous conditions. Anhydrous solvents were

commercially available and stored over molecular sieves. Flash column chromatography was conducted using Wakogel® C-300HG (Wako Pure Chemical Industries, Osaka, Japan) as the absorbent. NMR spectra were recorded on a Bruker AVANCE-400 or Bruker AVANCE-500 spectrometer. Tetramethylsilane was used as the internal standard for $^{1}$H NMR (0 ppm); deuterated solvent signals were used as the internal standard for $^{13}$C NMR (77.16 ppm for $CDCl_3$ and 39.52 ppm for DMSO-$d_6$); and α,α,α-trifluorotoluene was used as the external standard for $^{19}$F NMR (-64.00 ppm). Melting points were measured with a Yanaco melting point apparatus (Yanagimoto Seisakusho Co. Ltd., Kyoto, Japan) and are uncorrected. Elemental analyses were performed at the Microanalytical Center of Kyoto University. High-resolution mass spectra (HRMS) were recorded on a Thermo Fisher Scientific EXACTIVE spectrometer at Department of Synthetic Chemistry and Biological Chemistry of Kyoto University.

**2.1.2. Synthesis of 2-amino-1,3,4-thiadiazoles (Scheme 1)**

Scheme 1

*i) 2-Amino-5-(trifluoromethyl)-1,3,4-thiadiazole (Step a):* Phosphoryl chloride (27.5 mL, 300 mmol) was added dropwise to a mixture of thiosemicarbazide (13.7 g, 150 mmol) and trifluoroacetic acid (24.1 mL, 315 mmol) at 0°C, and the mixture was gradually heated to 70°C. Foaming was observed during the reaction. The mixture was kept at 70ºC for 1 hour after the foaming ceased. After cooling the reaction mixture to room temperature, it was treated with water (300 mL) and neutralized with saturated $Na_2CO_3$ solution. The resulting solid was filtered off, washed with water and dried *in vacuo* to yield an off-white solid (19.4 g, 77%). $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.07 (2H, s) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 119.8 (q, $J_{C\text{-}F}$ = 269 Hz), 143.8 (q, $J_{C\text{-}F}$ = 37 Hz), 171.7 ppm; $^{19}$F NMR (377 MHz, DMSO-$d_6$) $\delta$ -59.77 (3F, s) ppm.

2-Amino-1,3,4-thiadiazoles with $CF_2CF_3$ and $CF_2CF_2CF_3$ group were synthesized in a

similar manner as above.

*ii) 2-Amino-5-(methylthio)-1,3,4-thiadiazole (Step b)*: Potassium hydroxide (85%, 3.4 g, 51 mmol) was added in one portion to a suspension of 2-amino-5-mercapto-1,3,4-thiadiazole (6.7 g, 50 mmol) in 2-propanol (10 mL) and water (7.5 mL) at 0°C. When the starting materials completely dissolved, methyl iodide (3.3 mL, 53 mmol) was added dropwise to the reaction mixture maintaining the temperature below 15°C. It was stirred at room temperature overnight. The mixture was poured into water (200 mL) and the resulting solid was filtered off. This was washed with water and dried *in vacuo* to yield a white solid (5.6 g, 76%). $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.58 (3H, s), 7.21 (2H, s) ppm; $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 16.6, 151.9, 168.9 ppm.

### 2.1.3. Synthesis of 2-chlorophenacyl bromide

A solution of bromine (26.5 g, 166 mmol) in acetic acid (25 mL) was added dropwise to a solution of 2′-chloroacetophenone (25.1 g, 162 mmol) in acetic acid (175 mL) at room temperature. The mixture was stirred at room temperature for 2 hours. The mixture was diluted with water (250 mL) and extracted with $CH_2Cl_2$ (250 mL). The organic layer was washed successively with water (3×250 mL), saturated aqueous $NaHCO_3$ solution (250 mL) and brine (250 mL), and dried over anhydrous $Na_2SO_4$. The solvent was evaporated to give the crude 2-chlorophenacyl bromide (38.7 g, purity: *ca.* 83% determined by $^{1}$H NMR analysis), which was used for the next reaction without further purification.

### 2.1.4. Synthesis of (*E*)-3-(6-(2-chlorophenyl)-imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-acrylic acids (Scheme 2)

Scheme 2

*i) 6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazole (Step a):* A

mixture of 2-amino-5-(trifluoromethyl)-1,3,4-thiadiazole (19.2 g, 114 mmol) and 2-chlorophenacyl bromide (33.4 g, *ca.* 120 mmol) in ethanol (170 mL) was refluxed overnight. The mixture was then cooled in a freezer. The resulting crystalline solid was filtered off, washed with cold ethanol, and dried *in vacuo* to yield a pale yellow solid (19.3 g, 56%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.39 (1H, td, $J$ = 7.9, 1.8 Hz), 7.47 (1H, td, $J$ = 7.5, 1.3 Hz), 7.58 (1H, dd, $J$ = 7.9, 1.3 Hz), 8.11 (1H, dd, $J$ = 7.8, 1.8 Hz), 8.96 (1H, s) ppm.

Other imidazothiadiazole analogs with $CF_2CF_3$, $CF_2CF_2CF_3$, H, $CH_3$, and $SCH_3$ were synthesized in a similar manner as above.

*ii) 6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (Step b)*: Under an argon atmosphere, phosphoryl chloride (3.9 mL, 43 mmol) was added dropwise to anhydrous DMF (20 mL) at 0°C and stirred for 5 min. To this was added 6-(2-chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazole (3.91 g, 13 mmol) in anhydrous DMF (15 mL), and the mixture was heated to 70°C and stirred overnight. It was poured into ice-water (100 mL), neutralized with saturated aqueous $Na_2CO_3$ solution and then extracted with toluene (1×100 mL, 2×50 mL). The combined organic layer was washed with water (3×100 mL) and brine (100 mL), and dried over anhydrous $MgSO_4$. The solvent was evaporated and the crude product was purified by flash column chromatography (hexane/ethyl acetate = 95:5 – 50:50) to yield a pale yellow solid (2.91 g, 68%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.43-7.49 (2H, m), 7.55-7.59 (2H, m), 9.85 (1H, s) ppm.

Other imidazothiadiazole-5-carbaldehyde analogs with $CF_2CF_3$, $CF_2CF_2CF_3$, H, $CH_3$, and $SCH_3$ were synthesized in a similar manner as above.

*iii) (E)-3-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl) acrylic acid (Step c)*: 6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4] thiadiazole-5-carbaldehyde (2.01 g, 6.1 mmol) and piperidine (0.47 mL, 4.7 mmol) were

dissolved in pyridine (17 mL). To this, malonic acid (0.76 g, 7.3 mmol) was added and the mixture was stirred at 100°C for 4 h. After cooling, the reaction mixture was poured into 1 M HCl (70 mL) and acidified with concentrated HCl. The resulting solid was filtered off, washed with water, and dried *in vacuo*. This solid was triturated in hexane/ether (1:1) to give an off-white solid (1.89 g, 84%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 6.68 (1H, d, $J$ = 16.0 Hz), 7.31 (1H, d, $J$ = 16.0 Hz), 7.52-7.61 (3H, m), 7.67-7.71 (1H, m), 12.64 (1H, br. s) ppm.

Other (imidazothiadiazol-5-yl)acrylic acid analogs with $CF_2CF_3$, $CF_2CF_2CF_3$, H, $CH_3$, and $SCH_3$ were synthesized in a similar manner as above.

**2.1.5. Synthesis of (*E*)-3-(6-(2-chlorophenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl) acrylamides (Scheme 3)**

Scheme 3

*i) (E)-3-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl) acrylamide (1)*: Oxalyl chloride (0.17 mL, 2.0 mmol) was added to the suspension of (*E*)-3-(6-(2-chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)acrylic acid (377 mg, 1.0 mmol) in $CH_2Cl_2$ (5 mL) containing one drop of DMF. After the gas evolution ceased, the mixture was refluxed for 2 hours. After cooling, the solvent was evaporated to give the crude acid chloride. This was dissolved in $CH_2Cl_2$ (5 mL), and then added dropwise to vigorously stirred aqueous $NH_3$ solution (28%, 5 mL) at 0ºC. The mixture was stirred at room temperature overnight. It was diluted with water (30 mL) and extracted with $CH_2Cl_2$ (3×30 mL). The combined organic layer was washed with water (50 mL) and brine (50 mL), and dried over anhydrous $Na_2SO_4$. The solvent was evaporated and the crude product was recrystallized from ethyl acetate/hexane to give a white solid (276 mg, 73%). Mp: 232-234°C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.67 (2H, br

s), 6.96 (1H, d, $J$ = 15.6 Hz), 7.34-7.47 (3H, m), 7.51-7.58 (2H, m) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 118.7 (q, $J_{C\text{-}F}$ = 272 Hz), 119.0, 122.9, 126.2, 127.2, 130.5, 130.9, 131.5, 132.6, 134.0, 146.5, 148.5, 151.3 (q, $J_{C\text{-}F}$ = 42 Hz), 167.3 ppm; $^{19}$F NMR (377 MHz, $CDCl_3$) $\delta$ -62.46 (3F, s) ppm. Anal. Calcd for $C_{14}H_8ClF_3N_4OS$: C, 45.11; H, 2.16; N, 15.03. Found: C, 45.07; H, 2.20; N, 15.05.

*ii) (E)-3-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-N-isopropylacrylamide (**2**)*: To a suspension of compound **12** (374 mg, 1.0 mmol) in anhydrous $CH_2Cl_2$ (5 mL), EDC hydrochloride (227 mg, 1.2 mmol) and catalytic amount of DMAP were added. Then, isopropylamine (98 μL, 1.2 mmol) was added and the mixture was stirred at room temperature overnight. The mixture was diluted with $CH_2Cl_2$ (15 mL) and washed successively with saturated aqueous $Na_2CO_3$ solution, water, 1 M HCl, water, and brine (10 mL each). The organic layer was dried over $MgSO_4$ and concentrated to give compound **17** as pale yellow foam (387 mg, 93%). This was further recrystallized from ethyl acetate/hexane to afford white crystals, which were used for the bioassays. Mp: 174-175°C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.22 (6H, d, $J$ = 6.6 Hz), 4.14-4.27 (1H, m), 5.58 (1H, br d, $J$ = 7.7 Hz), 6.86 (1H, d, $J$ = 15.5 Hz), 7.33-7.45 (3H, m), 7.50-7.54 (2H, m) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 22.9, 41.9, 118.7 (q, $J_{C\text{-}F}$ = 272 Hz), 120.6, 123.2, 124.6, 127.1, 130.5, 130.7, 131.6, 132.6, 134.1, 146.1, 148.0, 151.0 (q, $J_{C\text{-}F}$ = 42 Hz), 164.6 ppm; $^{19}$F NMR (377 MHz, $CDCl_3$) $\delta$ -62.43 (3F, s) ppm. Anal. Calcd for $C_{17}H_{14}ClF_3N_4OS$: C, 49.22; H, 3.40; N, 13.51. Found: C, 49.16; H, 3.59; N, 13.65.

Other acrylamide analogs (**3** - **13**) were synthesized in a similar manner to that described for compound **2**. Analytical data for the compounds are shown below.

*iii) (E)-3-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-N-cyclobutylacrylamide (**3**)*: Mp: 199-200°C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.69-1.78 (2H, m), 1.88-2.00 (2H, m), 2.34-2.43 (2H, m), 4.51 (1H, quin, $J$ = 8.1 Hz), 5.92 (1H, br d,

243 $J$ = 7.7 Hz), 6.86 (1H, d, $J$ = 15.5 Hz), 7.33-7.44 (3H, m), 7.50-7.54 (2H, m) ppm; $^{13}$C
244 NMR (100 MHz, $CDCl_3$) $\delta$ 15.3, 31.4, 45.2, 118.7 (q, $J_{C\text{-}F}$ = 272 Hz), 120.3, 123.2, 124.9,
245 127.1, 130.5, 130.8, 131.6, 132.6, 134.1, 146.2, 148.1, 151.0 (q, $J_{C\text{-}F}$ = 42 Hz), 164.5
246 ppm; $^{19}$F NMR (377 MHz, $CDCl_3$) $\delta$ -62.43 (3F, s) ppm. Anal. Calcd for
247 $C_{18}H_{14}ClF_3N_4OS$: C, 50.65; H, 3.31; N, 13.13. Found: C, 50.52; H, 3.28; N, 13.25.
248
249 *iv) (E)-3-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)*
250 *-N-cyclohexylacrylamide (**4**)*: Mp: 221-223°C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.11-1.28
251 (3H, m), 1.32-1.46 (2H, m), 1.59-1.69 (1H, m), 1.60-1.79 (2H, m), 1.94-2.00 (2H, m),
252 3.83-3.94 (1H, m), 5.71 (1H, br d, $J$ = 8.1 Hz), 6.88 (1H, d, $J$ = 15.6 Hz), 7.32-7.44 (3H,
253 m), 7.49-7.55 (2H, m) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 25.0, 25.7, 33.3, 48.8, 118.7
254 (q, $J_{C\text{-}F}$ = 272 Hz), 120.8, 123.2, 124.5, 127.0, 130.5, 130.7, 131.6, 132.6, 134.0, 146.1,
255 148.0, 151.0 (q, $J_{C\text{-}F}$ = 42 Hz) ppm; 164.5, 164.5. $^{19}$F NMR (377 MHz, $CDCl_3$) $\delta$ -62.42
256 (3F, s) ppm. Anal. Calcd for $C_{20}H_{18}ClF_3N_4OS$: C, 52.81; H, 3.99; N, 12.32. Found: C,
257 52.82; H, 4.02; N, 12.32.
258
259 *v) (E)-3-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-*
260 *N-phenylacrylamide (**5**)*: Mp: 222-224°C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.03-7.15 (2H,
261 m), 7.29-7.47 (5H, m), 7.50-7.53 (1H, m), 7.57-7.69 (4H, m) ppm; $^{13}$C NMR (100 MHz,
262 $CDCl_3$) $\delta$ 118.7 (q, $J_{C\text{-}F}$ = 272 Hz), 119.9, 120.3, 123.1, 124.6, 126.0, 127.1, 129.2, 130.5,
263 130.9, 131.4, 132.5, 134.0, 138.1, 146.5, 148.6, 151.3 (q, $J_{C\text{-}F}$ = 42 Hz), 163.7 ppm; $^{19}$F
264 NMR (377 MHz, $CDCl_3$) $\delta$ -62.37 (3F, s) ppm. Anal. Calcd for $C_{20}H_{12}ClF_3N_4OS$: C,
265 53.52; H, 2.69; N, 12.48. Found: C, 53.25; H, 2.88; N, 12.43.
266
267 *vi) (E)-3-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)*
268 *-N-isopropyl-N-methylacrylamide (**6**)*: Mp: 144-145°C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$
269 (mixture of rotamers) 1.14 (3.3H, d, $J$ = 6.6 Hz), 1.26 (2.7H, d, $J$ =6.6 Hz), 2.89 (1.35H, s),
270 2.98 (1.65H, s), 4.27 (0.45H, sep, $J$ = 6.6 Hz), 4.96 (0.55H, sep, $J$ = 6.6 Hz), 7.32-7.53

(6H, m) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ (mixture of rotamers) 19.5, 20.7, 26.6, 28.5, 44.6, 28.5, 118.3, 118.5, 118.7 (q, $J_{C-F}$ = 272 Hz), 122.8, 123.7, 125.4, 125.7, 127.1, 130.4, 130.7, 131.7, 132.6, 134.1, 145.9, 146.0, 147.3, 147.6, 150.9 (q, $J_{C-F}$ = 42 Hz), 165.8, 166.1 ppm; $^{19}$F NMR (377 MHz, $CDCl_3$) $\delta$ (mixture of rotamers) -62.81 (1.35F, s), -62.71 (1.65F, s) ppm. Anal. Calcd for $C_{18}H_{16}ClF_3N_4OS$: C, 50.41; H, 3.76; N, 13.06. Found: C, 50.43; H, 3.76; N, 12.94.

*vii) (E)-3-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl) -1-(piperidin-1-yl)prop-2-en-1-one (7)*: Mp: 168-170°C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.55-1.75 (6H, m), 3.50-3.70 (4H, m), 7.33-7.46 (4H, m), 7.51-7.54 (2H, m) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 24.8, 25.7, 26.9, 43.6, 47.2, 117.6, 118.7 (q, $J_{C-F}$ = 271 Hz), 123.7, 125.8, 127.1, 130.4, 130.7, 131.7, 132.6, 134.1, 145.9, 147.5, 150.9 (q, $J_{C-F}$ = 42 Hz), 165.0 ppm; $^{19}$F NMR (377 MHz, $CDCl_3$) $\delta$ -62.67 (3F, s) ppm. Anal. Calcd for $C_{19}H_{16}ClF_3N_4OS$: C, 51.76; H, 3.66; N, 12.71. Found: C, 51.86; H, 3.81; N, 12.71.

*viii) (E)-3-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl) -1-morpholinoprop-2-en-1-one (**8**)*: Mp: 171-172°C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.60-3.70 (8H, m), 7.35-7.46 (4H, m), 7.52-7.54 (1H, m), 7.60 (1H, d, $J$ = 16.0 Hz) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 42.7, 46.3, 67.0, 116.1, 118.7 (q, $J_{C-F}$ = 272 Hz), 123.5, 126.7, 127.1, 130.5, 130.8, 131.6, 132.6, 134.0, 146.3, 148.1, 151.2 (q, $J_{C-F}$ = 42 Hz), 165.2 ppm. $^{19}$F NMR (377 MHz, $CDCl_3$) $\delta$ -62.61 (3F, s) ppm. Anal. Calcd for $C_{18}H_{14}ClF_3N_4O_2S$: C, 48.82; H, 3.19; N, 12.65. Found: C, 48.67; H, 3.27; N, 12.55.

*ix) (E)-3-(6-(2-Chlorophenyl)-2-(perfluoroethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-N-isopropylacrylamide (**9**)*: Mp: 165-166°C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.22 (6H, d, $J$ = 6.6 Hz), 4.12-4.27 (1H, m), 5.58 (1H, br d, $J$ = 7.8 Hz), 6.80 (1H, d, $J$ = 15.5 Hz), 7.33-7.48 (3H, m), 7.50-7.58 (2H, m) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 22.9, 41.9, 109.3 (tq, $J$ = 256, 41 Hz), 118.0 (qt, $J_{C-F}$ = 285, 36 Hz), 120.6, 123.1, 124.6, 127.1, 130.5,

130.8, 131.5, 132.6, 134.0, 146.2, 148.0, 150.7 (t, $J_{C\text{-}F}$ = 31 Hz), 164.6 ppm; $^{19}$F NMR (377 MHz, $CDCl_3$) $\delta$ -111.16 (2F, q, $J_{F\text{-}F}$ = 2.3 Hz), -84.20 (3F, t, $J_{F\text{-}F}$ = 2.3 Hz) ppm. Anal. Calcd for $C_{18}H_{14}ClF_5N_4OS$: C, 46.51; H, 3.04; N, 12.05. Found: C, 46.50; H, 3.08; N, 12.26.

*x) (E)-3-(6-(2-Chlorophenyl)-2-(perfluoropropyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-N-isopropylacrylamide (**10**):* Mp: 170-172°C. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.21 (6H, d, $J$ = 6.6 Hz), 4.15-4.26 (1H, m), 5.67 (1H, br d, $J$ = 7.8 Hz), 6.82 (1H, d, $J$ = 15.5 Hz), 7.33-7.45 (3H, m), 7.50-7.58 (2H, m) ppm; $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 22.9, 41.9, 105.5-111.2 (m), 111.3 (tt, $J_{C\text{-}F}$ = 285, 36 Hz), 117.6 (qt, $J_{C\text{-}F}$ = 286, 34 Hz), 120.7, 123.2, 124.6, 127.1, 130.5, 130.7, 131.6, 132.6, 134.1, 146.2, 148.0, 150.6 (t, $J_{C\text{-}F}$ = 31 Hz), 164.6 ppm; due to $^{13}$C-$^{19}$F coupling, the signals of the perfluoropropyl group were so weak that it was difficult to detect those signals and their coupling patterns. $^{19}$F NMR (471 MHz, $CDCl_3$) $\delta$ -126.61 (2F, t, $J_{F\text{-}F}$ = 8.5 Hz), -108.75 (2F, sex, $J_{F\text{-}F}$ = 9.4 Hz), -81.01 (3F, t, $J_{F\text{-}F}$ = 9.6 Hz) ppm. Anal. Calcd for $C_{19}H_{14}ClF_7N_4OS$: C, 44.33; H, 2.74; N, 10.88. Found: C, 44.18; H, 2.79; N, 10.89.

*xi) (E)-3-(6-(2-Chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-N-isopropylacrylamide (**11**):* Mp: 210-212°C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.19 (6H, d, $J$ = 6.6 Hz), 4.12-4.26 (1H, m), 5.65 (1H, br d, $J$ = 7.6 Hz), 6.92 (1H, d, $J$ = 15.5 Hz) 7.30-7.39 (2H, m), 7.42-7.47 (1H, m), 7.48-7.57 (2H, m), 8.69 (1H, s) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 22.9, 41.7, 119.5, 122.6, 125.2, 126.9, 130.30, 130.32, 132.2, 132.6, 134.0, 146.2, 147.3, 147.5, 165.1 ppm. HRMS (ESI) *m/z*: $C_{16}H_{16}ClN_4OS$ $[M+H]^+$, calcd 347.0728, found 347.0717.

*xii) (E)-3-(6-(2-Chlorophenyl)-2-methylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-N-isopropylacrylamide (**12**):* Mp: 214-215°C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.20 (6H, d, $J$ = 6.5 Hz), 2.78 (3H, s), 4.20 (1H, m), 5.59 (1H, br d, $J$ = 7.7 Hz), 6.89 (1H, d, $J$ = 15.4 Hz),

7.28-7.38 (2H, m), 7.40-7.54 (3H, m) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 18.0, 23.0, 41.7, 119.1, 122.3, 125.5, 126.9, 130.1, 130.3, 123.4, 132.7, 134.1, 146.2, 147.6, 160.2, 165.3 ppm. Anal. Calcd for $C_{17}H_{17}ClN_4OS$: C, 56.58; H, 4.75; N, 15.53. Found: C, 56.73; H, 4.65; N, 15.70.

*xiii) (E)-3-(6-(2-Chlorophenyl)-2-(methylthio)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-N-isopropylacrylamide (**13**)*: Mp: 229-230°C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.19 (6H, d, $J$ = 6.6 Hz), 2.79 (3H, s), 4.12-4.27 (1H, m), 5.62 (1H, br d, $J$ = 7.7 Hz), 6.80 (1H, d, $J$ = 15.4 Hz), 7.29-7.38 (2H, m), 7.39-7.55 (3H, m) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 16.7, 22.9, 41.7, 119.3, 122.4, 125.4, 126.9, 130.2, 130.3, 132.3, 132.6, 134.0, 145.7, 146.8, 162.6, 165.2 ppm. HRMS (ESI) *m/z*: $C_{17}H_{18}ClN_4OS_2$ $[M+H]^+$, calcd 393.0605, found 393.0594.

**2.1.6. Synthesis of imidazothiadiazole analogs with S(=O)$CH_3$ and $SO_2CH_3$ (Scheme 4)**

Sheme 4

*i) (E)-3-(6-(2-Chlorophenyl)-2-(methylsulfinyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-N-isopropylacrylamide (**14**)*: Compound **13** (394 mg, 1.0 mmol) was dissolved in $CH_2Cl_2$ (5 mL) and cooled to 0ºC. To this was added the solution of *m*-chloroperbenzoic acid (70%, 246 mg, 1.0 mmol) in $CH_2Cl_2$ (5 mL) within 30 min and the mixture was stirred at room temperature overnight. The reaction was quenched by adding saturated aqueous $Na_2CO_3$ solution (5 mL). The organic layer was washed with saturated aqueous $Na_2CO_3$ solution (5 mL) and brine (5mL), and dried over anhydrous $Na_2SO_4$. The solvent was evaporated and the crude product was purified by flash column chromatography (ethyl acetate 100%) to yield white foam (348 mg, 85%). This was further recrystallized from $CHCl_3$/hexane to afford white crystals, which were used for the bioassays. Mp: 236-237°C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.20 (6H, d, $J$ = 6.6 Hz), 3.17 (3H, s),

4.12-4.28 (1H, m), 5.65 (1H, br d, $J$ = 7.8 Hz), 6.77 (1H, d, $J$ = 15.7 Hz), 7.31-7.47 (3H, m), 7.47-7.57 (2H, m) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 22.9, 41.8, 43.9, 120.1, 122.6, 124.8, 127.1, 130.4, 130.6, 131.8, 132.6, 134.0, 146.8, 147.1, 164.8, 173.5 ppm. HRMS (ESI) *m/z*: $C_{17}H_{18}ClN_4O_2S_2$ $[M+H]^+$, calcd 409.0554, found 409.0546.

*ii) (E)-3-(6-(2-Chlorophenyl)-2-(methylsulfonyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-N-isopropylacrylamide (**15**)*: Compound **13** (398 mg, 1.0 mmol) was dissolved in THF/MeOH/water (1:1:1, 15 mL) and cooled to 0ºC. Oxone® (1.84 g, 3.0 mmol) was slowly added to the solution and the mixture was stirred at 0ºC for 5 min and at room temperature overnight. After largely evaporating the solvent, the mixture was diluted with water (20 mL) and extracted with $CH_2Cl_2$ (1×10 mL, 2×5 mL). The combined organic layer was washed with water (10 mL) and brine (10 mL), and dried over anhydrous $Na_2SO_4$. The solvent was evaporated and the crude product was purified by flash column chromatography (hexane/ethyl acetate = 3:7) to yield a pale yellow solid (340 mg, 79%). This was further recrystallized from $CHCl_3$/hexane to afford pale yellow crystals, which were used for the bioassays. Mp: 247-249°C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.20 (6H, d, $J$ = 6.6 Hz), 3.45 (3H, s), 4.12-4.27 (1H, m), 5.81 (1H, br d, $J$ = 7.6 Hz), 6.86 (1H, d, $J$ = 15.6 Hz), 7.33-7.47(3H, m), 7.48-7.59 (2H, m) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 22.9, 41.9, 44.0, 120.9, 123.1, 124.4, 127.1, 130.5, 130.8, 131.4, 132.6, 134.0, 147.3, 148.2, 162.1, 164.6 ppm. HRMS (ESI) *m/z*: $C_{17}H_{18}ClN_4O_3S_2$ $[M+H]^+$, calcd 425.0503, found 425.0492.

### 2.1.7. Synthesis of other imidazothiadiazole analogs

Scheme 5

*i) 3-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-N-isopropylpropanamide (**16**)*: To the solution of (*E*)-3-(6-(2-chlorophenyl)-2-

(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)acrylic acid (374 mg, 1.0 mmol) in MeOH (6 mL) was added hydrazine hydrate (2.0 mL, 41 mmol) and catalytic amount of acetic acid and saturated aqueous $CuSO_4$ solution. To this, the solution of $NaIO_4$ (1.09 g, 5.1 mmol) in water (10 mL) was added dropwise in 1 hour and the mixture was stirred at room temperature overnight. After largely evaporating the solvent, the mixture was diluted with 3 M HCl (20 mL) and extracted with ethyl acetate (3×20 mL). The combined organic layer was washed with water (20 mL) and brine (20 mL), and dried over anhydrous $Na_2SO_4$. The solvent was evaporated to give the crude propanoic acid. This was suspended in anhydrous $CH_2Cl_2$ (5 mL), and EDC hydrochloride (225 mg, 1.2 mmol) and catalytic amount of DMAP were added. Then, isopropylamine (98 µL, 1.2 mmol) was added and the mixture was stirred at room temperature overnight. Because TLC analysis indicated that the reaction was not complete, EDC hydrochloride (122 mg, 0.64 mmol), isopropylamine (50 µL, 0.61 mmol) and catalytic amount of DMAP were further added and the mixture was stirred at room temperature for 3 days. The mixture was diluted with $CH_2Cl_2$ (15 mL) and washed successively with saturated aqueous $Na_2CO_3$ solution, water, 1 M HCl, water, and brine (10 mL each). The organic layer was dried over $MgSO_4$ and filtered through a plug of silica gel, which was eluted with ethyl acetate. The filtrate was concentrated and the crude product was purified by flash column chromatography (hexane/ethyl acetate = 3:2) to yield a pale yellow solid (86 mg, 21%). This was further recrystallized from ethyl acetate to afford pale yellow crystals, which were used for the bioassays. Mp: 180-182°C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.09 (6H, d, $J$ = 6.6 Hz), 2.49-2.58 (2H, m), 3.22-3.31 (2H, m), 3.93-4.09 (1H, m), 5.16 (1H, br d, $J$ = 5.4 Hz), 7.32-7.40 (2H, m), 7.42-7.53 (2H, m) ppm; $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 20.6, 22.8, 34.0, 41.6, 118.8 (q, $J_{C\text{-}F}$ = 271 Hz), 125.0, 127.1, 130.10, 130.12, 132.5, 132.8, 133.9, 142.2, 142.4, 150.2 (q, $J_{C\text{-}F}$ = 42 Hz), 170.0 ppm. $^{19}$F NMR (377 MHz, $CDCl_3$) $\delta$ -62.63 (3F, s) ppm. Anal. Calcd for $C_{17}H_{16}ClF_3N_4OS$: C, 48.98; H, 3.87; N, 13.44. Found: C, 48.76; H, 4.01; N, 13.26.

Scheme 6

*ii) E)-3-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-N- isopropylprop-2-enethioamide (**17**)*: A mixture of compound **2** (349 mg, 0.84 mmol) and Lawesson's reagent (174 mg, 0.43 mmol) in toluene (20 mL) was refluxed for 2 hours. After cooling, the mixture was filtered through a plug of silica gel, which was eluted with ethyl acetate. The filtrate was concentrated and the crude product was purified by flash column chromatography (hexane/ethyl acetate = 3:1) and recrystallization from ethyl acetate/hexane to yield yellow crystals (282 mg, 78%). Mp: 203-204°C. $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.33 (6H, d, $J$ = 6.6 Hz), 4.77-4.91 (1H, m), 7.16-7.26 (2H, m), 7.36-7.49 (3H, m), 7.52-7.58 (1H, m), 7.87 (1H, d, $J$ = 15.1 Hz) ppm. $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 21.7, 47.6, 118.7 (q, $J_{C-F}$ = 272 Hz), 123.4, 126.6, 126.8, 127.1, 130.6, 130.8, 131.5, 132.6, 133.9, 146.2, 148.7, 151.1 (q, $J_{C-F}$ = 42 Hz), 192.7 ppm; $^{19}$F NMR (377 MHz, $CDCl_3$) $\delta$ -62.35 (3F, s) ppm. Anal. Calcd for $C_{17}H_{14}ClF_3N_4S_2$: C, 47.39; H, 3.28; N, 13.00. Found: C, 47.27; H, 3.29; N, 12.86.

Scheme 7

*iii) (E)-2-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4] thiadiazol-5-yl)-N-isopropylethenesulfonamide (**18**):* To a suspension of lithium chloride (319 mg, 7.5 mmol) and $Ph_2P(O)CH_2SO_2NHBoc$ (1.31 g, 3.3 mmol) in anhydrous $CH_3CN$ (25 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 1.1 mL, 7.4 mmol). To this solution was added 6-(2-chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]-thiadiazole-5-carbaldehyde (994 mg, 3.0 mmol) and the mixture was stirred at room temperature for 1.5 h. The resulting suspension was diluted with water (75 mL) and the pH was adjusted to 3 with 1 M HCl. This was extracted with ether (1×80 mL, 2×40 mL), and the combined organic layer was washed with brine (100 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated and the crude product was purified by

flash column chromatography (hexane/ethyl acetate = 95:5 – 50:50) to give the *N*-Boc vinyl sulfonamide as a pale yellow solid (1.11 g, 73%). $^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.37 (9H, s), 7.24 (1H, d, $J$ = 15.4 Hz), 7.36 (1H, d, $J$ = 15.4 Hz), 7.52-7.62 (3H, m), 7.68-7.72 (1H, m), 11.46 (1H, br s) ppm.

*N*-Boc vinyl sulfonamide obtained as above (511 mg, 1.0 mmol), isopropanol (0.19 mL, 2.5 mmol) and triphenylphosphine (668 mg, 2.6 mmol) were dissolved in anhydrous THF (15 mL). Diethyl azodicarboxylate (DEAD; 40% in toluene; 1.35 mL, 3.1 mmol) was slowly added to the solution and the mixture was stirred at room temperature for 1 h. The solvent was evaporated and the crude product was purified by flash column chromatography (hexane/ethyl acetate = 95:5 – 65:35) to give the *N*-Boc *N*-isopropyl vinyl sulfonamide as a pale yellow solid (416 mg, 76%). $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.41 (6H, d, $J$ = 6.9 Hz), 1.54 (9H, s), 4.55 (1H, sep, $J$ = 6.9 Hz), 7.38-7.48 (4H, m), 7.53-7.58 (1H, m), 7.69 (1H, d, $J$ = 15.5 Hz) ppm.

To a solution of *N*-Boc *N*-isopropyl vinyl sulfonamide obtained as above (377 mg, 0.68 mmol) in $CH_2Cl_2$ (5 mL) was added trifluoroacetic acid (TFA; 5 mL) and the mixture was stirred at room temperature for 1 h. The solvent was evaporated and the crude product was purified by flash column chromatography (hexane/ethyl acetate = 95:5 – 50: 50) to give a white solid (270 mg, 88%). This was further recrystallized from $CHCl_3$/hexane to afford white crystals, which were used for the bioassays. Mp: 157-158°C. $^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 1.21 (6H, d, $J$ = 6.6 Hz), 3.51-3.62 (1H, m), 4.42 (1H, d, $J$ = 7.7 Hz), 7.30 (1H, d, $J$ = 15.4 Hz), 7.35 (1H, d, $J$ = 15.4 Hz), 7.38-7.48 (3H, m), 7.52-7.56 (1H, m) ppm; $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 24.2, 46.4, 118.6 (q, $J_{C\text{-}F}$ = 271 Hz), 120.9, 124.4, 126.2, 127.3, 130.6, 131.1, 131.2, 132.5, 133.8, 147.1, 148.9, 151.9 (q, $J_{C\text{-}F}$ = 42 Hz) ppm; $^{19}$F NMR (471 MHz, $CDCl_3$) $\delta$ -62.52 (3F, s) ppm. Anal. Calcd for $C_{17}H_{14}ClF_3N_4S_2$: C, 47.39; H, 3.28; N, 13.00. Found: C, 47.27; H, 3.29; N, 12.86. HRMS (ESI) *m/z*: $C_{16}H_{15}ClF_3N_4O_2S_2$ $[M+H]^+$, calcd 451.0272, found 451.0261.

**2.2. Ligand-receptor binding assay**

Tritiated ponasterone A ([$^3$H]PonA, 140 Ci/mmol) was purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO, USA). PonA and 20E were purchased from Cosmo Bio Co., Ltd. (Tokyo, Japan) and Sigma-Aldrich Co. (St. Louis, MO, USA), respectively. RH-5849 and tebufenozide were from our stock samples. All insect cells are cultured in our laboratory. Originally, Sf-9 cell line was kindly gifted from Wakenyaku Co., Ltd. (Kyoto, Japan), NIAS-AeAl2 was given from NIAS Genebank (Tsukuba, Japan)[14], and BCIRL-Lepd-SL1 was kindly gifted from Dr. Cynthia Goodman (USDA-ARS, Columbia, MO, USA)[15].

The ligand binding assay using insect cells was performed as previously reported [16, 17]. In brief, insect cell suspension (400 μL; 2 - 3 × $10^6$ cells/mL) was incubated with DMSO solution of a test compound (1 μL) and 70% EtOH solution of [$^3$H]PonA (2 μL; *ca.* 60,000 dpm) at 25ºC for 30 min. The mixture was diluted with water (3 mL) and filtered through a glass filter GF-75 (ADVANTEC, Tokyo, Japan). The filter was washed 2 times with water (3 mL), dried, and placed in a vial containing 3 mL of Insta-Gel Plus (PerkinElmer, Inc., Waltham, MA, USA) to measure the radioactivity with a LSC-6100 liquid scintillation counter (Aloka, Tokyo, Japan). The concentration required for 50% inhibiton of [$^3$H]PonA binding ($IC_{50}$) was determined by probit analysis[18], and its reciprocal logarithm, $pIC_{50}$, was used as the index of binding activity.

**2.3. Larvicidal activity test**

*Spodoptera litura* was kindly provided from Ishihara Sangyo Kaisha, Ltd. (Kusatsu, Japan). Twenty 3$^{rd}$ instar larvae were put in the glass petri dish with paper filter. DMSO solution of a test compound (1 μL) was applied on the dorsal part of larvae. They were reared in an insectary at 25ºC. Insecta LFS (Nosan Corporation Life-Tech Department, Yokohama, Japan) was used to feed larvae. After one week rearing, the mortality was measured, and 50% lethal dose ($LD_{50}$) was determined by probit analysis[18]. Its reciprocal logarithm, $pLD_{50}$, was used as the index of larvicidal activity.

495 **2.4. QSAR analysis**

496 In order to examine the effect of the substituents at 2-position of the
497 imidazothiadiazole ring, the Hansch-Fujita QSAR analysis was performed using QREG
498 2.05[19]. ClogP values of synthesized chemicals, which were shown in Table 1, were
499 calculated by ClogP for Windows Ver. 4.0 (Biobyte Corp., Claremont, CA, USA). In all
500 equations, the number in parentheses are 95% confidence intervals of each coefficient, *n*
501 is the number of compounds used to analyze, *s* is the standard deviation, and *r* is the
502 correlation coefficient, and *F* is the value of ratio between regression and residual
503 variances.
504
505 **2.5. Receptor modeling and ligand-receptor docking**

506 Since no 3-D structure of *Spodoptera frugiperda* EcR (SfEcR) is available, we
507 constructed a 3-D structure model of the ligand binding domain (LBD) of SfEcR from the
508 X-ray structure of Lepidopteran EcR using a homology modeling software PDFAMS
509 (In-Silico Sciences Inc., Tokyo, Japan)[20]. We combined two partial primary sequences
510 of SfEcR (NCBI accession number: AAM54494 and CAD58232)[21, 22] to construct the
511 primary sequence of SfEcR-LBD. At present four X-ray structures of Lepidopteran EcR
512 bound to different ligands, namely, PonA (PDB ID: 1R1K) [23], 20E (2R40) [24], a
513 DAH-type agonist BYI06830 (1R20)[23] and an imidazole-type agonist BYI08346
514 (3IXP), are available. We used 1R1K, 1R20 and 3IXP as the templates for homology
515 modeling because the shapes of the ligand binding pockets (LBP) are different among
516 them. Thus, we obtained three homology models of SfEcR, which were stored as
517 complexes with PonA, BYI06830 or BYI08346 in order to compare the binding modes.
518 Ligand-receptor docking was conducted using OMEGA (ver. 2.5.1.4)[25] and
519 OEDocking (ver. 3.0.1) of Openeye Co. Ltd. (Santa Fe, NM, USA;
520 http://www.eyesopen.com). First, the LBPs of the SfEcR homology models were defined
521 using "MAKE RECEPTOR" tool of OEDocking. Next, the mol2 file of ITD (**10**) was
522 processed with OMEGA to generate the conformer libraries. Generated conformers are

aligned in the order of ascending energy and low energy conformers (maximum: 200) were compiled into a single file as a conformer library. Finally, these conformers were docked to the LBPs of SfEcR using "FRED"[26] tool of OEDocking. FRED uses Chemgauss4 scoring function, and this function ranks each binding mode in terms of shape interactions, hydrogen bonding interactions, metal-chelator interactions, and desolvation. The binding modes which gave the highest Chemgauss4 scores towards each LBP~~s~~ of SfEcR were shown in Fig. 4.

## 3. Results

### 3.1 Synthesis

ITDs were synthesized according to a previously described method with some modifications [27]. 2-Amino-1,3,4-thiadiazoles bearing $CF_3$, $CF_2CF_3$, and $CF_2CF_2CF_3$ groups were prepared using a reported method [28]. 2-Amino-5-(methylthio)-1,3,4-thiadiazole was also prepared according to a modified method described in the literature [29]. Formation of the imidazo[2,1-*b*][1,3,4]thiadiazole ring was accomplished by simply refluxing 2-amino-1,3,4-thiadiazoles and 2-chlorophenacyl bromide in ethanol. While compounds with electron-withdrawing substituents ($CF_3$, $CF_2CF_3$, and $CF_2CF_2CF_3$) did not form HBr salts, compounds with other substituents (H, $CH_3$, $SCH_3$) precipitated as HBr salts in the reaction mixture. In the next step, imidazothiadiazoles were subjected to the Vilsmeier-Haack reaction to afford imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehydes. Next, Knoevenagel condensation of aldehydes with malonic acid afforded pure (*E*)-acrylic acids. Finally, condensation of acrylic acids with various amines yielded ITDs (**1**–**13**). In further, compound **13** was oxidized with *m*-CPBA and Oxone® to yield sulfoxide **14** and sulfone **15**.

Next, we modified the acrylamide moiety. Hydrogenation of the double bond in the acrylic acid followed by condensation with isopropylamine afforded the propionamide **16**. Hydrogenation using Pd-C or Wilkinson's catalyst under a hydrogen atmosphere was unsuccessful, but diimide reduction afforded the desired compound,

551 although in poor yield. Compound **2** was successfully converted to thioamide **17** using
552 Lawesson's reagent. Vinyl sulfonamide **18** was then synthesized in three steps. First, the
553 intermediate aldehyde was subjected to the modified Horner-Wittig reaction [30] to
554 afford the *N*-Boc vinyl sulfonamide with complete *E* selectivity. Use of LiCl and DBU
555 [31] rather than strong bases was essential for preventing product degradation. Next,
556 *N*-Boc vinyl sulfonamide was alkylated at the acidic NH group through the Mitsunobu
557 reaction, and then Boc protecting group was removed by TFA to yield vinyl sulfonamide
558 **18**.
559
560 **3.2. Ligand binding activity**
561 The biological activities of various ITD analogs are summarized in Table 1. In
562 terms of the inhibition of [$^3$H]PonA incorporation to Sf-9 cells, compound **1** with the
563 primary acrylamide moiety at the 5-position of the imidazothiadiazole ring was inactive.
564 However, secondary amides with *i*-Pr (**2**), *c*-Bu (**3**), and *c*-Hex (**4**) were very potent. $IC_{50}$
565 values of these compounds were approximately 10–20 nM, which was 10–20 times more
566 potent than the natural molting hormone 20E ($IC_{50}$ = 200 nM). The Ph analog (**5**) was less
567 potent than the alkyl analogs (**2**–**4**) among secondary amides, but equipotent to 20E (**19**).
568 Further alkylation (tertiary acrylamide: **6**–**8**) drastically decreased the activity.
569 Conversion of the oxygen atom of the amide moiety to a sulfur atom (thioamide: **17**) did
570 not have a large impact on activity, but saturation of the olefin moiety (**16**) decreased
571 binding by 100-fold. Introduction of a sulfonamide moiety (**18**) drastically decreased
572 activity by approximately 1000-fold compared to compound **2**.
573
574 Table 1
575
576 Next, the substituent effect at the 2-position of the imidazothiadiazole ring was
577 examined for $CF_2CF_3$ (**9**), $CF_2CF_2CF_3$ (**10**), H (**11**), $CH_3$ (**12**), $SCH_3$ (**13**), $S(=O)CH_3$ (**14**),
578 and $SO_2CH_3$ (**15**). By introducing strong electron withdrawing fluorinated alkyl groups

579 such as $CF_3$, $CF_2CF_3$, and $CF_2CF_2CF_3$, the activity dramatically increased (more than
580 1000-fold compared to unsubstituted compound **11**). Other electron withdrawing groups
581 containing a sulfur atom such as $S(=O)CH_3$ and $SO_2CH_3$ did not enhance the activity. The
582 $SCH_3$ group enhanced the activity by 300-fold, although its electronic properties were
583 equivalent to H in terms of Hammett σ. The electron-donating $CH_3$ group increased the
584 activity by only 5-fold. Among TDI congeners, compound **10** showed the highest activity,
585 which was 3-fold higher than that of PonA and only 3-fold less potent than tebufenozide
586 in the binding assay against Sf-9 cells. The structure-activity relationship is summarized
587 in Fig. 3.
588
589 Fig. 3
590
591
592 The binding assay was also performed using other insect cell lines, including
593 Colorado potato beetle cells (BCIRL-Lepd-SL) and Asian tiger mosquito cells
594 (NIAS-AeAl2). As shown in Table 1, a few compounds showed moderate activity against
595 mosquito cells, but most compounds were weak or inactive against these cells.
596 Compound **9** was 2.5-fold more potent than tebufenozide against NIAS-AeAl2, while it
597 was approximately 30-fold less potent than PonA.
598
599 **3.3. Larvicidal activity**
600 Larvicidal activity of the synthesized compounds was measured against *S. litura*,
601 which is shown in Table 1. The $pLD_{50}$ values of compounds **2** and **9** were determined to
602 be 5.16 and 5.03, respectively, which were approximately 1/20 of tebufenozide, but
603 5-fold more toxic than RH-5849.
604
605 **3.4. QSAR analysis**
606 As shown in Table 1, activity was enhanced by introducing substituents at the

607 2-position of the imidazothiadiazole ring. To determine the physicochemical mechanism
608 of these substituents, binding activity was quantitatively analyzed using substituent
609 parameters. We previously demonstrated that the hydrophobicity of substituent is
610 important for the binding of DAHs to the ecdysone receptor of Sf-9 cells [32]. Therefore,
611 activity was quantitatively analyzed using hydrophobicity ΔClogP [ClogP (X) – ClogP
612 (H)] to formulate statistically significant values using Eq. 1.
613
614 $pIC_{50} = 1.326\ (\pm 0.772)\ \Delta\text{Clog P} + 6.162\ (\pm 0.828)$ (1)
615 $n = 8 \quad s = 0.930 \quad r = 0.864 \quad F_{1,6} = 17.688$
616
617 This equation suggests that the hydrophobic interaction between the substituents and the
618 ligand binding site of the receptor is important for binding. Although the equation was
619 significant according to the *F* test and the ΔClog P term was justified over 99% by t-test,
620 this correlation equation was not acceptable because of the large standard deviation.
621 Because the $pIC_{50}$ value was highly reproducible [32], the value of 0.930 is too large.
622 Therefore, the addition of another physicochemical parameter to Eq. 1 was considered,
623 although using two parameters may not be allowed for the analysis of eight compounds (n
624 = 8). Addition of an electronic parameter (Swain-Lupton F: field effect) drastically
625 improved the correlation, as shown in Eq. 2, although the standard deviation remained
626 large (s=0.349).
627
628 $pIC_{50} = 1.519\ (\pm 0.315)\ \Delta\text{Clog P} + 3.923\ (\pm 1.643)\ F + 4.872\ (\pm 0.631)$ (2)
629 $n = 8 \quad s = 0.349 \quad r = 0.985 \quad F_{2,5} = 81.754$
630
631 These results indicate that the electrostatic interaction between substituents and the
632 receptor surface surrounding the substituents is important for activity because the
633 correlation derived using F was better than that using Hammett σ. Physicochemical
634 parameter values and calculated $pIC_{50}$ values from Eq. 2 are listed in Table 2.

Table 2.

## 4. Discussion

We previously synthesized various non-steroidal ecdysone agonists, including diacylhydrazine (DAH) [33-35], *N*-alkyl-3,5-di-tert-butyl-4-hydroxybenzamides (DTBHIB) [36], tetrahydroquinoline (THQ)[14], oxadiazoline (ODZ) [3], and γ-methylene-γ-lactam (GML) (Akahane unpublished), and measured their biological activity against whole insects, insect tissue, insect cells, and *in vitro* translated EcR/USP proteins. Some DAH analogs (tebufenozide, methoxyfenozide, and chromafenozide) were very potent against lepidopteran tissues and proteins, were equipotent to ponasterone A, and were moderately potent against dipteran tissues and proteins, but not potent against Ceoleoptera~~n~~. In contrast, THQ-type compounds were reported to be potent against Diptera, particularly mosquitoes [4], but they were not very potent against Lepidoptera [37]. As shown in Table 1, the selectivity of ITD-type compound **9** was similar to that of the DAH-type compound **21** (tebufenozide). These results indicate that the binding mode of ITDs may be similar to that of DAHs, but different from those of THQs and steroidal agonists.

According to Holmwood and Schindler [9], the binding mode of ITDs differs from those of PonA and DAHs, but there is no data supporting the binding mode of ITDs. In contrast, the X-ray crystal structure of the EcR/USP complex bound to an IMD-type compound can be found in the Protein Data Bank (PDB ID: 3IXP). We performed docking simulation of ITD compounds to predict the ligand-receptor binding modes, and two crystal structures of receptor complexes bound to PonA and a DAH-type compound (BYI06833) were used as template 3-D structures (Fig. 4).

Fig. 4

663 As shown in Fig. 4, compound **10** fits snugly in the DAH-type pocket. In this
664 model, the perfluoropropyl group is surrounded by the hydrophobic region of the LBP.
665 This model is consistent with the results of the QSAR study, which showed that
666 hydrophobic and electrostatic interactions between the substituents at 2-position and the
667 receptor surface are important for activity.
668 Docking simulation of compound **10** was also performed against SfEcR, which
669 was constructed from the X-ray crystal structure bound to the IMD-type compound (PDB
670 ID: 3IXP). Although compounds **2** and **9** can dock to the LBP of SfEcR constructed from
671 3IXP, compound **10** containing the perfluoropropyl group could not be accommodated in
672 the corresponding pocket. This is likely because of the slightly smaller size of the LBP of
673 3IXP compared to the DAH binding pocket. Because the protein was treated as a rigid
674 body in the docking simulation using FRED, the initial size of the ligand binding pocket
675 is thought to be critical for docking.
676 As shown above, compound **2** and **9** were moderately toxic to *S. litura*. The dead
677 larva of *S. liura* treated with compound **2** is shown in Fig. 5. In our previous studies, we
678 synthesized DAH analogs with various substituents at both benzene rings and
679 quantitatively analyzed the structure-activity relationship to identify the essential
680 physicochemical properties for larvicidal activity [33, 34]. QSAR equations showed that
681 when molecular hydrophobicity was high, larvicidal activity against the lepidopteran rice
682 stem borer *Chilo suppressalis* was also high. Although the optimum hydrophobicity was
683 not derived for the DAH-type compounds, which had limited hydrophobicity (varied
684 A-ring moiety: 2.04–4.68; varied B-ring moiety: 1.99–4.53), there may be an optimum
685 value for the expanded set of compounds with supra-optimum hydrophobicity. In fact,
686 optimum hydrophobicity ($\log P_{opt}$ = 5.15) was evaluated for activity against *C.*
687 *suppressalis* [38]. Although this insect species was different from *S. frugiperda* used in
688 this study, log P values of compounds **4** and **5** exceeded 5.15, suggesting the presence of
689 optimum hydrophobicity.
690

691 Fig. 5

692

693 Compound **13** containing the $SCH_3$ group showed relatively high binding activity
694 ($pIC_{50}$ = 7.39) against Sf-9 cells, but was not toxic to *S. litura*. This may be because of the
695 facile oxidation of the sulfide moiety to sulfone/sulfoxide through metabolism. We
696 reported that RH-5849 was 10-fold less toxic than tebufenozide against Lepidopteran *C.*
697 *suppressalis* (Pyralidae; $pLD_{50}$ = 6.27 vs. 7.32) and 100-fold less toxic than tebufenozide
698 against *S. exigua* (Noctuidae; $pLD_{50}$ = 4.91 vs. 7.06) [39]. The difference in $pLD_{50}$ values
699 between RH-5849 and tebufenozide was 100-fold against *S. litura* (Noctuidae), which is
700 consistent with the toxicity results for *S. exigua*.

701

702 **5. Conclusion**

703 Among 18 synthesized imidazothiadiazole analogs, two compounds with $CF_2CF_3$
704 and $CF_2CF_2CF_3$ groups showed higher receptor binding activity than ponasterone A
705 against Lepidoptera Sf-9 cells. The larvicidal activity of the $CF_2CF_3$ analog was
706 determined against *S. litura* larvae in terms of $pLD_{50}$. It was 5 times more toxic than
707 RH5849, but 20 times less potent than tebufenozide. All compounds, however, did not
708 show strong binding activity against mosquito cells (Diptera) and beetle cells
709 (Coleoptera). This selective toxicity profile among insect orders is similar to that for
710 DAHs. In the structure-activity relationship study, a compound with a primary
711 acrylamide moiety was inactive, but the mono-alkylation of terminal nitrogen of
712 acrylamides (secondary amides) drastically enhanced the activity. Among secondary
713 amides, compounds with isopropyl, *cyc*-butyl and *cyc*-hexyl groups have similar receptor
714 binding activity, but the further alkylation (tertiary amide) was detrimental for the
715 binding. The conversion of amide to thioamide did not have much impact to the activity,
716 but the saturation of olefin moiety and conversion of amide to sulfonamide were also
717 detrimental to the activity. QSAR analysis of the substituent effect at 2-position of the
718 imidazothiadiazole ring indicated that the electron withdrawing and hydrophobic

719 substituents at this position are favorable for the ligand-receptor binding.

720

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728

729

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**Figure Legends**

Fig. 1. Chemical structures of ecdysone agonists

Fig. 2. Imidazothiadiazole-type compounds synthesized for SAR study

Fig. 3. Summary of structure-activity relationship for the binding activity against Sf-9

Fig. 4. Docking simulation of compound **10** (colored with green) against SfEcR bound to PonA (left) and DAH (BYI06833; right). Structure colored with gray is PonA and that colored with light brown is BYI06833. Ligand binding domains were modeled from 1R1K (left) and IR20 (right).

Fig. 5. *S. litura* larva treated with compound **2** ($10^{-5}$ mmol/insect)

Scheme 1. Construction of 2-amino-1,3,4-thiadiazole moiety: (a) $POCl_3$; (b) $CH_3I$, KOH, 2-propanol/$H_2O$

Scheme 2. Construction of (imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)acrylic acid moiety: (a) EtOH; (b) $POCl_3$, DMF; (c) $CH_2(COOH)_2$, piperidine, pyridine

Scheme 3. Synthesis of (imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)acrylamides: (a) i. $(COCl)_2$, DMF, $CH_2Cl_2$ ii. $NH_3$ aq., $CH_2Cl_2$; (b) amine, EDC, DMAP, $CH_2Cl_2$

Scheme 4. Synthesis of sulfoxide **14** and sulfone **15** (a) *m*-CPBA, $CH_2Cl_2$; (b) Oxone®, THF/MeOH/$H_2O$

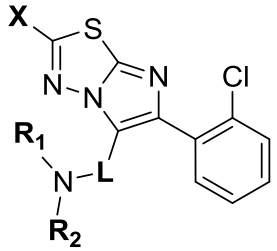
Scheme 5. Synthesis of propionamide **16**: (a) $N_2H_4$, $NaIO_4$, MeOH/$H_2O$ (b) isopropylamine, EDC, DMAP, $CH_2Cl_2$

Scheme 6. Synthesis of thioamide **17:** (a) Lawesson's reagent, toluene

Scheme 7. Synthesis of vinyl sulfonamide **18**: (a) $Ph_2P(O)CH_2SO_2NHBoc$, LiCl, DBU, $CH_3CN$; (b) *i*-PrOH, DEAD, $PPh_3$, THF; (c) TFA, $CH_2Cl_2$

**Table(s)**

Table 1. Biological activity of synthesized compounds.




| No | X | L | $R_1$ | $R_2$ | $pIC_{50}$(M) Sf-9 | $pIC_{50}$(M) NIAS-AeAl2 | $pIC_{50}$(M) BCIRL-Lepd-SL1 | $pLD_{50}$ (mmol/insect) *S. litura* | ClogP |
|---|---|---|---|---|---|---|---|---|---|
| **1** | $CF_3$ | *trans* –CH=CH-C(=O)- | H | H | < 4.9 (12%) | n.d.[a] | n.d. | n.d. | 3.56 |
| **2** | $CF_3$ | *trans* –CH=CH-C(=O)- | H | *i*-Pr | 8.03[d] | 7.10 | 4.88 | 5.16 | 4.63 |
| **3** | $CF_3$ | *trans* –CH=CH-C(=O)- | H | *c*-Bu | 7.71 | 6.74 | ≈ 4.08 (49%) | < 4.00 (45%) | 4.70 |
| **4** | $CF_3$ | *trans* –CH=CH-C(=O)- | H | *c*-Hex | 7.65 | 6.05 | < 4.60 (27%) | < 4.30 (10%) | 5.82 |
| **5** | $CF_3$ | *trans* –CH=CH-C(=O)- | H | Ph | 7.08 | 5.94 | < 4.38 (10%) | < 4.30 (0%) | 5.65 |
| **6** | $CF_3$ | *trans* –CH=CH-C(=O)- | $CH_3$ | *i*-Pr | 5.20 | ≈ 4.38 (51%) | < 4.38 (39%) | < 4.30 (25%) | 4.52 |
| **7** | $CF_3$ | *trans* –CH=CH-C(=O)- | $-(CH_2)_5-$ | | 5.24 | ≈ 4.08 (53%) | ≈ 4.08 (55%) | < 4.48 (0%) | 4.88 |
| **8** | $CF_3$ | *trans* –CH=CH-C(=O)- | $-(CH_2)_2-O-(CH_2)_2-$ | | 5.02 | < 4.08 (24%) | < 4.08 (17%) | < 4.48 (10%) | 3.75 |
| **9** | $CF_2CF_3$ | *trans* –CH=CH-C(=O)- | H | *i*-Pr | 8.35[e] | 7.52 | 4.72 | 5.03 | 5.00 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **10** | $CF_2CF_2CF_3$ | *trans* –CH=CH-C(=O)- | H | *i*-Pr | 8.48 | n.d. | n.d. | n.d. | 5.23 |
| **11** | H | *trans* –CH=CH-C(=O)- | H | *i*-Pr | 4.79 | n.d. | < 4.60 (4.6%) | n.d. | 3.74 |
| **12** | $CH_3$ | *trans* –CH=CH-C(=O)- | H | *i*-Pr | 5.44 | < 4.38 (42%) | ≈ 4.38 (47%) | < 4.30 (0%) | 4.24 |
| **13** | $SCH_3$ | *trans* –CH=CH-C(=O)- | H | *i*-Pr | 7.39[f] | 6.39 | < 4.90 (35%) | < 4.30 (0%) | 4.44 |
| **14** | $S(=O)CH_3$ | *trans* –CH=CH-C(=O)- | H | *i*-Pr | 4.79 | < 4.60 (38%) | < 4.90 (16%) | < 4.00 (0%) | 2.44 |
| **15** | $SO_2CH_3$ | *trans* –CH=CH-C(=O)- | H | *i*-Pr | 4.76 | > 4.38 (97%) | < 4.90 (11%) | < 4.30 (0%) | 2.26 |
| **16** | $CF_3$ | $–CH_2$-$CH_2$-C(=O)- | H | *i*-Pr | 5.83 | ≈ 4.08 (56%) | < 4.08 (25%) | < 4.48 (5%) | 3.54 |
| **17** | $CF_3$ | *trans* –CH=CH-C(=S)- | H | *i*-Pr | 7.51 | > 4.60 (99%) | < 4.90 (38%) | n.d. | 4.85 |
| **18** | $CF_3$ | *trans* –CH=CH-$SO_2$- | H | *i*-Pr | 4.98 | n.d. | n.d. | n.d. | 4.23 |
| **19** | Ponasterone A | | | | 8.05 | 9.01[b] | 8.13[c] | n.d. | 0.49 |
| **20** | 20-Hydroxyecdysone | | | | 6.78 | n.d. | 6.36[c] | n.d. | -1.72 |
| **21** | Tebufenozide | | | | 8.81 | 7.12 | 5.18 | 6.47[g] | 4.51 |
| **22** | RH-5849 | | | | 6.44 | n.d. | n.d. | 4.41[g] | 2.45 |

[a] Not determined

[b] From Ref. [11]

[c] Against the in vitro translated EcR/USP heterodimers. From Ref. [40]

[d] Mean of 7.78, 8.04 and 8.27

[e] Mean of 8.32 and 8.38

[f] Mean of 7.37 and 7.40

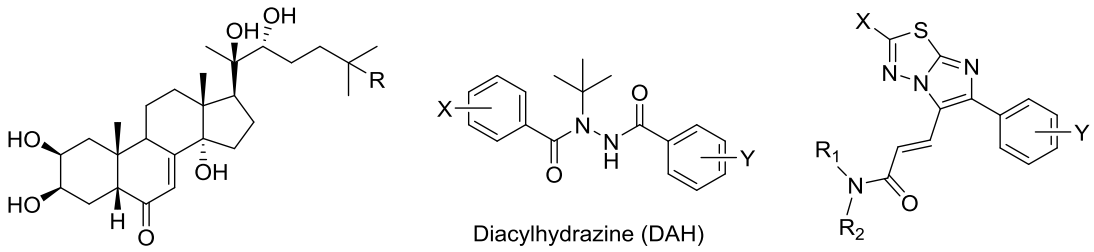
[g] Dose-response relationship was derived using ten larvae for each dose.

Table 2. Physicochemical parameters for QSAR calculation and prediction of the binding activity by Eq. 2.

| Compounds | | Physicochemical parameter | | Binding affinity ($pIC_{50}$) | | |
|---|---|---|---|---|---|---|
| No | Substituents | ΔClogP | F | Obsd | Calcd (Eq.2) | Δ[a] |
| **11** | H | 0.00 | 0.00 | 4.79 | 4.87 | -0.08 |
| **12** | $CH_3$ | 0.50 | 0.01 | 5.44 | 5.67 | -0.23 |
| **13** | $SCH_3$ | 0.70 | 0.23 | 7.39 | 6.84 | 0.55 |
| **14** | $SOCH_3$ | -1.30 | 0.52 | 4.79 | 4.94 | -0.15 |
| **15** | $SO_2CH_3$ | -1.48 | 0.53 | 4.76 | 4.70 | -0.06 |
| **2** | $CF_3$ | 0.89 | 0.38 | 8.03 | 7.72 | 0.31 |
| **9** | $CF_2CF_3$ | 1.26 | 0.44 | 8.35 | 8.51 | -0.16 |
| **10** | $CF_2CF_2CF_3$ | 1.49 | 0.42 | 8.48 | 8.78 | -0.30 |

[a] The difference between observed and calculated value [$pIC_{50}$(obsd) – $pIC_{50}$(calcd by Eq. 2)].

**Figure(s)**




20-Hydroxyecdysone (R=OH)
Ponasterone A (R=H)

Diacylhydrazine (DAH)

Tebufenozide (X=3,5-$(CH_3)_2$, Y=4-Et)
RH-5849 (X=Y=H)

Imidazothiadiazole (ITD)

Fig. 1. Chemical structures of ecdysone agonists

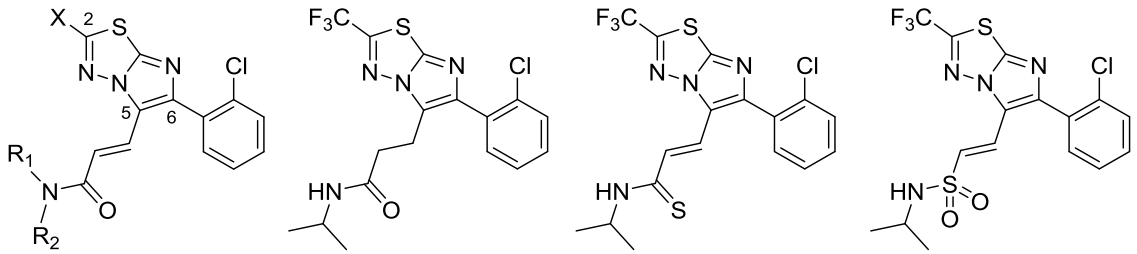



Fig. 2. Imidazothiadiazole-type compounds synthesized for SAR study

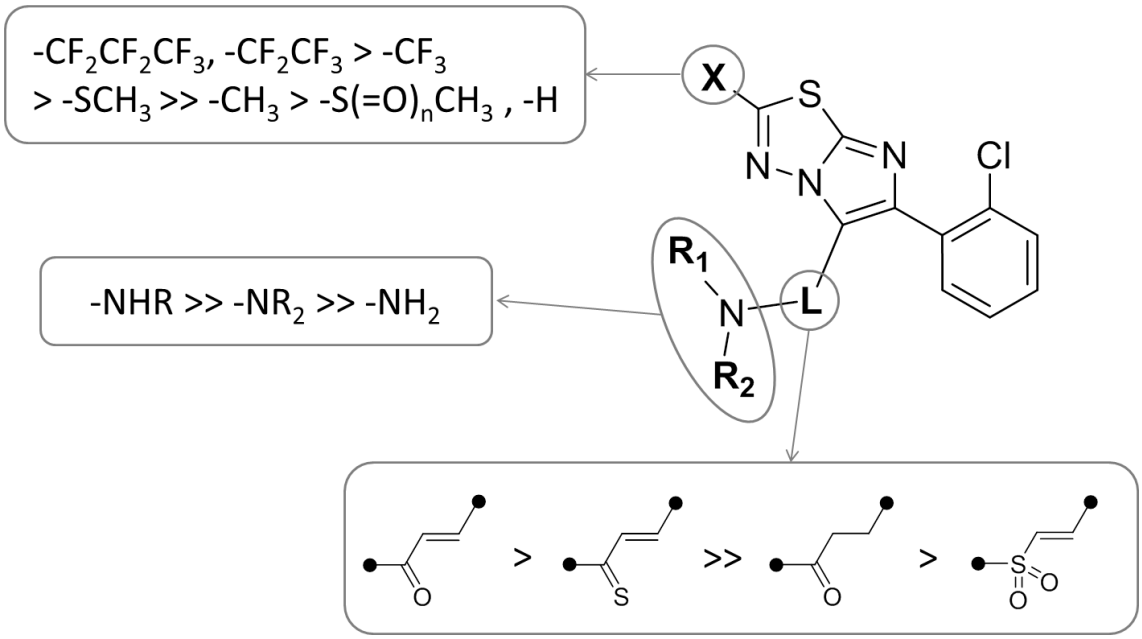



Fig. 3. Summary of structure-activity relationship for the binding activity against Sf-9

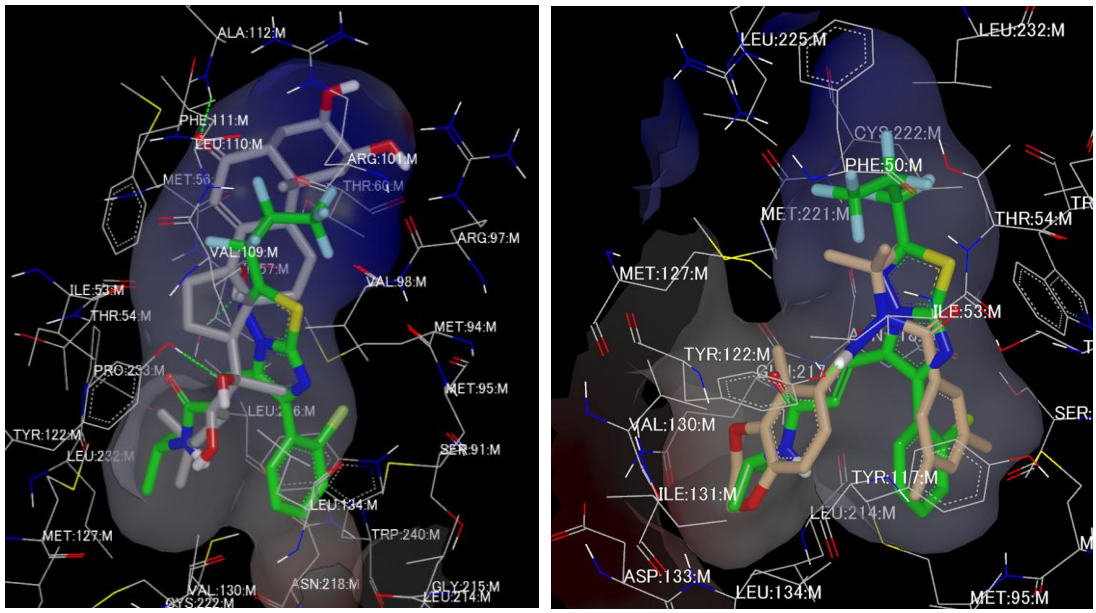
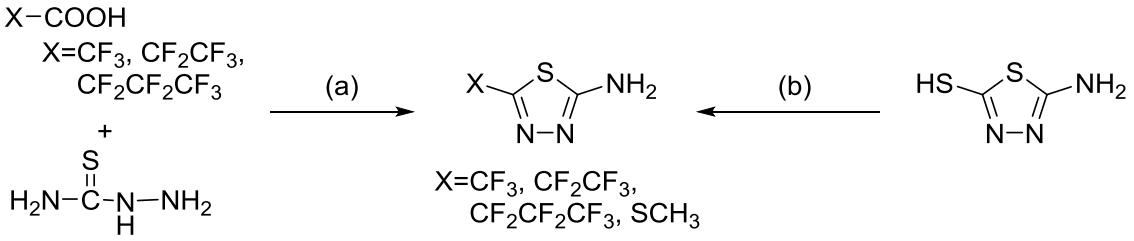
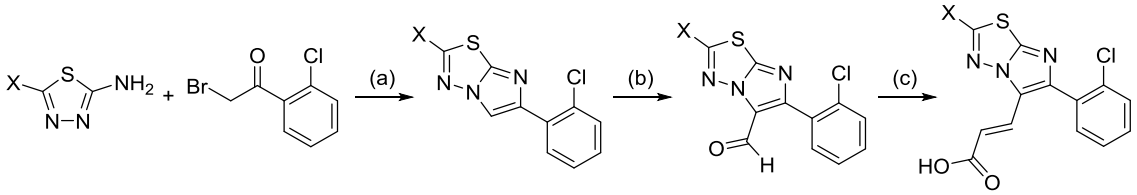



Fig. 4. Docking simulation of compound **10** (colored with green) against SfEcR bound to PonA (left) and DAH (BYI06833; right). Structure colored with gray is PonA and that colored with light brown is BYI06833. Ligand binding domains were modeled from 1R1K (left) and IR20 (right).

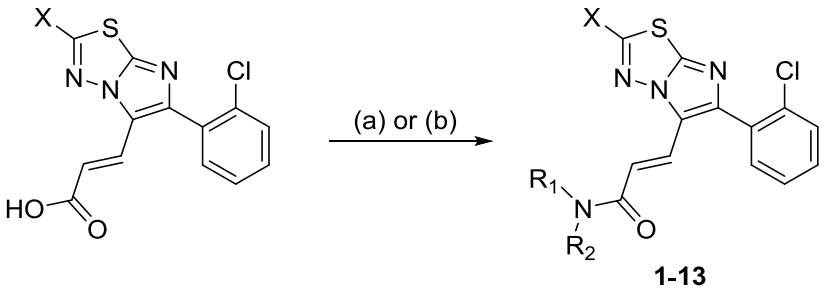
Fig. 5 *S. litura* Larva treated with compound **2** ($10^{-5}$ mmol/insect)



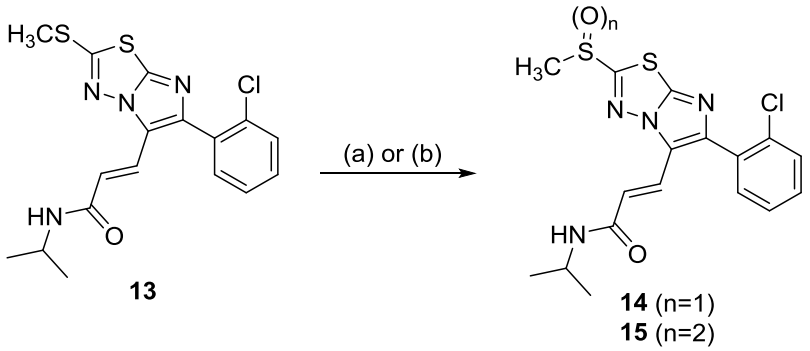

Scheme 1. Construction of 2-amino-1,3,4-thiadiazole moiety: (a) $POCl_3$; (b) $CH_3I$, KOH, 2-propanol/$H_2O$



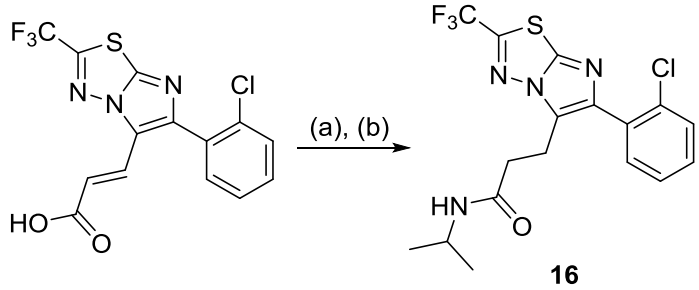

Scheme 2. Construction of (imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)acrylic acid moiety: (a) EtOH; (b) $POCl_3$, DMF; (c) $CH_2(COOH)_2$, piperidine, pyridine



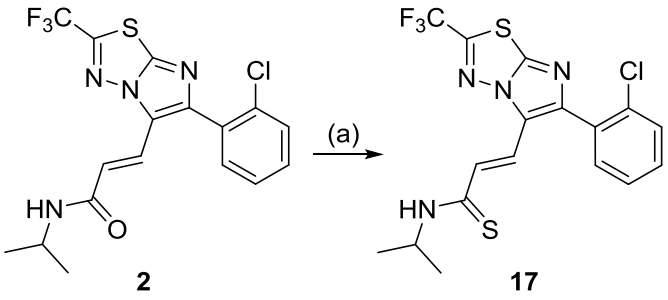

Scheme 3. Synthesis of (imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)acrylamides: (a) i. $(COCl)_2$, DMF, $CH_2Cl_2$ ii. $NH_3$ aq., $CH_2Cl_2$; (b) amine, EDC, DMAP, $CH_2Cl_2$



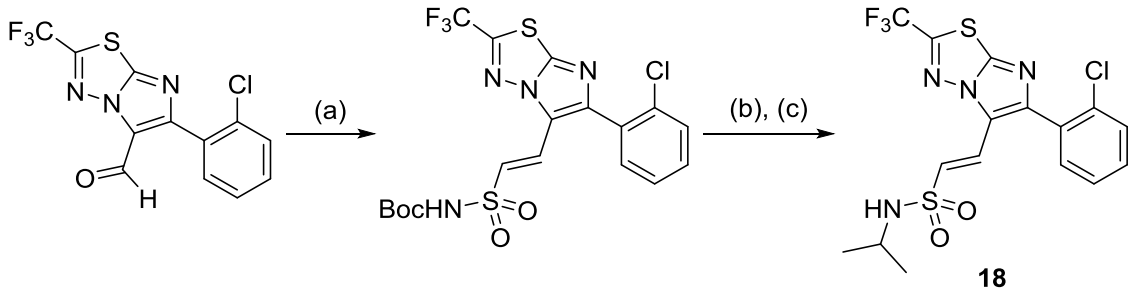

Scheme 4. Synthesis of sulfoxide **14** and sulfone **15**: (a) *m*-CPBA, $CH_2Cl_2$; (b) Oxone®, THF/MeOH/$H_2O$




Scheme 5. Synthesis of propionamide **16**: (a) $N_2H_4$, $NaIO_4$, MeOH/$H_2O$ (b) isopropylamine, EDC, DMAP, $CH_2Cl_2$




Scheme 6. Synthesis of thioamide **17**: (a) Lawesson's reagent, toluene




Scheme 7. Synthesis of vinyl sulfonamide **18**: (a) $Ph_2P(O)CH_2SO_2NHBoc$, LiCl, DBU, $CH_3CN$; (b) *i*-PrOH, DEAD, $PPh_3$, THF; (c) TFA, $CH_2Cl_2$