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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 [³H]ponasterone A incorporation [IC₅₀ (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: –CONR₂) decreased the activity dramatically. Additionally, a primary amide analog (–CONH₂) 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 CF₂CF₂CF₃ 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.

44 **Keywords:**

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

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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 pInd₅₀ (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-inflammatory [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, CH₃, CF₃, CF₂CF₃, CF₂CF₂CF₃, SCH₃, S(=O)CH₃, SO₂CH₃, 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 -CH₂CH₂- (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 ¹H NMR (0 ppm); deuterated solvent signals were used as the internal standard for ¹³C NMR (77.16 ppm for CDCl₃ and 39.52 ppm for DMSO-*d*₆); and α,α,α -trifluorotoluene was used as the external standard for ¹⁹F 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 70°C 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 Na₂CO₃ solution. The resulting solid was filtered off, washed with water and dried *in vacuo* to yield an off-white solid (19.4 g, 77%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.07 (2H, s) ppm; ¹³C NMR (100 MHz, DMSO-*d*₆) δ 119.8 (q, J_{C-F} = 269 Hz), 143.8 (q, J_{C-F} = 37 Hz), 171.7 ppm; ¹⁹F NMR (377 MHz, DMSO-*d*₆) δ -59.77 (3F, s) ppm. 2-Amino-1,3,4-thiadiazoles with CF₂CF₃ and CF₂CF₂CF₃ 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%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.58 (3H, s), 7.21 (2H, s) ppm; ¹³C NMR (100 MHz, DMSO-*d*₆) δ 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 CH₂Cl₂ (250 mL). The organic layer was washed successively with water (3×250 mL), saturated aqueous NaHCO₃ solution (250 mL) and brine (250 mL), and dried over anhydrous Na₂SO₄. The solvent was evaporated to give the crude 2-chlorophenacyl bromide (38.7 g, purity: *ca.* 83% determined by ¹H 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%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 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 CF₂CF₃, CF₂CF₂CF₃, H, CH₃, and SCH₃ 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 Na₂CO₃ 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 MgSO₄. 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%). ¹H NMR (400 MHz, CDCl₃) δ 7.43-7.49 (2H, m), 7.55-7.59 (2H, m), 9.85 (1H, s) ppm.

Other imidazothiadiazole-5-carbaldehyde analogs with CF₂CF₃, CF₂CF₂CF₃, H, CH₃, and SCH₃ 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%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 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 CF₂CF₃, CF₂CF₂CF₃, H, CH₃, and SCH₃ 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 CH₂Cl₂ (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 CH₂Cl₂ (5 mL), and then added dropwise to vigorously stirred aqueous NH₃ solution (28%, 5 mL) at 0°C. The mixture was stirred at room temperature overnight. It was diluted with water (30 mL) and extracted with CH₂Cl₂ (3×30 mL). The combined organic layer was washed with water (50 mL) and brine (50 mL), and dried over anhydrous Na₂SO₄. 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. ¹H NMR (400 MHz, CDCl₃) δ 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) δ 118.7 (q, $J_{\text{C-F}} = 272$ 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, $J_{\text{C-F}} = 42$ Hz), 167.3 ppm; ^{19}F NMR (377 MHz, CDCl_3) δ -62.46 (3F, s) ppm. Anal. Calcd for $\text{C}_{14}\text{H}_8\text{ClF}_3\text{N}_4\text{OS}$: C, 45.11; H, 2.16; N, 15.03. Found: C, 45.07; H, 2.20; N, 15.05.

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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. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 22.9, 41.9, 118.7 (q, $J_{\text{C-F}} = 272$ 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, $J_{\text{C-F}} = 42$ Hz), 164.6 ppm; ^{19}F NMR (377 MHz, CDCl_3) δ -62.43 (3F, s) ppm. Anal. Calcd for $\text{C}_{17}\text{H}_{14}\text{ClF}_3\text{N}_4\text{OS}$: 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.

239

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. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 15.3, 31.4, 45.2, 118.7 (q, $J_{\text{C-F}} = 272$ 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, $J_{\text{C-F}} = 42$ Hz), 164.5
 246 ppm; ^{19}F NMR (377 MHz, CDCl_3) δ -62.43 (3F, s) ppm. Anal. Calcd for
 247 $\text{C}_{18}\text{H}_{14}\text{ClF}_3\text{N}_4\text{OS}$: 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. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 25.0, 25.7, 33.3, 48.8, 118.7
 254 (q, $J_{\text{C-F}} = 272$ 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, $J_{\text{C-F}} = 42$ Hz) ppm; 164.5, 164.5. ^{19}F NMR (377 MHz, CDCl_3) δ -62.42
 256 (3F, s) ppm. Anal. Calcd for $\text{C}_{20}\text{H}_{18}\text{ClF}_3\text{N}_4\text{OS}$: 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. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 118.7 (q, $J_{\text{C-F}} = 272$ 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, $J_{\text{C-F}} = 42$ Hz), 163.7 ppm; ^{19}F
 264 NMR (377 MHz, CDCl_3) δ -62.37 (3F, s) ppm. Anal. Calcd for $\text{C}_{20}\text{H}_{12}\text{ClF}_3\text{N}_4\text{OS}$: 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. ^1H NMR (400 MHz, CDCl_3) δ
 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

271 (6H, m) **ppm**; ^{13}C NMR (100 MHz, CDCl_3) δ (mixture of rotamers) 19.5, 20.7, 26.6, 28.5,
 272 44.6, 28.5, 118.3, 118.5, 118.7 (q, $J_{\text{C-F}} = 272$ Hz), 122.8, 123.7, 125.4, 125.7, 127.1, 130.4,
 273 130.7, 131.7, 132.6, 134.1, 145.9, 146.0, 147.3, 147.6, 150.9 (q, $J_{\text{C-F}} = 42$ Hz), 165.8,
 274 166.1 **ppm**; ^{19}F NMR (377 MHz, CDCl_3) δ (mixture of rotamers) -62.81 (1.35F, s), -62.71
 275 (1.65F, s) **ppm**. Anal. Calcd for $\text{C}_{18}\text{H}_{16}\text{ClF}_3\text{N}_4\text{OS}$: C, 50.41; H, 3.76; N, 13.06. Found: C,
 276 50.43; H, 3.76; N, 12.94.

277

278 vii) (E)-3-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)
 279 -1-(piperidin-1-yl)prop-2-en-1-one (7): Mp: 168-170°C. ^1H NMR (400 MHz, CDCl_3) δ
 280 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
 281 NMR (100 MHz, CDCl_3) δ 24.8, 25.7, 26.9, 43.6, 47.2, 117.6, 118.7 (q, $J_{\text{C-F}} = 271$ Hz),
 282 123.7, 125.8, 127.1, 130.4, 130.7, 131.7, 132.6, 134.1, 145.9, 147.5, 150.9 (q, $J_{\text{C-F}} = 42$
 283 Hz), 165.0 **ppm**; ^{19}F NMR (377 MHz, CDCl_3) δ -62.67 (3F, s) **ppm**. Anal. Calcd for
 284 $\text{C}_{19}\text{H}_{16}\text{ClF}_3\text{N}_4\text{OS}$: C, 51.76; H, 3.66; N, 12.71. Found: C, 51.86; H, 3.81; N, 12.71.

285

286 viii) (E)-3-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)
 287 -1-morpholinoprop-2-en-1-one (8): Mp: 171-172°C. ^1H NMR (400 MHz, CDCl_3) δ
 288 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**;
 289 ^{13}C NMR (100 MHz, CDCl_3) δ 42.7, 46.3, 67.0, 116.1, 118.7 (q, $J_{\text{C-F}} = 272$ Hz), 123.5,
 290 126.7, 127.1, 130.5, 130.8, 131.6, 132.6, 134.0, 146.3, 148.1, 151.2 (q, $J_{\text{C-F}} = 42$ Hz),
 291 165.2 **ppm**. ^{19}F NMR (377 MHz, CDCl_3) δ -62.61 (3F, s) **ppm**. Anal. Calcd for
 292 $\text{C}_{18}\text{H}_{14}\text{ClF}_3\text{N}_4\text{O}_2\text{S}$: C, 48.82; H, 3.19; N, 12.65. Found: C, 48.67; H, 3.27; N, 12.55.

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294 ix) (E)-3-(6-(2-Chlorophenyl)-2-(perfluoroethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-
 295 N-isopropylacrylamide (9): Mp: 165-166°C. ^1H NMR (400 MHz, CDCl_3) δ 1.22 (6H, d, J
 296 = 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),
 297 7.33-7.48 (3H, m), 7.50-7.58 (2H, m) **ppm**; ^{13}C NMR (100 MHz, CDCl_3) δ 22.9, 41.9,
 298 109.3 (tq, $J = 256, 41$ Hz), 118.0 (qt, $J_{\text{C-F}} = 285, 36$ Hz), 120.6, 123.1, 124.6, 127.1, 130.5,

299 130.8, 131.5, 132.6, 134.0, 146.2, 148.0, 150.7 (t, $J_{\text{C-F}} = 31$ Hz), 164.6 ppm; ^{19}F NMR
 300 (377 MHz, CDCl_3) δ -111.16 (2F, q, $J_{\text{F-F}} = 2.3$ Hz), -84.20 (3F, t, $J_{\text{F-F}} = 2.3$ Hz) ppm. Anal.
 301 Calcd for $\text{C}_{18}\text{H}_{14}\text{ClF}_5\text{N}_4\text{OS}$: C, 46.51; H, 3.04; N, 12.05. Found: C, 46.50; H, 3.08; N,
 302 12.26.

303

304 x) (E)-3-(6-(2-Chlorophenyl)-2-(perfluoropropyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-
 305 N-isopropylacrylamide (10): Mp: 170-172°C. ^1H NMR (500 MHz, CDCl_3) δ 1.21 (6H, d,
 306 $J = 6.6$ Hz), 4.15-4.26 (1H, m), 5.67 (1H, br d, $J = 7.8$ Hz), 6.82 (1H, d, $J = 15.5$ Hz),
 307 7.33-7.45 (3H, m), 7.50-7.58 (2H, m) ppm; ^{13}C NMR (125 MHz, CDCl_3) δ 22.9, 41.9,
 308 105.5-111.2 (m), 111.3 (tt, $J_{\text{C-F}} = 285$, 36 Hz), 117.6 (qt, $J_{\text{C-F}} = 286$, 34 Hz), 120.7, 123.2,
 309 124.6, 127.1, 130.5, 130.7, 131.6, 132.6, 134.1, 146.2, 148.0, 150.6 (t, $J_{\text{C-F}} = 31$ Hz),
 310 164.6 ppm; due to ^{13}C - ^{19}F coupling, the signals of the perfluoropropyl group were so
 311 weak that it was difficult to detect those signals and their coupling patterns. ^{19}F NMR
 312 (471 MHz, CDCl_3) δ -126.61 (2F, t, $J_{\text{F-F}} = 8.5$ Hz), -108.75 (2F, sex, $J_{\text{F-F}} = 9.4$ Hz), -81.01
 313 (3F, t, $J_{\text{F-F}} = 9.6$ Hz) ppm. Anal. Calcd for $\text{C}_{19}\text{H}_{14}\text{ClF}_7\text{N}_4\text{OS}$: C, 44.33; H, 2.74; N, 10.88.
 314 Found: C, 44.18; H, 2.79; N, 10.89.

315

316 xi) (E)-3-(6-(2-Chlorophenyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-N-isopropylacryl-
 317 amide (11): Mp: 210-212°C. ^1H NMR (400 MHz, CDCl_3) δ 1.19 (6H, d, $J = 6.6$ Hz),
 318 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,
 319 m), 7.42-7.47 (1H, m), 7.48-7.57 (2H, m), 8.69 (1H, s) ppm; ^{13}C NMR (100 MHz,
 320 CDCl_3) δ 22.9, 41.7, 119.5, 122.6, 125.2, 126.9, 130.30, 130.32, 132.2, 132.6, 134.0,
 321 146.2, 147.3, 147.5, 165.1 ppm. HRMS (ESI) m/z : $\text{C}_{16}\text{H}_{16}\text{ClN}_4\text{OS}$ $[\text{M}+\text{H}]^+$, calcd
 322 347.0728, found 347.0717.

323

324 xii) (E)-3-(6-(2-Chlorophenyl)-2-methylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)-N-
 325 isopropylacrylamide (12): Mp: 214-215°C. ^1H NMR (400 MHz, CDCl_3) δ 1.20 (6H, d, J
 326 $= 6.5$ 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) δ 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 $\text{C}_{17}\text{H}_{17}\text{ClN}_4\text{OS}$: 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. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 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 : $\text{C}_{17}\text{H}_{18}\text{ClN}_4\text{OS}_2$ $[\text{M}+\text{H}]^+$, calcd 393.0605, found 393.0594.

2.1.6. Synthesis of imidazothiadiazole analogs with $\text{S}(=\text{O})\text{CH}_3$ and SO_2CH_3 (Scheme 4)

Scheme 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 0°C. 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 (5 mL), 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. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 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 : $\text{C}_{17}\text{H}_{18}\text{ClN}_4\text{O}_2\text{S}_2$ $[\text{M}+\text{H}]^+$, 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 0°C. Oxone[®] (1.84 g, 3.0 mmol) was slowly added to the solution and the mixture was stirred at 0°C 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. ^1H NMR (400 MHz, CDCl_3) δ 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) δ 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 : $\text{C}_{17}\text{H}_{18}\text{ClN}_4\text{O}_3\text{S}_2$ $[\text{M}+\text{H}]^+$, 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 CuSO₄ solution. To this, the solution of NaIO₄ (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 Na₂SO₄. The solvent was evaporated to give the crude propanoic acid. This was suspended in anhydrous CH₂Cl₂ (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 CH₂Cl₂ (15 mL) and washed successively with saturated aqueous Na₂CO₃ solution, water, 1 M HCl, water, and brine (10 mL each). The organic layer was dried over MgSO₄ 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. ¹H NMR (400 MHz, CDCl₃) δ 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; ¹³C NMR (100 MHz, CDCl₃) δ 20.6, 22.8, 34.0, 41.6, 118.8 (q, *J*_{C-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-F} = 42 Hz), 170.0 ppm. ¹⁹F NMR (377 MHz, CDCl₃) δ -62.63 (3F, s) ppm. Anal. Calcd for C₁₇H₁₆ClF₃N₄OS: 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. ¹H NMR (400 MHz, CDCl₃) δ 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. ¹³C NMR (100 MHz, CDCl₃) δ 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; ¹⁹F NMR (377 MHz, CDCl₃) δ -62.35 (3F, s) ppm. Anal. Calcd for C₁₇H₁₄ClF₃N₄S₂: 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*-isopropylethanesulfonamide (18):* To a suspension of lithium chloride (319 mg, 7.5 mmol) and Ph₂P(O)CH₂SO₂NHBoc (1.31 g, 3.3 mmol) in anhydrous CH₃CN (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 MgSO₄. 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%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 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%). ¹H NMR (400 MHz, CDCl₃) δ 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 CH₂Cl₂ (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 CHCl₃/hexane to afford white crystals, which were used for the bioassays. Mp: 157-158°C. ¹H NMR (500 MHz, CDCl₃) δ 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; ¹³C NMR (125 MHz, CDCl₃) δ 24.2, 46.4, 118.6 (q, *J*_{C-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-F} = 42 Hz) ppm; ¹⁹F NMR (471 MHz, CDCl₃) δ -62.52 (3F, s) ppm. Anal. Calcd for C₁₇H₁₄ClF₃N₄S₂: C, 47.39; H, 3.28; N, 13.00. Found: C, 47.27; H, 3.29; N, 12.86. HRMS (ESI) *m/z*: C₁₆H₁₅ClF₃N₄O₂S₂ [M+H]⁺, calcd 451.0272, found 451.0261.

2.2. Ligand-receptor binding assay

Tritiated ponasterone A ($[^3\text{H}]\text{PonA}$, 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 ($400\ \mu\text{L}$; $2 - 3 \times 10^6\ \text{cells/mL}$) was incubated with DMSO solution of a test compound ($1\ \mu\text{L}$) and 70% EtOH solution of $[^3\text{H}]\text{PonA}$ ($2\ \mu\text{L}$; *ca.* 60,000 dpm) at 25°C 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% inhibition of $[^3\text{H}]\text{PonA}$ 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 3rd instar larvae were put in the glass petri dish with paper filter. DMSO solution of a test compound ($1\ \mu\text{L}$) was applied on the dorsal part of larvae. They were reared in an insectary at 25°C . 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.

2.4. QSAR analysis

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

2.5. Receptor modeling and ligand-receptor docking

Since no 3-D structure of *Spodoptera frugiperda* EcR (SfEcR) is available, we constructed a 3-D structure model of the ligand binding domain (LBD) of SfEcR from the X-ray structure of **Lepidopteran EcR** using a homology modeling software PDFAMS (In-Silico Sciences Inc., Tokyo, Japan)[20]. We combined two partial primary sequences of SfEcR (NCBI accession number: AAM54494 and CAD58232)[21, 22] to construct the primary sequence of SfEcR-LBD. At present **four** X-ray structures of **Lepidopteran EcR bound to different ligands, namely, PonA (PDB ID: 1R1K) [23], 20E (2R40) [24], a DAH-type agonist BYI06830 (1R20)[23] and an imidazole-type agonist BYI08346 (3IXP),** are available. We used **1R1K, 1R20 and 3IXP** as the templates for homology modeling because the shapes of the ligand binding pockets (LBP) are different among them. Thus, we obtained three homology models of SfEcR, which were stored as complexes with PonA, BYI06830 or BYI08346 in order to compare the binding modes.

Ligand-receptor docking was conducted using OMEGA (ver. 2.5.1.4)[25] and OEDocking (ver. 3.0.1) of Openeye Co. Ltd. (Santa Fe, NM, USA; <http://www.eyesopen.com>). First, the LBPs of the SfEcR homology models were defined using “MAKE RECEPTOR” tool of OEDocking. Next, the mol2 file of ITD (**10**) was 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 LBPs 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 CF₃, CF₂CF₃, and CF₂CF₂CF₃ 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 (CF₃, CF₂CF₃, and CF₂CF₂CF₃) did not form HBr salts, compounds with other substituents (H, CH₃, SCH₃) 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,

although in poor yield. Compound **2** was successfully converted to thioamide **17** using Lawesson's reagent. Vinyl sulfonamide **18** was then synthesized in three steps. First, the intermediate aldehyde was subjected to the modified Horner-Wittig reaction [30] to afford the *N*-Boc vinyl sulfonamide with complete *E* selectivity. Use of LiCl and DBU [31] rather than strong bases was essential for preventing product degradation. Next, *N*-Boc vinyl sulfonamide was alkylated at the acidic NH group through the Mitsunobu reaction, and then Boc protecting group was removed by TFA to yield vinyl sulfonamide **18**.

3.2. Ligand binding activity

The biological activities of various ITD analogs are summarized in Table 1. In terms of the inhibition of [³H]PonA incorporation to Sf-9 cells, compound **1** with the primary acrylamide moiety at the 5-position of the imidazothiadiazole ring was inactive. However, secondary amides with *i*-Pr (**2**), *c*-Bu (**3**), and *c*-Hex (**4**) were very potent. IC₅₀ values of these compounds were approximately 10–20 nM, which was 10–20 times more potent than the natural molting hormone 20E (IC₅₀ = 200 nM). The Ph analog (**5**) was less potent than the alkyl analogs (**2–4**) among secondary amides, but equipotent to 20E (**19**). Further alkylation (tertiary acrylamide: **6–8**) drastically decreased the activity. Conversion of the oxygen atom of the amide moiety to a sulfur atom (thioamide: **17**) did not have a large impact on activity, but saturation of the olefin moiety (**16**) decreased binding by 100-fold. Introduction of a sulfonamide moiety (**18**) drastically decreased activity by approximately 1000-fold compared to compound **2**.

Table 1

Next, the substituent effect at the 2-position of the imidazothiadiazole ring was examined for CF₂CF₃ (**9**), CF₂CF₂CF₃ (**10**), H (**11**), CH₃ (**12**), SCH₃ (**13**), S(=O)CH₃ (**14**), and SO₂CH₃ (**15**). By introducing strong electron withdrawing fluorinated alkyl groups

such as CF₃, CF₂CF₃, and CF₂CF₂CF₃, the activity dramatically increased (more than 1000-fold compared to unsubstituted compound **11**). Other electron withdrawing groups containing a sulfur atom such as S(=O)CH₃ and SO₂CH₃ did not enhance the activity. The SCH₃ group enhanced the activity by 300-fold, although its electronic properties were equivalent to H in terms of Hammett σ . The electron-donating CH₃ group increased the activity by only 5-fold. Among TDI congeners, compound **10** showed the highest activity, which was 3-fold higher than that of PonA and only 3-fold less potent than tebufenozide in the binding assay against Sf-9 cells. The structure-activity relationship is summarized in Fig. 3.

Fig. 3

The binding assay was also performed using other insect cell lines, including Colorado potato beetle cells (BCIRL-Lepd-SL) and Asian tiger mosquito cells (NIAS-AeAl2). As shown in Table 1, a few compounds showed moderate activity against mosquito cells, but most compounds were weak or inactive against these cells. Compound **9** was 2.5-fold more potent than tebufenozide against NIAS-AeAl2, while it was approximately 30-fold less potent than PonA.

3.3. Larvicidal activity

Larvicidal activity of the synthesized compounds was measured against *S. litura*, which is shown in Table 1. The pLD₅₀ values of compounds **2** and **9** were determined to be 5.16 and 5.03, respectively, which were approximately 1/20 of tebufenozide, but 5-fold more toxic than RH-5849.

3.4. QSAR analysis

As shown in Table 1, activity was enhanced by introducing substituents at the

2-position of the imidazothiadiazole ring. To determine the physicochemical mechanism of these substituents, binding activity was quantitatively analyzed using substituent parameters. We previously demonstrated that the hydrophobicity of substituent is important for the binding of DAHs to the ecdysone receptor of Sf-9 cells [32]. Therefore, activity was quantitatively analyzed using hydrophobicity ΔClogP [$\text{ClogP}(\text{X}) - \text{ClogP}(\text{H})$] to formulate statistically significant values using Eq. 1.

$$\text{pIC}_{50} = 1.326 (\pm 0.772) \Delta\text{Clog P} + 6.162 (\pm 0.828) \quad (1)$$

$$n = 8 \quad s = 0.930 \quad r = 0.864 \quad F_{1,6} = 17.688$$

This equation suggests that the hydrophobic interaction between the substituents and the ligand binding site of the receptor is important for binding. Although the equation was significant according to the F test and the $\Delta\text{Clog P}$ term was justified over 99% by t -test, this correlation equation was not acceptable because of the large standard deviation. Because the pIC_{50} value was highly reproducible [32], the value of 0.930 is too large. Therefore, the addition of another physicochemical parameter to Eq. 1 was considered, although using two parameters may not be allowed for the analysis of eight compounds ($n = 8$). Addition of an electronic parameter (Swain-Lupton F : field effect) drastically improved the correlation, as shown in Eq. 2, although the standard deviation remained large ($s=0.349$).

$$\text{pIC}_{50} = 1.519 (\pm 0.315) \Delta\text{Clog P} + 3.923 (\pm 1.643) F + 4.872 (\pm 0.631) \quad (2)$$

$$n = 8 \quad s = 0.349 \quad r = 0.985 \quad F_{2,5} = 81.754$$

These results indicate that the electrostatic interaction between substituents and the receptor surface surrounding the substituents is important for activity because the correlation derived using F was better than that using Hammett σ . Physicochemical 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 *Ce*oleoptera. 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

As shown in Fig. 4, compound **10** fits snugly in the DAH-type pocket. In this model, the perfluoropropyl group is surrounded by the hydrophobic region of the LBP. This model is consistent with the results of the QSAR study, which showed that hydrophobic and electrostatic interactions between the substituents at 2-position and the receptor surface are important for activity.

Docking simulation of compound **10** was also performed against SfEcR, which was constructed from the X-ray crystal structure bound to the IMD-type compound (PDB ID: 3IXP). Although compounds **2** and **9** can dock to the LBP of SfEcR constructed from 3IXP, compound **10** containing the perfluoropropyl group could not be accommodated in the corresponding pocket. This is likely because of the slightly smaller size of the LBP of 3IXP compared to the DAH binding pocket. Because the protein was treated as a rigid body in the docking simulation using FRED, the initial size of the ligand binding pocket is thought to be critical for docking.

As shown above, compound **2** and **9** were moderately toxic to *S. litura*. The dead larva of *S. liura* treated with compound **2** is shown in Fig. 5. In our previous studies, we synthesized DAH analogs with various substituents at both benzene rings and quantitatively analyzed the structure-activity relationship to identify the essential physicochemical properties for larvicidal activity [33, 34]. QSAR equations showed that when molecular hydrophobicity was high, larvicidal activity against the lepidopteran rice stem borer *Chilo suppressalis* was also high. Although the optimum hydrophobicity was not derived for the DAH-type compounds, which had limited hydrophobicity (varied A-ring moiety: 2.04–4.68; varied B-ring moiety: 1.99–4.53), there may be an optimum value for the expanded set of compounds with supra-optimum hydrophobicity. In fact, optimum hydrophobicity ($\log P_{\text{opt}} = 5.15$) was evaluated for activity against *C. suppressalis* [38]. Although this insect species was different from *S. frugiperda* used in this study, log P values of compounds **4** and **5** exceeded 5.15, suggesting the presence of optimum hydrophobicity.

Fig. 5

Compound **13** containing the SCH₃ group showed relatively high binding activity (pIC₅₀ = 7.39) against Sf-9 cells, but was not toxic to *S. litura*. This may be because of the facile oxidation of the sulfide moiety to sulfone/sulfoxide through metabolism. We reported that RH-5849 was 10-fold less toxic than tebufenozide against Lepidopteran *C. suppressalis* (Pyralidae; pLD₅₀ = 6.27 vs. 7.32) and 100-fold less toxic than tebufenozide against *S. exigua* (Noctuidae; pLD₅₀ = 4.91 vs. 7.06) [39]. The difference in pLD₅₀ values between RH-5849 and tebufenozide was 100-fold against *S. litura* (Noctuidae), which is consistent with the toxicity results for *S. exigua*.

5. Conclusion

Among 18 synthesized imidazothiadiazole analogs, two compounds with CF₂CF₃ and CF₂CF₂CF₃ groups showed higher receptor binding activity than ponasterone A against Lepidoptera Sf-9 cells. The larvicidal activity of the CF₂CF₃ analog was determined against *S. litura* larvae in terms of pLD₅₀. It was 5 times more toxic than RH5849, but 20 times less potent than tebufenozide. All compounds, however, did not show strong binding activity against mosquito cells (Diptera) and beetle cells (Coleoptera). This selective toxicity profile among insect orders is similar to that for DAHs. In the structure-activity relationship study, a compound with a primary acrylamide moiety was inactive, but the mono-alkylation of terminal nitrogen of acrylamides (secondary amides) drastically enhanced the activity. Among secondary amides, compounds with isopropyl, cyc-butyl and cyc-hexyl groups have similar receptor binding activity, but the further alkylation (tertiary amide) was detrimental for the binding. The conversion of amide to thioamide did not have much impact to the activity, but the saturation of olefin moiety and conversion of amide to sulfonamide were also detrimental to the activity. QSAR analysis of the substituent effect at 2-position of the imidazothiadiazole ring indicated that the electron withdrawing and hydrophobic

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

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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 1R20 (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) POCl₃; (b) CH₃I, KOH, 2-propanol/H₂O

Scheme 2. Construction of (imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)acrylic acid moiety: (a) EtOH; (b) POCl₃, DMF; (c) CH₂(COOH)₂, piperidine, pyridine

Scheme 3. Synthesis of (imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)acrylamides: (a) i. (COCl)₂, DMF, CH₂Cl₂ ii. NH₃ aq., CH₂Cl₂; (b) amine, EDC, DMAP, CH₂Cl₂

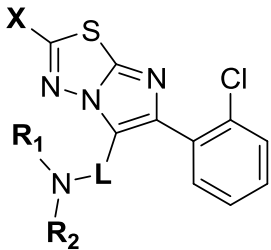
Scheme 4. Synthesis of sulfoxide **14** and sulfone **15** (a) *m*-CPBA, CH₂Cl₂; (b) Oxone[®], THF/MeOH/H₂O

Scheme 5. Synthesis of propionamide **16**: (a) N₂H₄, NaIO₄, MeOH/H₂O (b) isopropylamine, EDC, DMAP, CH₂Cl₂

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

Scheme 7. Synthesis of vinyl sulfonamide **18**: (a) Ph₂P(O)CH₂SO₂NHBoc, LiCl, DBU, CH₃CN; (b) *i*-PrOH, DEAD, PPh₃, THF; (c) TFA, CH₂Cl₂

Table 1. Biological activity of synthesized compounds.



No	X	L	R ₁	R ₂	pIC ₅₀ (M)			pLD ₅₀ (mmol/insect)	ClogP
					Sf-9	NIAS-AeAl2	BCIRL-Lepd-SL1	<i>S. litura</i>	
1	CF ₃	<i>trans</i> -CH=CH-C(=O)-	H	H	< 4.9 (12%)	n.d. ^a	n.d.	n.d.	3.56
2	CF ₃	<i>trans</i> -CH=CH-C(=O)-	H	<i>i</i> -Pr	8.03 ^d	7.10	4.88	5.16	4.63
3	CF ₃	<i>trans</i> -CH=CH-C(=O)-	H	<i>c</i> -Bu	7.71	6.74	≈ 4.08 (49%)	< 4.00 (45%)	4.70
4	CF ₃	<i>trans</i> -CH=CH-C(=O)-	H	<i>c</i> -Hex	7.65	6.05	< 4.60 (27%)	< 4.30 (10%)	5.82
5	CF ₃	<i>trans</i> -CH=CH-C(=O)-	H	Ph	7.08	5.94	< 4.38 (10%)	< 4.30 (0%)	5.65
6	CF ₃	<i>trans</i> -CH=CH-C(=O)-	CH ₃	<i>i</i> -Pr	5.20	≈ 4.38 (51%)	< 4.38 (39%)	< 4.30 (25%)	4.52
7	CF ₃	<i>trans</i> -CH=CH-C(=O)-	-(CH ₂) ₅ -		5.24	≈ 4.08 (53%)	≈ 4.08 (55%)	< 4.48 (0%)	4.88
8	CF ₃	<i>trans</i> -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 ₂ CF ₃	<i>trans</i> -CH=CH-C(=O)-	H	<i>i</i> -Pr	8.35 ^e	7.52	4.72	5.03	5.00

10	CF ₂ CF ₂ CF ₃	<i>trans</i> –CH=CH-C(=O)-	H	<i>i</i> -Pr	8.48	n.d.	n.d.	n.d.	5.23
11	H	<i>trans</i> –CH=CH-C(=O)-	H	<i>i</i> -Pr	4.79	n.d.	< 4.60 (4.6%)	n.d.	3.74
12	CH ₃	<i>trans</i> –CH=CH-C(=O)-	H	<i>i</i> -Pr	5.44	< 4.38 (42%)	≈ 4.38 (47%)	< 4.30 (0%)	4.24
13	SCH ₃	<i>trans</i> –CH=CH-C(=O)-	H	<i>i</i> -Pr	7.39 ^f	6.39	< 4.90 (35%)	< 4.30 (0%)	4.44
14	S(=O)CH ₃	<i>trans</i> –CH=CH-C(=O)-	H	<i>i</i> -Pr	4.79	< 4.60 (38%)	< 4.90 (16%)	< 4.00 (0%)	2.44
15	SO ₂ CH ₃	<i>trans</i> –CH=CH-C(=O)-	H	<i>i</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>i</i> -Pr	5.83	≈ 4.08 (56%)	< 4.08 (25%)	< 4.48 (5%)	3.54
17	CF ₃	<i>trans</i> –CH=CH-C(=S)-	H	<i>i</i> -Pr	7.51	> 4.60 (99%)	< 4.90 (38%)	n.d.	4.85
18	CF ₃	<i>trans</i> –CH=CH-SO ₂ -	H	<i>i</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	Δ ClogP	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 ₂ CH 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 ₂ CF ₃	1.26	0.44	8.35	8.51	-0.16
10	CF ₂ CF ₂ CF ₃	1.49	0.42	8.48	8.78	-0.30

^a The difference between observed and calculated value [pIC₅₀(obsd) – pIC₅₀(calcd by Eq. 2)].

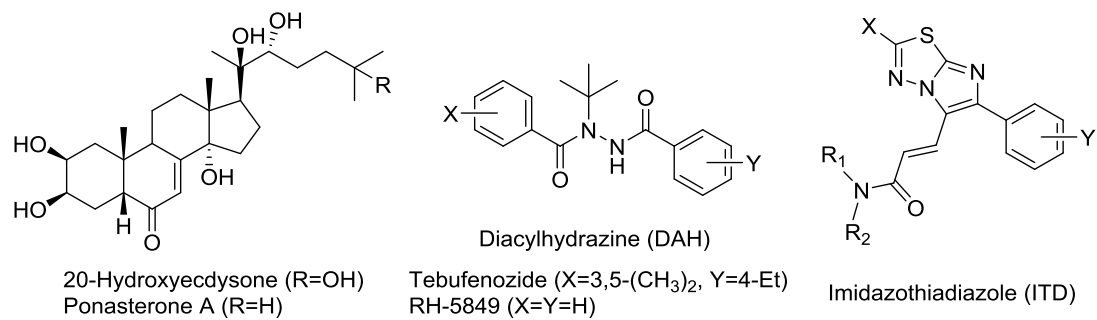


Fig. 1. Chemical structures of ecdysone agonists

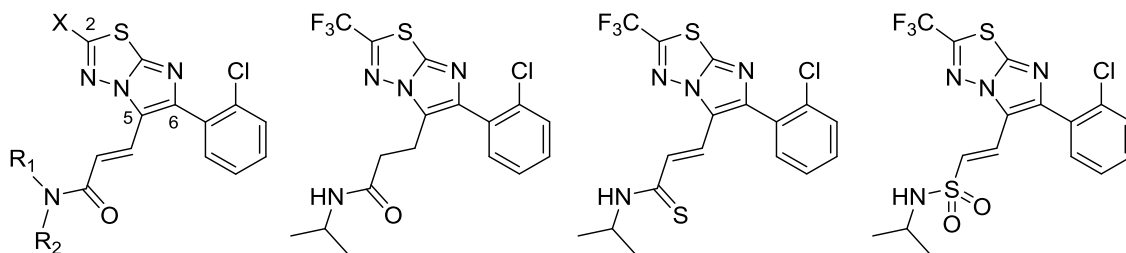


Fig. 2. Imidazothiadiazole-type compounds synthesized for SAR study

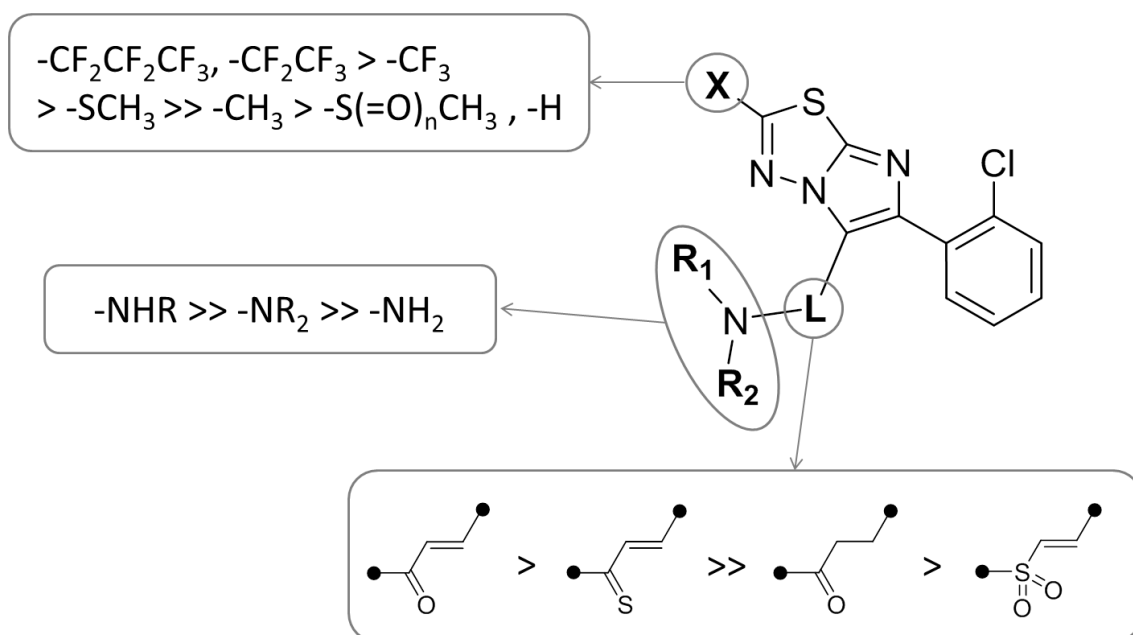


Fig. 3. Summary of structure-activity relationship for the binding activity against Sf-9

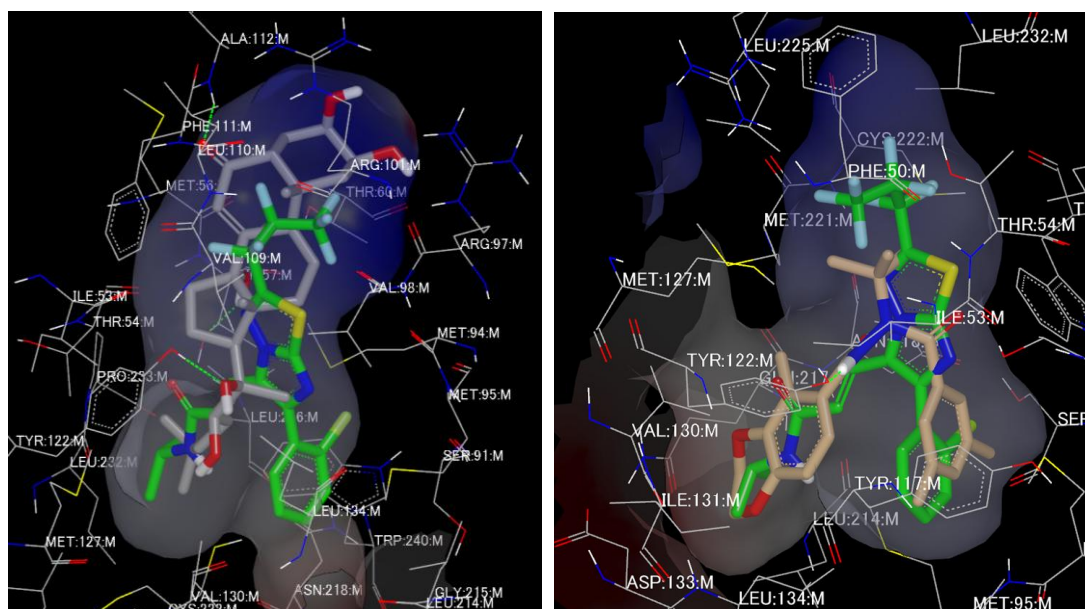
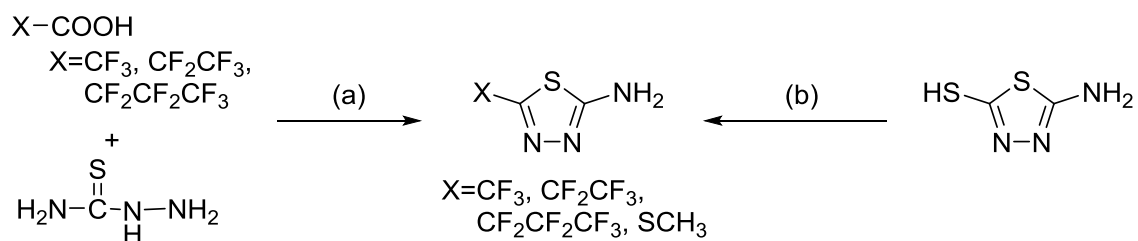


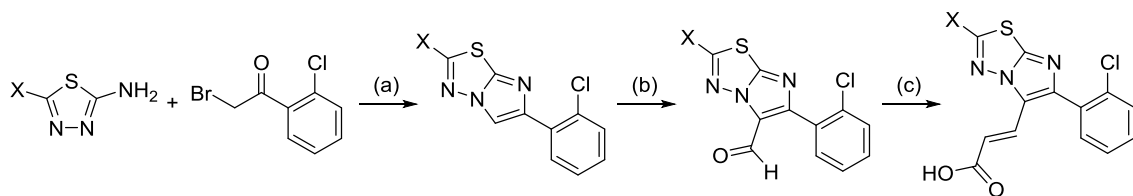
Fig. 4. Docking simulation of compound **10** (colored with green) against SfEcR bound to PonA (left) and DAH (BYI06833; right). Structure colored with gray is PonA and that colored with light brown is BYI06833. Ligand binding domains were modeled from 1R1K (left) and IR20 (right).



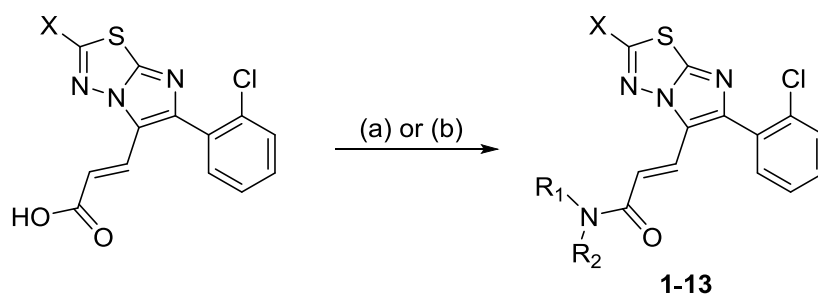
Fig. 5 *S. litura* Larva treated with compound **2** (10^{-5} mmol/insect)



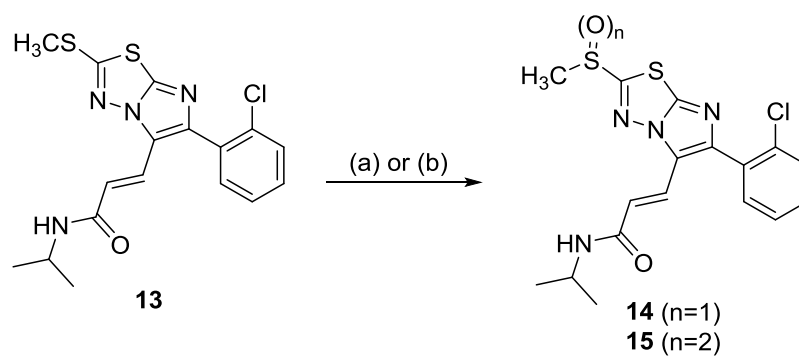
Scheme 1. Construction of 2-amino-1,3,4-thiadiazole moiety: (a) POCl_3 ; (b) CH_3I , KOH , 2-propanol/ H_2O



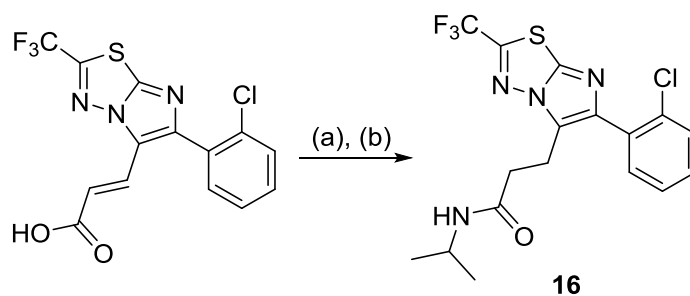
Scheme 2. Construction of (imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)acrylic acid moiety: (a) EtOH ; (b) POCl_3 , DMF ; (c) $\text{CH}_2(\text{COOH})_2$, piperidine, pyridine



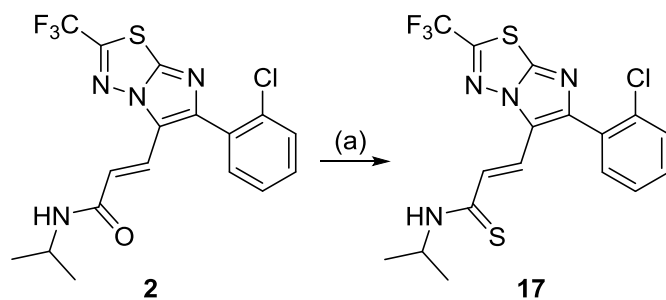
Scheme 3. Synthesis of (imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)acrylamides: (a) i. (COCl)₂, DMF, CH₂Cl₂ ii. NH₃ aq., CH₂Cl₂; (b) amine, EDC, DMAP, CH₂Cl₂



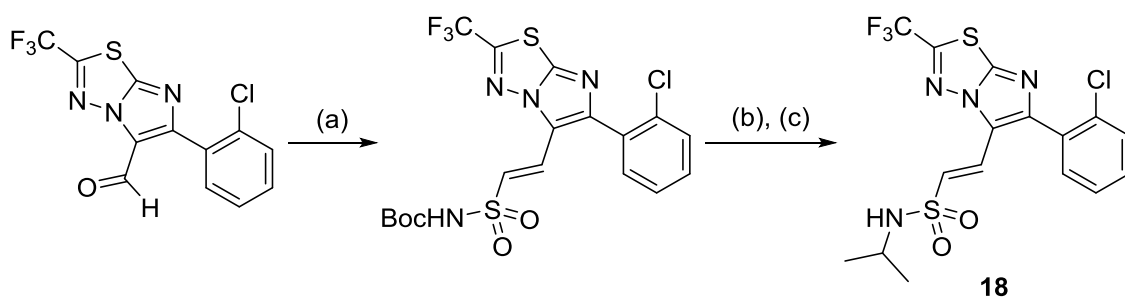
Scheme 4. Synthesis of sulfoxide **14** and sulfone **15**: (a) *m*-CPBA, CH₂Cl₂; (b) Oxone[®], THF/MeOH/H₂O



Scheme 5. Synthesis of propionamide **16**: (a) N₂H₄, NaIO₄, MeOH/H₂O (b) isopropylamine, EDC, DMAP, CH₂Cl₂



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



Scheme 7. Synthesis of vinyl sulfonamide **18**: (a) $\text{Ph}_2\text{P}(\text{O})\text{CH}_2\text{SO}_2\text{NHBoc}$, LiCl, DBU, CH_3CN ; (b) *i*-PrOH, DEAD, PPh_3 , THF; (c) TFA, CH_2Cl_2