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2 **Structure-activity relationship of imidazothiadiazole analogs for the**
3 **binding to the ecdysone receptor of insect cells**

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15

16 **Abstract**

17 Diacylhydrazines are the first non-steroidal ecdysone agonists, and five compounds are
18 used as insecticides in agriculture. After the discovery of diacylhydrazine-type
19 compounds, numerous non-steroidal structures were reported as ecdysone agonists.
20 Among various ecdysone agonists, imidazothiadiazoles are reported to be very potent *in*
21 *vitro*; however the experimental detail for the structure identification and bioassays are
22 not stated in the paper (Holmwood and Schindler, *Bioorg. Med. Chem.*, **17**, 4064-4070,
23 2009). In our present study, we synthesized 18 imidazothiadiazole-type compounds and
24 confirmed the chemical structures by spectrometric analyses. The binding activity of the
25 synthesized compounds to the ecdysone receptor was evaluated in terms of the
26 concentration required for 50% inhibition of [³H]ponasterone A incorporation [IC₅₀ (M)]
27 into lepidopteran (Sf-9), coleopteran (BCRL-Lepd-SL1), and dipteran (NIAS-AeAl2)
28 cells. 6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-*b*]
29 [1,3,4]-thiadiazolyl-5-yl)acrylamide analogs with –CONHR (secondary amide) were
30 very potent against Sf-9 cells, but further alkylation (tertiary amide: –CONR₂) decreased
31 the activity dramatically. Additionally, a primary amide analog (–CONH₂) was inactive.
32 The activity also decreased 150-fold by the saturation of olefin region of the acrylamide
33 moiety. In addition, various substituents were introduced at the 2-position of the
34 imidazothiadiazole ring to disclose the physicochemical properties of the substituents
35 which are important for receptor binding. The activity increased by 7500-fold with the
36 introduction of the CF₂CF₂CF₃ group compared to the unsubstituted compound against
37 Sf-9 cells. Quantitative structure-activity relationship analysis for these substituents
38 indicated that hydrophobic and electron-withdrawing groups were favorable for binding.
39 Some of the compounds with strong receptor binding activity showed good larvicidal
40 activity against *Spodoptera litura*. In contrast, the binding affinity of imidazothiadiazole
41 analogs was low or not observed against dipteran and coleopteran cells.

42

43

44 **Keywords:**

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

46

47 1. Introduction

48 Arthropods, including insects, grow by repeated molting, which is regulated by
49 molting hormones such as 20-hydroxyecdysone (20E; Fig. 1). Steroidal compounds with
50 20E-like activity are categorized as ecdysteroids, and have been identified in plants,
51 animals, and microorganisms. To date, more than 400 ecdysteroids have been
52 characterized (<http://ecdybase.org>), **but no ecdysteroids have been launched as**
53 **insecticides**. Using steroids as insecticides may not be practical because of their **high cost**
54 **and synthesis difficulty**. In addition, steroids do not easily penetrate the integument and
55 are rapidly excreted from insects.

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

61

62 Fig. 1

63

64 Because the insecticidal spectrum of DAHs is narrow, other chemical structures
65 have been screened as ecdysone agonists [3]. Among them, tetrahydroquinoline (THQ)
66 [4], *N*-alkyl-3,5-di-*tert*-butyl-4-hydroxy-benzamide [5], α -acylaminoketone [6],
67 oxadiazoline [7], and γ -methylene- γ -lactam [8] have been described over the past two
68 decades. In 2009, Holmwood and Schindler reported that imidazole (IMD) and
69 imidazothiadiazole (ITD)-type compounds are ecdysone agonists (Fig. 2) [9]. Although
70 the biological activity was evaluated quantitatively in terms of pInd₅₀ (EcR induction
71 assay), experimental procedures and target insect species have not been described.
72 Analytical data for the synthesized chemicals were not reported.

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

75 however, thought to differ from those of DAHs and steroidal agonists such as ponasterone
76 A (PonA). The ITD substructure is very interesting, because some ITD-type compounds
77 are reported to show anti-inflammatory [10], anticancer [11] and antitubercular activity
78 [12].

79 The aim of this study was to quantitatively measure the ligand-receptor binding
80 activity of ITD analogs and discuss the structure-activity relationship (SAR). For the
81 SAR study, various ITD analogs were chemically synthesized. The substituents X at
82 2-position of imidazothiadiazole ring (Fig. 2) were substituted with H, CH₃, CF₃, CF₂CF₃,
83 CF₂CF₂CF₃, SCH₃, S(=O)CH₃, SO₂CH₃, and the amide moiety to vary primary,
84 secondary, and tertiary structure (Fig. 2). The linker between the imidazole ring and
85 amide moiety was fixed as either *trans* -CH=CH- or -CH₂CH₂- (Fig. 2). Thioamide and
86 sulfonamide analogs were also synthesized (Fig. 2). The binding affinity of these
87 compounds was measured to the ecdysone receptors of three insect cells. The effect of
88 substituents X on ligand-receptor binding against Sf-9 was quantitatively analyzed using
89 classical quantitative structure-activity relationship (QSAR) analysis (Hansch-Fujita
90 method) [13]. Docking simulation was also performed to predict the ligand-receptor
91 interaction of ITDs.

92

93 Fig. 2

94

95

96 2. Materials and Methods

97 2.1. Synthesis

98 2.1.1. Chemicals

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

103 commercially available and stored over molecular sieves. Flash column chromatography
104 was conducted using Wakogel[®] C-300HG (Wako Pure Chemical Industries, Osaka,
105 Japan) as the absorbent. NMR spectra were recorded on a Bruker AVANCE-400 or
106 Bruker AVANCE-500 spectrometer. Tetramethylsilane was used as the internal standard
107 for ¹H NMR (0 ppm); deuterated solvent signals were used as the internal standard for ¹³C
108 NMR (77.16 ppm for CDCl₃ and 39.52 ppm for DMSO-*d*₆); and α,α,α -trifluorotoluene
109 was used as the external standard for ¹⁹F NMR (-64.00 ppm). Melting points were
110 measured with a Yanaco melting point apparatus (Yanagimoto Seisakusho Co. Ltd.,
111 Kyoto, Japan) and are uncorrected. Elemental analyses were performed at the
112 Microanalytical Center of Kyoto University. High-resolution mass spectra (HRMS) were
113 recorded on a Thermo Fisher Scientific EXACTIVE spectrometer at Department of
114 Synthetic Chemistry and Biological Chemistry of Kyoto University.

115

116 2.1.2. Synthesis of 2-amino-1,3,4-thiadiazoles (Scheme 1)

117

118

Scheme 1

119

120 *i) 2-Amino-5-(trifluoromethyl)-1,3,4-thiadiazole (Step a):* Phosphoryl chloride (27.5 mL,
121 300 mmol) was added dropwise to a mixture of thiosemicarbazide (13.7 g, 150 mmol)
122 and trifluoroacetic acid (24.1 mL, 315 mmol) at 0°C, and the mixture was gradually
123 heated to 70°C. Foaming was observed during the reaction. The mixture was kept at 70°C
124 for 1 hour after the foaming ceased. After cooling the reaction mixture to room
125 temperature, it was treated with water (300 mL) and neutralized with saturated Na₂CO₃
126 solution. The resulting solid was filtered off, washed with water and dried *in vacuo* to
127 yield an off-white solid (19.4 g, 77%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.07 (2H, s)
128 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),
129 171.7 ppm; ¹⁹F NMR (377 MHz, DMSO-*d*₆) δ -59.77 (3F, s) ppm.

130 2-Amino-1,3,4-thiadiazoles with CF₂CF₃ and CF₂CF₂CF₃ group were synthesized in a

131 similar manner as above.

132

133 ii) 2-Amino-5-(methylthio)-1,3,4-thiadiazole (Step b): Potassium hydroxide (85%, 3.4 g,
134 51 mmol) was added in one portion to a suspension of 2-amino-5-mercapto-
135 1,3,4-thiadiazole (6.7 g, 50 mmol) in 2-propanol (10 mL) and water (7.5 mL) at 0°C.
136 When the starting materials completely dissolved, methyl iodide (3.3 mL, 53 mmol) was
137 added dropwise to the reaction mixture maintaining the temperature below 15°C. It was
138 stirred at room temperature overnight. The mixture was poured into water (200 mL) and
139 the resulting solid was filtered off. This was washed with water and dried *in vacuo* to
140 yield a white solid (5.6 g, 76%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.58 (3H, s), 7.21 (2H,
141 s) ppm; ¹³C NMR (100 MHz, DMSO-*d*₆) δ 16.6, 151.9, 168.9 ppm.

142

143 **2.1.3. Synthesis of 2-chlorophenacyl bromide**

144 A solution of bromine (26.5 g, 166 mmol) in acetic acid (25 mL) was added dropwise to a
145 solution of 2'-chloroacetophenone (25.1 g, 162 mmol) in acetic acid (175 mL) at room
146 temperature. The mixture was stirred at room temperature for 2 hours. The mixture was
147 diluted with water (250 mL) and extracted with CH₂Cl₂ (250 mL). The organic layer was
148 washed successively with water (3×250 mL), saturated aqueous NaHCO₃ solution (250
149 mL) and brine (250 mL), and dried over anhydrous Na₂SO₄. The solvent was evaporated
150 to give the crude 2-chlorophenacyl bromide (38.7 g, purity: *ca.* 83% determined by ¹H
151 NMR analysis), which was used for the next reaction without further purification.

152

153 **2.1.4. Synthesis of (*E*)-3-(6-(2-chlorophenyl)-imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)-** 154 **acrylic acids (Scheme 2)**

155

156 Scheme 2

157

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

159 mixture of 2-amino-5-(trifluoromethyl)-1,3,4-thiadiazole (19.2 g, 114 mmol) and
160 2-chlorophenacyl bromide (33.4 g, *ca.* 120 mmol) in ethanol (170 mL) was refluxed
161 overnight. The mixture was then cooled in a freezer. The resulting crystalline solid was
162 filtered off, washed with cold ethanol, and dried *in vacuo* to yield a pale yellow solid
163 (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,
164 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,
165 s) ppm.

166 Other imidazothiadiazole analogs with CF₂CF₃, CF₂CF₂CF₃, H, CH₃, and SCH₃ were
167 synthesized in a similar manner as above.

168

169 ii) 6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbalde-
170 hyde (Step b): Under an argon atmosphere, phosphoryl chloride (3.9 mL, 43 mmol) was
171 added dropwise to anhydrous DMF (20 mL) at 0°C and stirred for 5 min. To this was
172 added 6-(2-chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazole (3.91 g,
173 13 mmol) in anhydrous DMF (15 mL), and the mixture was heated to 70°C and stirred
174 overnight. It was poured into ice-water (100 mL), neutralized with saturated aqueous
175 Na₂CO₃ solution and then extracted with toluene (1×100 mL, 2×50 mL). The combined
176 organic layer was washed with water (3×100 mL) and brine (100 mL), and dried over
177 anhydrous MgSO₄. The solvent was evaporated and the crude product was purified by
178 flash column chromatography (hexane/ethyl acetate = 95:5 – 50:50) to yield a pale yellow
179 solid (2.91 g, 68%). ¹H NMR (400 MHz, CDCl₃) δ 7.43-7.49 (2H, m), 7.55-7.59 (2H, m),
180 9.85 (1H, s) ppm.

181 Other imidazothiadiazole-5-carbaldehyde analogs with CF₂CF₃, CF₂CF₂CF₃, H, CH₃,
182 and SCH₃ were synthesized in a similar manner as above.

183

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

187 dissolved in pyridine (17 mL). To this, malonic acid (0.76 g, 7.3 mmol) was added and the
188 mixture was stirred at 100°C for 4 h. After cooling, the reaction mixture was poured into
189 1 M HCl (70 mL) and acidified with concentrated HCl. The resulting solid was filtered
190 off, washed with water, and dried *in vacuo*. This solid was triturated in hexane/ether (1:1)
191 to give an off-white solid (1.89 g, 84%). ¹H NMR (400 MHz, DMSO-*d*₆) δ 6.68 (1H, d, *J*
192 = 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.
193 s) ppm.

194 Other (imidazothiadiazol-5-yl)acrylic acid analogs with CF₂CF₃, CF₂CF₂CF₃, H, CH₃,
195 and SCH₃ were synthesized in a similar manner as above.

196

197 **2.1.5. Synthesis of (*E*)-3-(6-(2-chlorophenyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)** 198 **acrylamides (Scheme 3)**

199

200 Scheme 3

201

202

203 *i)* (*E*)-3-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)
204 acrylamide (**1**): Oxalyl chloride (0.17 mL, 2.0 mmol) was added to the suspension of
205 (*E*)-3-(6-(2-chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)acry-
206 lic acid (377 mg, 1.0 mmol) in CH₂Cl₂ (5 mL) containing one drop of DMF. After the gas
207 evolution ceased, the mixture was refluxed for 2 hours. After cooling, the solvent was
208 evaporated to give the crude acid chloride. This was dissolved in CH₂Cl₂ (5 mL), and then
209 added dropwise to vigorously stirred aqueous NH₃ solution (28%, 5 mL) at 0°C. The
210 mixture was stirred at room temperature overnight. It was diluted with water (30 mL) and
211 extracted with CH₂Cl₂ (3×30 mL). The combined organic layer was washed with water
212 (50 mL) and brine (50 mL), and dried over anhydrous Na₂SO₄. The solvent was
213 evaporated and the crude product was recrystallized from ethyl acetate/hexane to give a
214 white solid (276 mg, 73%). Mp: 232-234°C. ¹H NMR (400 MHz, CDCl₃) δ 5.67 (2H, br

215 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
216 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,
217 132.6, 134.0, 146.5, 148.5, 151.3 (q, $J_{\text{C-F}} = 42$ Hz), 167.3 ppm; ^{19}F NMR (377 MHz,
218 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,
219 15.03. Found: C, 45.07; H, 2.20; N, 15.05.

220

221 ii) (E)-3-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-
222 N-isopropylacrylamide (2): To a suspension of compound **12** (374 mg, 1.0 mmol) in
223 anhydrous CH_2Cl_2 (5 mL), EDC hydrochloride (227 mg, 1.2 mmol) and catalytic amount
224 of DMAP were added. Then, isopropylamine (98 μL , 1.2 mmol) was added and the
225 mixture was stirred at room temperature overnight. The mixture was diluted with CH_2Cl_2
226 (15 mL) and washed successively with saturated aqueous Na_2CO_3 solution, water, 1 M
227 HCl, water, and brine (10 mL each). The organic layer was dried over MgSO_4 and
228 concentrated to give compound **17** as pale yellow foam (387 mg, 93%). This was further
229 recrystallized from ethyl acetate/hexane to afford white crystals, which were used for the
230 bioassays. Mp: 174-175°C. ^1H NMR (400 MHz, CDCl_3) δ 1.22 (6H, d, $J = 6.6$ Hz),
231 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,
232 m), 7.50-7.54 (2H, m) ppm; ^{13}C NMR (100 MHz, CDCl_3) δ 22.9, 41.9, 118.7 (q, $J_{\text{C-F}} =$
233 272 Hz), 120.6, 123.2, 124.6, 127.1, 130.5, 130.7, 131.6, 132.6, 134.1, 146.1, 148.0,
234 151.0 (q, $J_{\text{C-F}} = 42$ Hz), 164.6 ppm; ^{19}F NMR (377 MHz, CDCl_3) δ -62.43 (3F, s) ppm.
235 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;
236 N, 13.65.

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

239

240 iii) (E)-3-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-
241 N-cyclobutylacrylamide (3): Mp: 199-200°C. ^1H NMR (400 MHz, CDCl_3) δ 1.69-1.78
242 (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; ¹³C NMR (100 MHz, CDCl₃) 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*_{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*_{C-F} = 42 Hz), 165.8,
274 166.1 ppm; ¹⁹F NMR (377 MHz, CDCl₃) δ (mixture of rotamers) -62.81 (1.35F, s), -62.71
275 (1.65F, s) ppm. Anal. Calcd for C₁₈H₁₆ClF₃N₄OS: 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, CDCl₃) 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; ¹³C
281 NMR (100 MHz, CDCl₃) δ 24.8, 25.7, 26.9, 43.6, 47.2, 117.6, 118.7 (q, *J*_{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*_{C-F} = 42
283 Hz), 165.0 ppm; ¹⁹F NMR (377 MHz, CDCl₃) δ -62.67 (3F, s) ppm. Anal. Calcd for
284 C₁₉H₁₆ClF₃N₄OS: 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, CDCl₃) 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 ¹³C NMR (100 MHz, CDCl₃) δ 42.7, 46.3, 67.0, 116.1, 118.7 (q, *J*_{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*_{C-F} = 42 Hz),
291 165.2 ppm. ¹⁹F NMR (377 MHz, CDCl₃) δ -62.61 (3F, s) ppm. Anal. Calcd for
292 C₁₈H₁₄ClF₃N₄O₂S: C, 48.82; H, 3.19; N, 12.65. Found: C, 48.67; H, 3.27; N, 12.55.

293

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, CDCl₃) 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; ¹³C NMR (100 MHz, CDCl₃) δ 22.9, 41.9,
298 109.3 (tq, *J* = 256, 41 Hz), 118.0 (qt, *J*_{C-F} = 285, 36 Hz), 120.6, 123.1, 124.6, 127.1, 130.5,

299 130.8, 131.5, 132.6, 134.0, 146.2, 148.0, 150.7 (t, $J_{C-F} = 31$ Hz), 164.6 ppm; ^{19}F NMR
300 (377 MHz, CDCl_3) δ -111.16 (2F, q, $J_{F-F} = 2.3$ Hz), -84.20 (3F, t, $J_{F-F} = 2.3$ 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, $J_{C-F} = 285, 36$ Hz), 117.6 (qt, $J_{C-F} = 286, 34$ 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, $J_{C-F} = 31$ 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, $J_{F-F} = 8.5$ Hz), -108.75 (2F, sex, $J_{F-F} = 9.4$ Hz), -81.01
313 (3F, t, $J_{F-F} = 9.6$ 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),

327 7.28-7.38 (2H, m), 7.40-7.54 (3H, m) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 18.0, 23.0,
328 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,
329 165.3 ppm. Anal. Calcd for C₁₇H₁₇ClN₄OS: C, 56.58; H, 4.75; N, 15.53. Found: C, 56.73;
330 H, 4.65; N, 15.70.

331

332 *xiii* (E)-3-(6-(2-Chlorophenyl)-2-(methylthio)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-
333 N-isopropylacrylamide (13): Mp: 229-230°C. ¹H NMR (400 MHz, CDCl₃) δ 1.19 (6H,
334 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*
335 = 15.4 Hz), 7.29-7.38 (2H, m), 7.39-7.55 (3H, m) ppm; ¹³C NMR (100 MHz, CDCl₃) δ
336 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,
337 146.8, 162.6, 165.2 ppm. HRMS (ESI) *m/z*: C₁₇H₁₈ClN₄OS₂ [M+H]⁺, calcd 393.0605,
338 found 393.0594.

339

340 **2.1.6. Synthesis of imidazothiadiazole analogs with S(=O)CH₃ and SO₂CH₃ (Scheme**
341 **4)**

342

Scheme 4

343

344 *i* (E)-3-(6-(2-Chlorophenyl)-2-(methylsulfinyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-
345 N-isopropylacrylamide (14): Compound **13** (394 mg, 1.0 mmol) was dissolved in CH₂Cl₂
346 (5 mL) and cooled to 0°C. To this was added the solution of *m*-chloroperbenzoic acid
347 (70%, 246 mg, 1.0 mmol) in CH₂Cl₂ (5 mL) within 30 min and the mixture was stirred at
348 room temperature overnight. The reaction was quenched by adding saturated aqueous
349 Na₂CO₃ solution (5 mL). The organic layer was washed with saturated aqueous Na₂CO₃
350 solution (5 mL) and brine (5 mL), and dried over anhydrous Na₂SO₄. The solvent was
351 evaporated and the crude product was purified by flash column chromatography (ethyl
352 acetate 100%) to yield white foam (348 mg, 85%). This was further recrystallized from
353 CHCl₃/hexane to afford white crystals, which were used for the bioassays. Mp:
354 236-237°C. ¹H NMR (400 MHz, CDCl₃) δ 1.20 (6H, d, *J* = 6.6 Hz), 3.17 (3H, s),

355 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,
356 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,
357 124.8, 127.1, 130.4, 130.6, 131.8, 132.6, 134.0, 146.8, 147.1, 164.8, 173.5 ppm. HRMS
358 (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.

359

360 ii) (E)-3-(6-(2-Chlorophenyl)-2-(methylsulfonyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-
361 N-isopropylacrylamide (15): Compound **13** (398 mg, 1.0 mmol) was dissolved in
362 THF/MeOH/water (1:1:1, 15 mL) and cooled to 0°C. Oxone[®] (1.84 g, 3.0 mmol) was
363 slowly added to the solution and the mixture was stirred at 0°C for 5 min and at room
364 temperature overnight. After largely evaporating the solvent, the mixture was diluted
365 with water (20 mL) and extracted with CH_2Cl_2 (1×10 mL, 2×5 mL). The combined
366 organic layer was washed with water (10 mL) and brine (10 mL), and dried over
367 anhydrous Na_2SO_4 . The solvent was evaporated and the crude product was purified by
368 flash column chromatography (hexane/ethyl acetate = 3:7) to yield a pale yellow solid
369 (340 mg, 79%). This was further recrystallized from CHCl_3 /hexane to afford pale yellow
370 crystals, which were used for the bioassays. Mp: 247-249°C. ^1H NMR (400 MHz, CDCl_3)
371 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),
372 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,
373 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,
374 147.3, 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
375 425.0503, found 425.0492.

376

377 2.1.7. Synthesis of other imidazothiadiazole analogs

378

379

Scheme 5

380

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

383 (trifluoromethyl)imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)acrylic acid (374 mg, 1.0 mmol) in
384 MeOH (6 mL) was added hydrazine hydrate (2.0 mL, 41 mmol) and catalytic amount of
385 acetic acid and saturated aqueous CuSO₄ solution. To this, the solution of NaIO₄ (1.09 g,
386 5.1 mmol) in water (10 mL) was added dropwise in 1 hour and the mixture was stirred at
387 room temperature overnight. After largely evaporating the solvent, the mixture was
388 diluted with 3 M HCl (20 mL) and extracted with ethyl acetate (3×20 mL). The combined
389 organic layer was washed with water (20 mL) and brine (20 mL), and dried over
390 anhydrous Na₂SO₄. The solvent was evaporated to give the crude propanoic acid. This
391 was suspended in anhydrous CH₂Cl₂ (5 mL), and EDC hydrochloride (225 mg, 1.2 mmol)
392 and catalytic amount of DMAP were added. Then, isopropylamine (98 μL, 1.2 mmol)
393 was added and the mixture was stirred at room temperature overnight. Because TLC
394 analysis indicated that the reaction was not complete, EDC hydrochloride (122 mg, 0.64
395 mmol), isopropylamine (50 μL, 0.61 mmol) and catalytic amount of DMAP were further
396 added and the mixture was stirred at room temperature for 3 days. The mixture was
397 diluted with CH₂Cl₂ (15 mL) and washed successively with saturated aqueous Na₂CO₃
398 solution, water, 1 M HCl, water, and brine (10 mL each). The organic layer was dried over
399 MgSO₄ and filtered through a plug of silica gel, which was eluted with ethyl acetate. The
400 filtrate was concentrated and the crude product was purified by flash column
401 chromatography (hexane/ethyl acetate = 3:2) to yield a pale yellow solid (86 mg, 21%).
402 This was further recrystallized from ethyl acetate to afford pale yellow crystals, which
403 were used for the bioassays. Mp: 180-182°C. ¹H NMR (400 MHz, CDCl₃) δ 1.09 (6H, d,
404 *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* =
405 5.4 Hz), 7.32-7.40 (2H, m), 7.42-7.53 (2H, m) ppm; ¹³C NMR (100 MHz, CDCl₃) δ 20.6,
406 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,
407 133.9, 142.2, 142.4, 150.2 (q, *J*_{C-F} = 42 Hz), 170.0 ppm. ¹⁹F NMR (377 MHz, CDCl₃) δ
408 -62.63 (3F, s) ppm. Anal. Calcd for C₁₇H₁₆ClF₃N₄OS: C, 48.98; H, 3.87; N, 13.44. Found:
409 C, 48.76; H, 4.01; N, 13.26.

410

411 Scheme 6

412

413 ii) (E)-3-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl)-
414 N-isopropylprop-2-enethioamide (17): A mixture of compound **2** (349 mg, 0.84 mmol)
415 and Lawesson's reagent (174 mg, 0.43 mmol) in toluene (20 mL) was refluxed for 2 hours.
416 After cooling, the mixture was filtered through a plug of silica gel, which was eluted with
417 ethyl acetate. The filtrate was concentrated and the crude product was purified by flash
418 column chromatography (hexane/ethyl acetate = 3:1) and recrystallization from ethyl
419 acetate/hexane to yield yellow crystals (282 mg, 78%). Mp: 203-204°C. ¹H NMR (400
420 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
421 (3H, m), 7.52-7.58 (1H, m), 7.87 (1H, d, *J* = 15.1 Hz) ppm. ¹³C NMR (100 MHz, CDCl₃)
422 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,
423 132.6, 133.9, 146.2, 148.7, 151.1 (q, *J*_{C-F} = 42 Hz), 192.7 ppm; ¹⁹F NMR (377 MHz,
424 CDCl₃) δ -62.35 (3F, s) ppm. Anal. Calcd for C₁₇H₁₄ClF₃N₄S₂: C, 47.39; H, 3.28; N,
425 13.00. Found: C, 47.27; H, 3.29; N, 12.86.

426

427 Scheme 7

428

429 iii) (E)-2-(6-(2-Chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4] thiadiazol-5-
430 yl)-N-isopropylethanesulfonamide (18): To a suspension of lithium chloride (319 mg, 7.5
431 mmol) and Ph₂P(O)CH₂SO₂NHBoc (1.31 g, 3.3 mmol) in anhydrous CH₃CN (25 mL)
432 was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 1.1 mL, 7.4 mmol). To this
433 solution was added 6-(2-chlorophenyl)-2-(trifluoromethyl)imidazo[2,1-b][1,3,4]-
434 thiadiazole-5-carbaldehyde (994 mg, 3.0 mmol) and the mixture was stirred at room
435 temperature for 1.5 h. The resulting suspension was diluted with water (75 mL) and the
436 pH was adjusted to 3 with 1 M HCl. This was extracted with ether (1×80 mL, 2×40 mL),
437 and the combined organic layer was washed with brine (100 mL) and dried over
438 anhydrous MgSO₄. The solvent was evaporated and the crude product was purified by

439 flash column chromatography (hexane/ethyl acetate = 95:5 – 50:50) to give the *N*-Boc
440 vinyl sulfonamide as a pale yellow solid (1.11 g, 73%). ¹H NMR (400 MHz, DMSO-*d*₆) δ
441 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),
442 7.68-7.72 (1H, m), 11.46 (1H, br s) ppm.

443 *N*-Boc vinyl sulfonamide obtained as above (511 mg, 1.0 mmol), isopropanol
444 (0.19 mL, 2.5 mmol) and triphenylphosphine (668 mg, 2.6 mmol) were dissolved in
445 anhydrous THF (15 mL). Diethyl azodicarboxylate (DEAD; 40% in toluene; 1.35 mL,
446 3.1 mmol) was slowly added to the solution and the mixture was stirred at room
447 temperature for 1 h. The solvent was evaporated and the crude product was purified by
448 flash column chromatography (hexane/ethyl acetate = 95:5 – 65:35) to give the *N*-Boc
449 *N*-isopropyl vinyl sulfonamide as a pale yellow solid (416 mg, 76%). ¹H NMR (400 MHz,
450 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,
451 m), 7.53-7.58 (1H, m), 7.69 (1H, d, *J* = 15.5 Hz) ppm.

452 To a solution of *N*-Boc *N*-isopropyl vinyl sulfonamide obtained as above (377 mg,
453 0.68 mmol) in CH₂Cl₂ (5 mL) was added trifluoroacetic acid (TFA; 5 mL) and the
454 mixture was stirred at room temperature for 1 h. The solvent was evaporated and the
455 crude product was purified by flash column chromatography (hexane/ethyl acetate = 95:5
456 – 50: 50) to give a white solid (270 mg, 88%). This was further recrystallized from
457 CHCl₃/hexane to afford white crystals, which were used for the bioassays. Mp:
458 157-158°C. ¹H NMR (500 MHz, CDCl₃) δ 1.21 (6H, d, *J* = 6.6 Hz), 3.51-3.62 (1H, m),
459 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
460 (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}
461 = 271 Hz), 120.9, 124.4, 126.2, 127.3, 130.6, 131.1, 131.2, 132.5, 133.8, 147.1, 148.9,
462 151.9 (q, *J*_{C-F} = 42 Hz) ppm; ¹⁹F NMR (471 MHz, CDCl₃) δ -62.52 (3F, s) ppm. Anal.
463 Calcd for C₁₇H₁₄ClF₃N₄S₂: C, 47.39; H, 3.28; N, 13.00. Found: C, 47.27; H, 3.29; N,
464 12.86. HRMS (ESI) *m/z*: C₁₆H₁₅ClF₃N₄O₂S₂ [M+H]⁺, calcd 451.0272, found 451.0261.

465

466 **2.2. Ligand-receptor binding assay**

467 Tritiated ponasterone A ($[^3\text{H}]\text{PonA}$, 140 Ci/mmol) was purchased from American
468 Radiolabeled Chemicals Inc. (St. Louis, MO, USA). PonA and 20E were purchased
469 from Cosmo Bio Co., Ltd. (Tokyo, Japan) and Sigma-Aldrich Co. (St. Louis, MO, USA),
470 respectively. RH-5849 and tebufenozide were from our stock samples. All insect cells are
471 cultured in our laboratory. Originally, Sf-9 cell line was kindly gifted from Wakenyaku
472 Co., Ltd. (Kyoto, Japan), NIAS-AeA12 was given from NIAS Genebank (Tsukuba,
473 Japan)[14], and BCIRL-Lepd-SL1 was kindly gifted from Dr. Cynthia Goodman
474 (USDA-ARS, Columbia, MO, USA)[15].

475 The ligand binding assay using insect cells was performed as previously reported [16,
476 17]. In brief, insect cell suspension (400 μL ; $2 - 3 \times 10^6$ cells/mL) was incubated with
477 DMSO solution of a test compound (1 μL) and 70% EtOH solution of $[^3\text{H}]\text{PonA}$ (2 μL ;
478 *ca.* 60,000 dpm) at 25°C for 30 min. The mixture was diluted with water (3 mL) and
479 filtered through a glass filter GF-75 (ADVANTEC, Tokyo, Japan). The filter was washed
480 2 times with water (3 mL), dried, and placed in a vial containing 3 mL of Insta-Gel Plus
481 (PerkinElmer, Inc., Waltham, MA, USA) to measure the radioactivity with a LSC-6100
482 liquid scintillation counter (Aloka, Tokyo, Japan). The concentration required for 50%
483 inhibition of $[^3\text{H}]\text{PonA}$ binding (IC_{50}) was determined by probit analysis[18], and its
484 reciprocal logarithm, pIC_{50} , was used as the index of binding activity.

485

486 **2.3. Larvicidal activity test**

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

494

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

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

530

531 3. Results

532 3.1 Synthesis

533 ITDs were synthesized according to a previously described method with some
534 modifications [27]. 2-Amino-1,3,4-thiadiazoles bearing CF₃, CF₂CF₃, and CF₂CF₂CF₃
535 groups were prepared using a reported method [28].
536 2-Amino-5-(methylthio)-1,3,4-thiadiazole was also prepared according to a modified
537 method described in the literature [29]. Formation of the imidazo[2,1-*b*][1,3,4]thiadiazole
538 ring was accomplished by simply refluxing 2-amino-1,3,4-thiadiazoles and
539 2-chlorophenacyl bromide in ethanol. While compounds with electron-withdrawing
540 substituents (CF₃, CF₂CF₃, and CF₂CF₂CF₃) did not form HBr salts, compounds with
541 other substituents (H, CH₃, SCH₃) precipitated as HBr salts in the reaction mixture. In the
542 next step, imidazothiadiazoles were subjected to the Vilsmeier-Haack reaction to afford
543 imidazo[2,1-*b*][1,3,4]thiadiazole-5-carbaldehydes. Next, Knoevenagel condensation of
544 aldehydes with malonic acid afforded pure (*E*)-acrylic acids. Finally, condensation of
545 acrylic acids with various amines yielded ITDs (**1–13**). In further, compound **13** was
546 oxidized with *m*-CPBA and Oxone[®] to yield sulfoxide **14** and sulfone **15**.

547 Next, we modified the acrylamide moiety. Hydrogenation of the double bond in
548 the acrylic acid followed by condensation with isopropylamine afforded the
549 propionamide **16**. Hydrogenation using Pd-C or Wilkinson’s catalyst under a hydrogen
550 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 [³H]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. IC₅₀
565 values of these compounds were approximately 10–20 nM, which was 10–20 times more
566 potent than the natural molting hormone 20E (IC₅₀ = 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 CF₂CF₃ (**9**), CF₂CF₂CF₃ (**10**), H (**11**), CH₃ (**12**), SCH₃ (**13**), S(=O)CH₃ (**14**),
578 and SO₂CH₃ (**15**). By introducing strong electron withdrawing fluorinated alkyl groups

579 such as CF₃, CF₂CF₃, and CF₂CF₂CF₃, 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)CH₃ and SO₂CH₃ did not enhance the activity. The
582 SCH₃ group enhanced the activity by 300-fold, although its electronic properties were
583 equivalent to H in terms of Hammett σ . The electron-donating CH₃ 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-AeA12). 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-AeA12, 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 pLD₅₀ 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 [$\text{ClogP}(\text{X}) - \text{ClogP}$
 612 (H)] to formulate statistically significant values using Eq. 1.

613

$$614 \quad \text{pIC}_{50} = 1.326 (\pm 0.772) \Delta\text{Clog P} + 6.162 (\pm 0.828) \quad (1)$$

$$615 \quad n = 8 \quad s = 0.930 \quad r = 0.864 \quad F_{1,6} = 17.688$$

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 $\Delta\text{Clog P}$ 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

$$628 \quad \text{pIC}_{50} = 1.519 (\pm 0.315) \Delta\text{Clog P} + 3.923 (\pm 1.643) F + 4.872 (\pm 0.631) \quad (2)$$

$$629 \quad n = 8 \quad s = 0.349 \quad r = 0.985 \quad F_{2,5} = 81.754$$

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.**

635

636

Table 2.

637

638 4. Discussion

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

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

660

661

Fig. 4

662

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. litura* 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 ($\log P_{\text{opt}} = 5.15$) 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 SCH₃ group showed relatively high binding activity
694 (pIC₅₀ = 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; pLD₅₀ = 6.27 vs. 7.32) and 100-fold less toxic than tebufenozide
698 against *S. exigua* (Noctuidae; pLD₅₀ = 4.91 vs. 7.06) [39]. The difference in pLD₅₀ 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 CF₂CF₃
704 and CF₂CF₂CF₃ groups showed higher receptor binding activity than ponasterone A
705 against Lepidoptera Sf-9 cells. The larvicidal activity of the CF₂CF₃ analog was
706 determined against *S. litura* larvae in terms of pLD₅₀. 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) $\text{CH}_2(\text{COOH})_2$, piperidine, pyridine

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

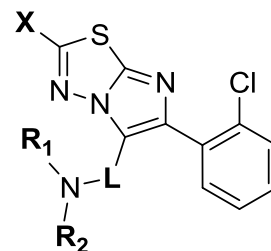
Scheme 4. Synthesis of sulfoxide **14** and sulfone **15** (a) *m*-CPBA, CH₂Cl₂; (b) Oxone[®], THF/MeOH/H₂O

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	ClogP
					Sf-9	NIAS-AeA12	BCIRL-Lepd-SL1	(mmol/insect)	
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	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 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)].

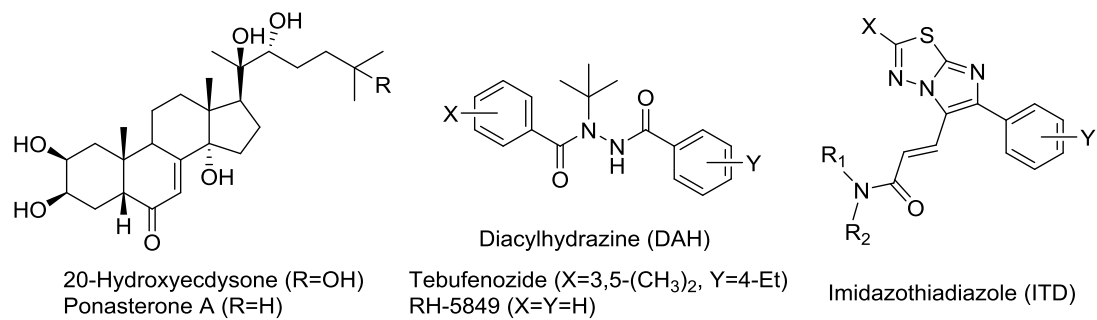


Fig. 1. Chemical structures of ecdysone agonists

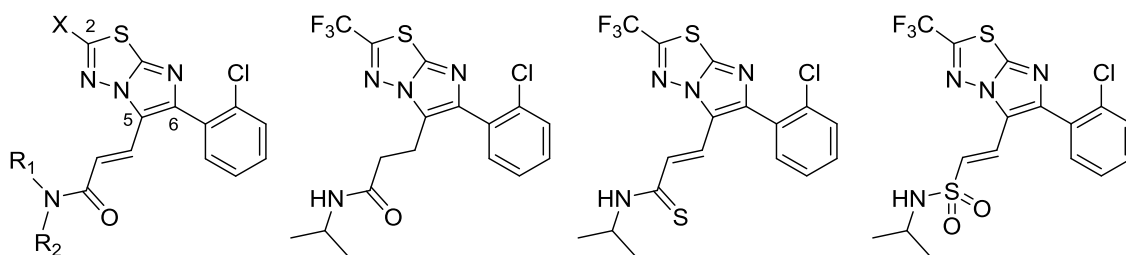


Fig. 2. Imidazothiadiazole-type compounds synthesized for SAR study

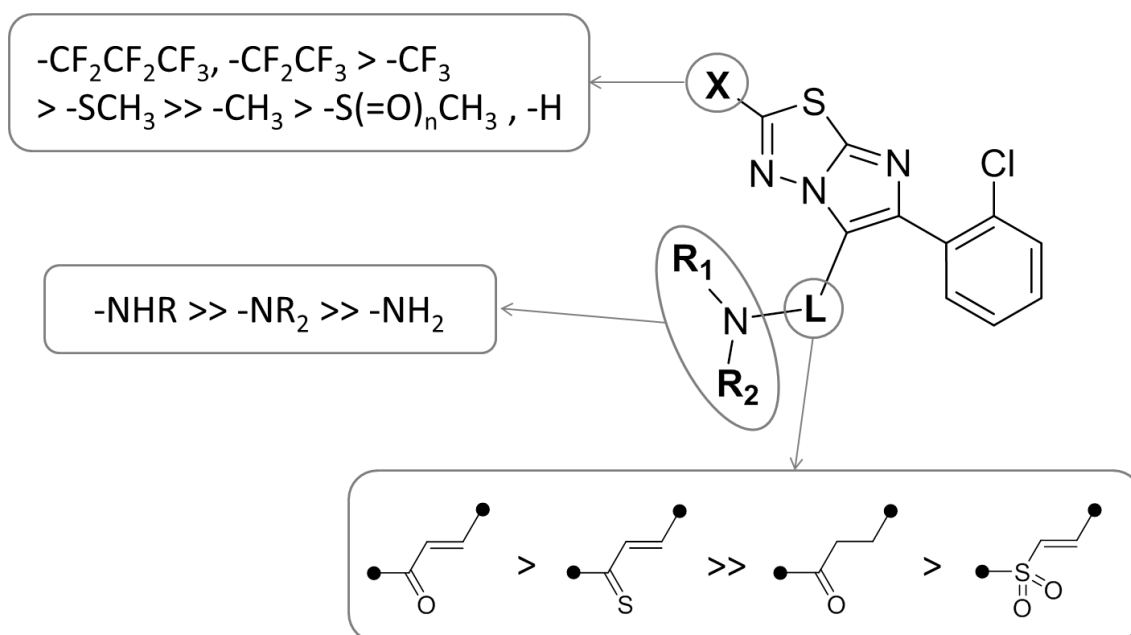


Fig. 3. Summary of structure-activity relationship for the binding activity against Sf-9

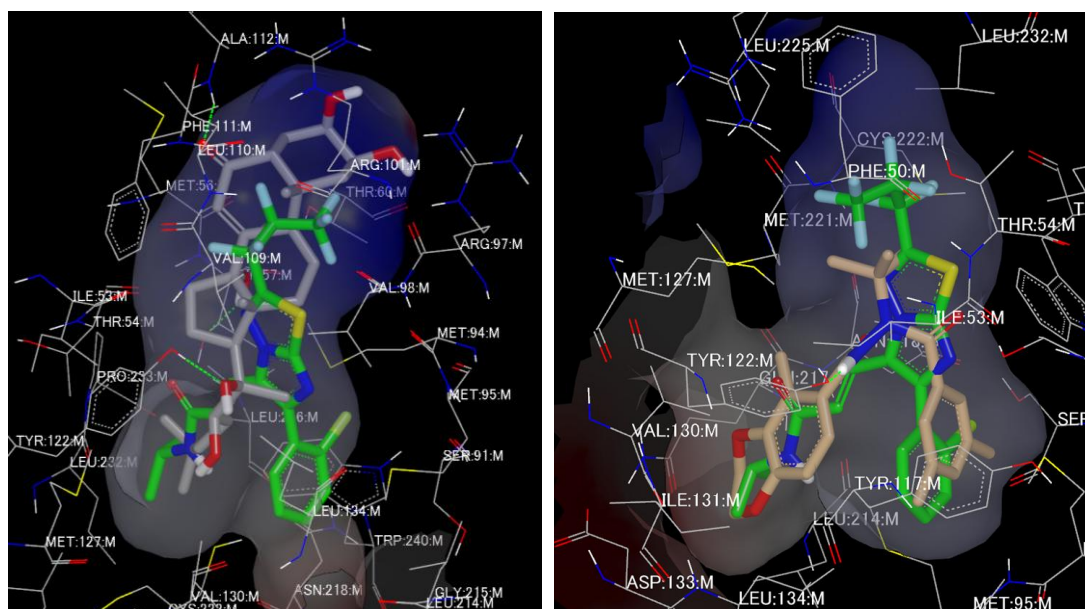
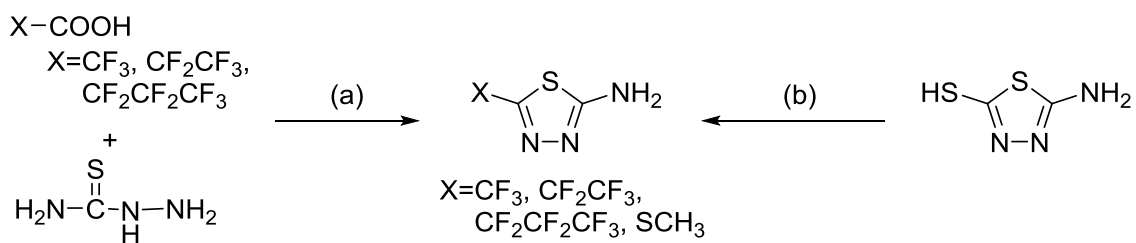


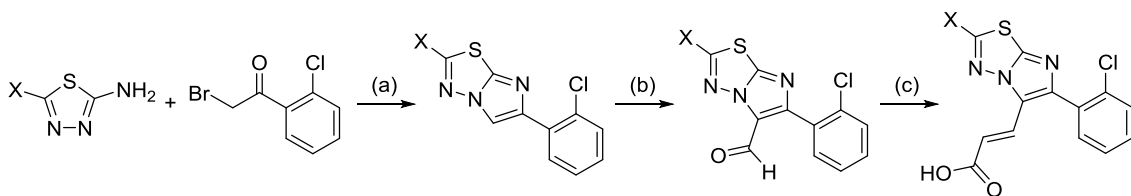
Fig. 4. Docking simulation of compound **10** (colored with green) against SfEcR bound to PonA (left) and DAH (BYI06833; right). Structure colored with gray is PonA and that colored with light brown is BYI06833. Ligand binding domains were modeled from 1R1K (left) and IR20 (right).



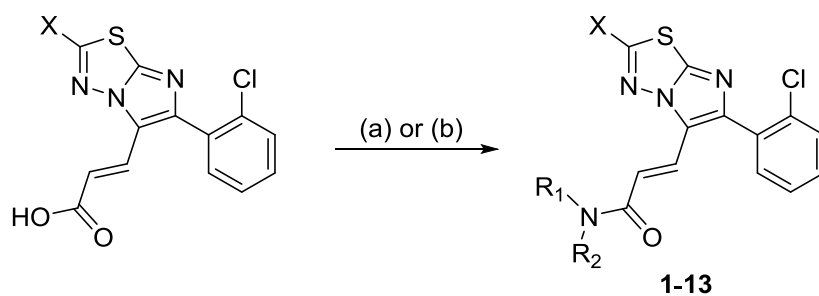
Fig. 5 *S. litura* Larva treated with compound **2** (10^{-5} mmol/insect)



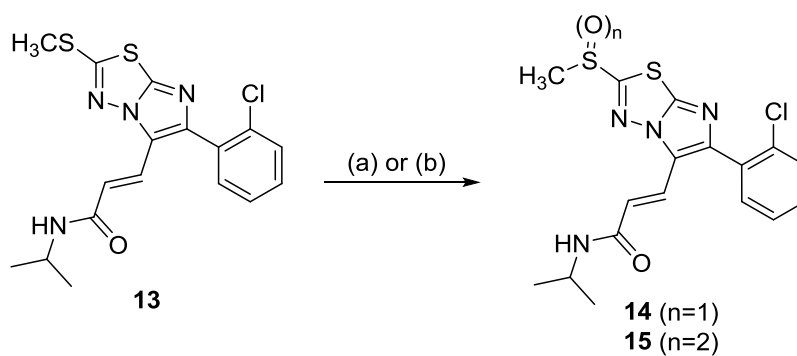
Scheme 1. Construction of 2-amino-1,3,4-thiadiazole moiety: (a) POCl_3 ; (b) CH_3I , KOH , 2-propanol/ H_2O



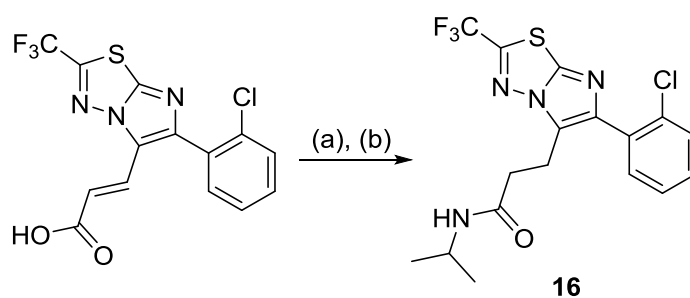
Scheme 2. Construction of (imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)acrylic acid moiety: (a) EtOH ; (b) POCl_3 , DMF ; (c) $\text{CH}_2(\text{COOH})_2$, piperidine, pyridine



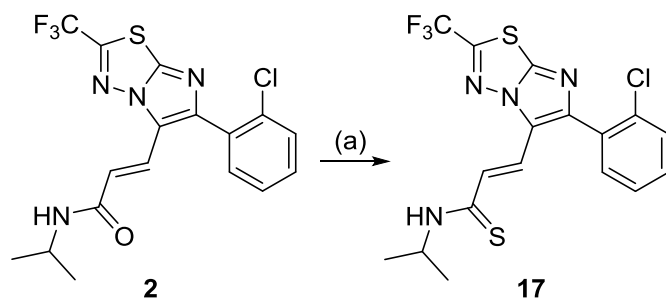
Scheme 3. Synthesis of (imidazo[2,1-*b*][1,3,4]thiadiazol-5-yl)acrylamides: (a) i. (COCl)₂, DMF, CH₂Cl₂ ii. NH₃ aq., CH₂Cl₂; (b) amine, EDC, DMAP, CH₂Cl₂



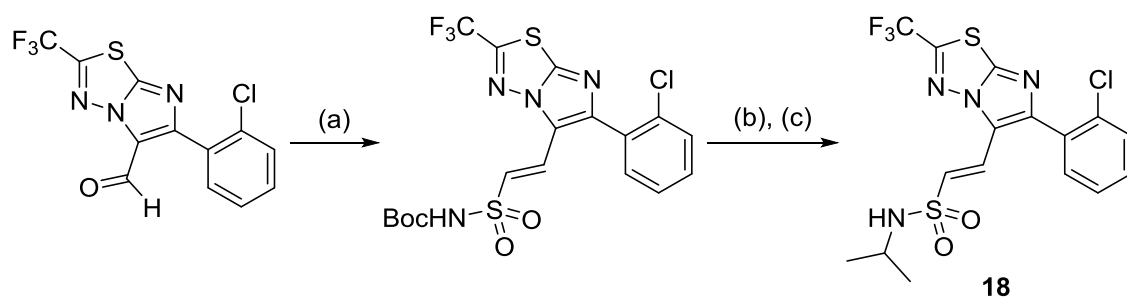
Scheme 4. Synthesis of sulfoxide **14** and sulfone **15**: (a) *m*-CPBA, CH₂Cl₂; (b) Oxone[®], THF/MeOH/H₂O



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