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

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ABSTRACT

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

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.

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-hydroxybenzamide [5], α-acylaminoketone [6], oxadiazoline [7], and γ-methylene-γ-lactam [8] have been described over the past two decades. In 2009, Holmwood and Schindler reported that imidazole (IMD) and imidazothiadiazole (ITD)-type compounds are ecdysone agonists (Fig. 2) [9]. Although the biological activity was evaluated quantitatively in terms of $pInd_{50}$ (EcR induction assay), experimental procedures and target insect species have not been described. Analytical data for the synthesized chemicals were not reported.

The binding mode of IMD-type compounds was reported to be similar to that of DAHs based on crystal structure analysis. The binding mode of ITD-type compounds is, however, thought to differ from those of DAHs and steroidal agonists such as ponasterone A

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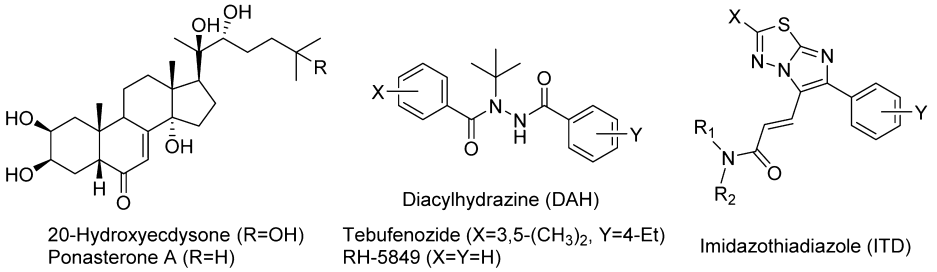


Fig. 1. Chemical structures of ecdysone agonists.

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

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

2.1.2. Synthesis of 2-amino-1,3,4-thiadiazoles (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 Na_2CO_3

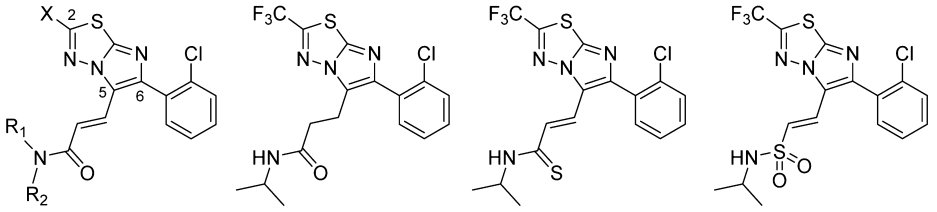
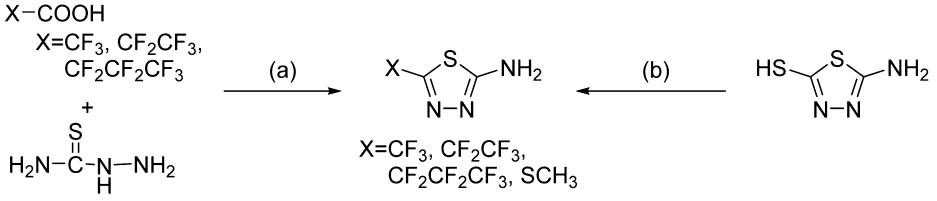


Fig. 2. Imidazothiadiazole-type compounds synthesized for SAR study.



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