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Virtual screening for the ligands of molting hormonal receptor

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1. Introduction

Insects grow by repeating molting and metamorphosis, which are regulated by the steroidal molting hormone, 20-hydroxyecdysone (20E; Fig.1). On the other hand, vertebrates do not have such physiological events, and therefore, 20E and its analogs have become candidates for safe insecticides. However, the low hydrophobicity of these steroidal compounds and the high synthetic cost prevented the development of ecdysteroids for practical use. In 1988, \(\text{N-tert-butyl-N',N'-dibenzoylhydrazines (DBHs)}\) (Fig.1) were discovered as molting hormonal agonists, which cause incomplete molting in insects leading to death. Then, a number of DBH analogs with various substituents at benzene rings were synthesized and the structure-activity relationship (SAR) studies have been performed. To date, four DBH compounds including tebufenozide, methoxyfenozide, chromafenozide and halofenozide have been commercialized. Even though 20E is commonly used as molting hormone in most of insects and SARs of ecdysteroids are similar among insects, SARs of non-steroidal ecdysone agonists varied among insect species. The reason for the difference of these SARs is disclosed by the three dimensional structure analysis of ligand-bound EcR. Ponasterone A (PonA), one of the most potent ecdysteroids, only partially occupy the binding pocket of a chromafenozide analog (BYI06830), meaning that the interaction between EcR and DBHs is species-specific.

Fig.1 The structures of a natural molting hormone and the agonists

In this study, we designed the novel ecdysone agonists based on the ecdysteroid structure, because SARs for ecdysteroids were supposed to be similar among various insects. The target structures were selected from the database using virtual screening methods, in which shape, electric charge distribution and configurations of functional groups were considered.

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2. Results and Discussion

Virtual screening

The molecular modeling softwares package comprised of OMEGA, ROCS and EON (OpenEye Scientific Software; Santa Fe) was used for virtual screening. In the first step, about 2.1 million compounds with the molecular weight ranging from 300 to 500 were selected from the structure database provided Namiki Shoji Co. Ltd. In the second step, each compound was submitted to OMEGA to find the conformations with local minimum energy. In the third step, the compounds with similar conformations to the structure of EcR-bound PonA were selected by ROCS using Shape Tanimoto scores. In the fourth step, the selected compounds were submitted to EON screening by the similarities of electrostatic potential distributions to PonA using Electrostatic Tanimoto scores. Finally, 24 commercially available compounds having similar positional relationships of functional groups with that of PonA were selected as candidates.

Binding assay

The activity was evaluated by measuring the inhibition of the incorporation of $[^{3}H]$ PonA into Lepidoptera Sf-9 cells and Coleoptera BCIRL-Lepd-SL1 cells. The concentrations required for 50% inhibition of the incorporation of $[^{3}H]$ PonA (IC$_{50}$) were determined for all compounds. Consequently, 3 compounds inhibited the incorporation by more than 50% at the concentration of 250 µM (Fig.2). The IC$_{50}$ values of these 3 compounds were 11, 74, 115 µM against Sf-9 cells and 28, 46, 250 µM against BCIRL-Lepd-SL1 cells, respectively. The potencies of these candidates are similar between Lepidoptera and Coleoptera being consistent with the similar SARs of ecdysteroids among various insect species.

Fig.2 Inhibitions of incorporation of PonA to Lepidoptera (A) and Coleoptera (B) cells

Number : inhibition (%)