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Original article

Target-based design, synthesis and biological activity of new pyrazole amide derivatives

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ABSTRACT

Based on the similarities in the conformation of VS008 (*N*-(4-methylphenyl)-3-(*tert*-butyl)-1-(phenylmethyl)-1*H*-pyrazole-5-carboxamide) and BYIO6830 (*N*′-(3,5-dimethylbenzoyl)-*N*′-*tert*-butyl-5-methyl-2,3-dihydro-1,4-benzodioxine-6-carbohydrazide) bound to the active site of the EcR subunit of the ecdysone receptor (EcR)-ultraspiracle protein (USP) heterodimeric receptor, a series of new pyrazole amide derivatives were designed and synthesized. Their structures were confirmed by IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. Results from a preliminary bioassay revealed that two of the pyrazole derivatives exhibited promising insecticidal activity. Specifically, compounds **6e** and **6i** exhibited good activity against *Helicoverpa armigera* (cotton bollworm) at low concentration. Symptoms displayed by tebufenozide-treated *H. armigera* were identical with those displayed by its treated counterpart. **6i** showed the same poisoning symptoms as those of tebufenozide. In addition, results from molecular docking result indicated that the binding modes of **6e** and **6i** at the active site of the EcR subunit of the heterodimeric receptor were similar to that of the bound tebufenozide.

1. Introduction

20-Hydroxyecdysone (20E), the molting hormone of several insects, initiates molting through the heterodimeric ecdysone receptor (EcR)-ultraspiracle protein (USP) receptor [1,2]. Design of other ligands targeting the EcR-USP heterodimer remains a prominent approach toward the development of environmentally benign insect growth regulators [3]. For example, non-steroidal ecdysone agonists, dibenzoylhydrazine insecticides (DBHs), bind to the EcR subunit of the heterodimer and induce the insect molting. These agents, which bear the common dibenzoylhydrazine core structure, exhibit an excellent activity against lepidopteran pests but have no effect on mammals and environment [4–7].

However, their relatively narrow spectrum of activity and emergence of DBH-resistant insects have prompted the efforts toward the design of compounds containing different structural motifs or identification of newer molecular targets. With the progress toward the structural resolution of target EcRs over last few decades, it is now possible to adopt a target structure-guided approach toward the design of new compounds with molting hormone activity. Such efforts have led to the identification of highly active and novel ecdysone agonists (I–IV, Fig. 1) in recent years [8,9].

Previously, using a technique of virtual screen against the EcR-USP crystal structure, we obtained a compound library and identified ligands that interact with the EcR subunit of the heterodimer. Analysis of the binding results revealed that the identified pyrazole-based compound VS008 (*N*-(4-methylphenyl)-3-(*tert*-butyl)-1-(phenylmethyl)-1*H*-pyrazole-5-carboxamide) has an active conformation that is similar to that of BYIO6830 (*N*′-(3,5-dimethylbenzoyl)-*N*′-*tert*-butyl-5-methyl-2,3-dihydro-1,4-benzodioxine-6-carbohydrazide), a DBH analog (Fig. 2), at the target site of the EcR subunit [10]. Inspired by these observations, a series of pyrazole amides based on the lead compound, VS008, were designed and synthesized in this study. Subsequently, their insecticidal activity against a few lepidopterans was evaluated. Furthermore, interactions between these newly synthesized compounds and the target site on the EcR subunit were also investigated by molecular docking.

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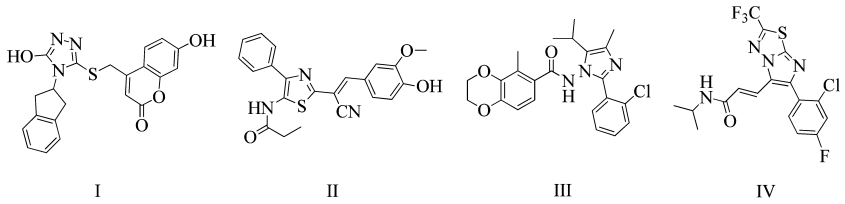

Fig. 1. Structures of new ecdysone agonists.

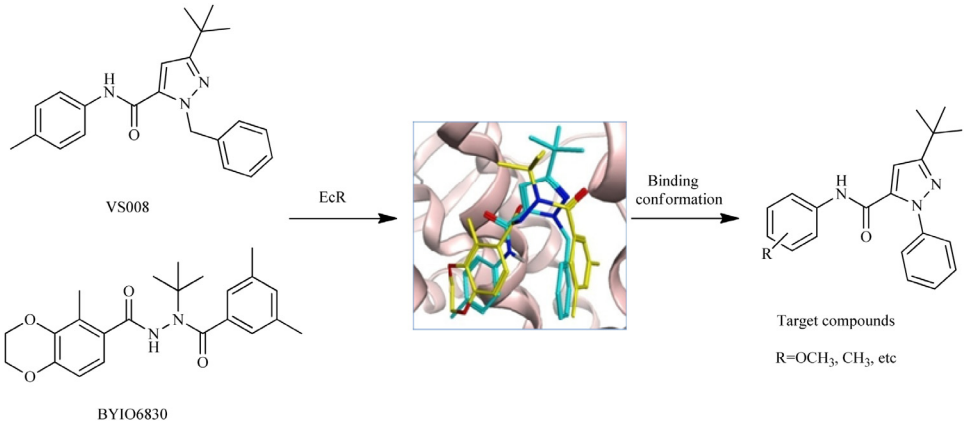

Fig. 2. The design strategy of target compound **6** based on the binding conformation of VS008 and BYIO6830 with EcR.

2. Experimental

Melting points of all compounds were determined on an X-5 binocular (Fukai Instrument Co., Beijing, China), and were not corrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-300 spectrometer with $CDCl_3$ as the solvent and TMS as the internal standard. Chemical shifts were reported in δ (parts per million) values. IR spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometor (KBr presser method). Elemental Analysis was obtained with an ST-Carloerba. Co instrument. All the reagents were obtained commercially and used after further purification. VS008 was bought from J&K Scientific. Column chromatography purification was carried out using sillca gel.

2.1. General procedure for synthesis of compounds *6a–p*

Pinacolone **1** (0.087 mol) and diethyl oxalate **2** (0.087 mol) were added dropwise to a solution of ethanol (55 mL) containing thinly sliced sodium (0.047 mol) at 0 °C. The mixture was stirred overnight at room temperature. Next morning, the mixture was acidified (pH 3.0, with 20% H_2SO_4) and filtered to remove the formed solid. The filtrate was extracted with dichloromethane, dried, and concentrated under vacuum to yield an orange red viscous liquid **3** (12.98 g; 74.6% y). A solution of the 1,3-diketone **3** (0.025 mol) in methanol (10 mL) was added dropwise to a cooled solution (0 °C) of hydrazinobenzene (0.025 mol) in methanol (30 mL). The mixture was warmed to room temperature by stirring for an hour and then refluxed for 2 h. The resulting cooled mixture was concentrated under vacumm and the pyrazole ester **4** was obtained after purification by column chromatography (a 5% gradient of ethyl acetate in hexanes over a column of silica gel). To saponify the ester, a solution of **4** (0.007 mol) was combined with an aliquot of 6 mol/L NaOH(aq) (7 mL) and the mixture was stirred at 80 °C. Ice water (50 mL) was added at the end of 2 h and the mixture was acidified (pH 1–2) with concentrated HCl. The formed solid was collected by filtration and the filter-cake was dried. The carboxylic acid was purified by recrystallization (methanol:water, 1:1) to afford **5** (3.57 g; 83.2% yield).

The amide derivatives **6a–p** were prepared through the acyl chlorides derived from **5**. A solution of **5** (0.004 mol) in thionyl chloride (10 mL) was refluxed for 5 h [11] and then concentrated under vacuum. The formed crude acyl chloride was added dropwise to a cooled solution (0 °C) of substituted aniline (0.004 mol) and TEA (0.008 mol) in dichloromethane (10 mL). The resulting mixture was stirred overnight at room temperature to produce the crude product, which was purified on a column of silica using a gradient of ethyl acetate in hexanes to afford the pure products **6a–p**.

2.2. Insecticidal test of target compounds *6a–p*

Their biological activities against *Mythimna separata*, *Helicoverpa armigera* and *Pyrausta nubilalis* were evaluated using the reference method [12,13]. The poisoning symptoms of *H. armigera* treated with **6i** were tested *using* the reference method [14].

2.3. Molecular docking

A Molecular Operating Environment (MOE) software [15] was used for the molecular docking. All synthesized compounds were built and optimized using the MMFF94 force field and charges. The low energy conformation of each compound was selected as the initial docking conformation. A crystal structure of ecdysone receptor (EcR) complexed with BYIO8346, which was obtained at 2.85 Å, was downloaded from the protein data bank (PDB ID: 3IXP). Waters and other solvent molecules (such as phosphatidylethanolamines) were removed and the modified structure was protonated. The active site was defined by the residues with a radius of 6 Å around BYIO8346. The ligand molecules were placed in the site with the Triangle Matcher method and ranked with the London dG scoring function. 30 Docking poses per ligand molecules were retained and further refined by energy minimization in the pocket. Then they were rescored with the GBVI/WSA dG scoring function, a force field-based scoring function, which was used to estimate the binding free energy of these ligands with EcR.