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Exploring the resistance-developing mutations on Ryanodine receptor in diamondback moth and binding mechanism of its activators using computational study

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ABSTRACT

Insect Ryanodine receptor (RyR) is an intracellular calcium release channels that play a key role in calcium signaling in numerous cell types. Targeting Ryanodine receptor is considered as efficient treatment option for the control of diamondback moth, *Plutella xylostella*, an important pest of cruciferous crops. The present study was carried out to identify potential RyR modulators through pharmacophore modeling and virtual screening. A total of 23 experimentally proven activators of RyR were used in the development of pharmacophore model. The resulting pharmacophore consisted of one hydrogen bond acceptor site (A), one hydrophobic feature site (H) and three aromatic ring sites (R). The model AHRRR was used as a query to find effective activators through database screening and AHRRR was validated to check its reliability using enrichment calculations. ADME properties were predicted to confirm the safety profile of the identified virtual hits. Furthermore, a structural modeling approach combining computational mutagenesis, induced fit docking, MM/GBSA and DFT calculations was used to evaluate the binding mode and structural basis of the two activators screened from pharmacophore-based virtual screening. Thus, the results could provide more knowledge on the activation of RyR and helpful in the development of more potent insecticides to overcome diamide insecticide resistance.

1. Introduction

Diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), is an extremely destructive pest of cruciferous crops of *Brassica olaracea* L., including cabbage, cauliflower, broccoli, brussels sprout and turnip. It has been causing an estimated global annual loss of US $1 billion in economic crops since the early 1990s and now it is estimated to cost the world economy US$4–5 billion annually on damage and management of *P. xylostella* [1,2]. According to Arthropod Pesticide Resistance Database in 2016, *P. xylostella* has developed robust resistance to at least 93 chemical and biological active pesticides due to intensive use or misuse of insecticides and its unique biological properties, including short life cycle, high fecundity and genetic plasticity [3]. In most cases, insecticide resistance is governed by increased metabolic detoxification, reduced uptake or penetration of the insecticides at the target site and alterations in the target site due to amino acid mutations [4,5]. Therefore, to address the issue of insecticide resistance, there is an urgent need for the development of new potent insecticides against biochemical targets of *P. xylostella.*

Insect Ryanodine receptors (RyR) are large tetrameric calcium release channel protein with a molecular mass of ~2–2.5 MDa that are located in the endoplasmic reticulum/sacroplasmic reticulum in nervous and muscle tissue [6]. The release of the universal intracellular messenger, calcium, from intracellular stores to the cytosol of muscle and non-muscle cells is modulated by the Ca^{2+} channels, including RyR. As a result of calcium release, many cellular processes, including hormone secretion, muscle contraction, neurotransmitter release and gene expression are mediated by RyR [7–9]. RyR is represented as a new potential insecticidal target to understand the integrated strategies for pest management, including insect toxicology and synthesis of novel insecticides [10]. Recently, many economically relevant compounds were developed

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to target RyR proteins in *P. xylostella.* The insecticides selectively bind to insect RyR in muscle cell and strongly interfere with an uncontrolled release of internal Ca^{2+} in the cell, and exhibiting feeding cessation, lethargy, muscle paralysis and eventually death of target insects [11,12].

In the year 2006, a new class of synthetic compounds, namely diamide, was successfully commercialized and introduced to the market [13]. Phthalic diamides [14] and anthranilic diamides [15] are the two major classes of synthetic diamides that are widely applied for the control of lepidopteran pests. Anthranilic diamides consist of two compounds namely, chlorantraniliprole [16,17] and cyantraniliprole [18]. The difference between chlorantraniliprole and cyantraniliprole is the substitution of the chlorine present at the 5th position of the phenyl moiety to a cyano group [19]. In anthranilic diamides, modifications are categorized into three substructures, including the anthraniloyl moiety, aliphatic amide moiety and *N*-pyridylpyrazole moiety [20]. More recently, a series of 23 novel acetamido derivatives containing *N*-pyridylpyrazole moiety were designed and synthesized against *P. xylostella* RyR by increasing the amide bridge via introduction of an acetamido moiety [21]. The diamide insecticides activate and regulate uncontrolled intracellular Ca^{2+} release to the cell through their favourable binding with insect RyR [22]. Thus, diamides have paid close attention in the designing of new compounds with better insecticidal activity.

Based on the literature background, the present work mainly focuses on the identification of potent activators of insect RyR in order to control *P. xylostella* through a combined computational method consisting of pharmacophore model development and virtual screening. Based on the structural features of 23 novel acetamido derivatives containing *N*-pyridylpyrazole carboxamides, a unique and effective pharmacophore model was developed. Pharmacophore-based virtual screening approach was utilized to identify potent RyR activators from an in-house database of organic compounds. The quality of the developed pharmacophore model was confirmed with enrichment factor calculations. The hit compounds identified from the pharmacophore screen were subsequently used for absorption, distribution, metabolism and excretion (ADME) and density functional theory (DFT) analysis in order to understand the pharmacological and chemical properties, respectively. In the absence of experimental structure of RyR, computational methods were used to predict the three dimensional structure to provide insight into the structure, function and binding mode of the protein. Further, computational mutagenesis carried out in the study may provide better understanding of two possible diamide binding site of RyR in *P. xylostella.*

2. Materials and methods

All computational analyses were carried out on a RedHat 6.2 (Santiago) running on Linux platform in High Performance Computer with Intel-Xeon 12 due processor (16-node) and 94.5 GB RAM.

2.1. Biological activity dataset

A dataset of 23 acetamido derivatives containing *N*-pyridylpyrazole carboxamides with wide range of experimentally known activators for RyR were collected from the literature [21]. Pharmacophore hypotheses generation has been established with this dataset. Two dimensional structures of all 23 reported compounds were constructed using ChemSketch v12.01 from ACD/Labs.

2.2. Preparation of ligands and generation of conformers

Conversion of 2D into 3D structures and minimization was performed using LigPrep v3.5 module incorporated in PHASE (Schrödinger, LLC, New York, 2015). The MacroModel torsional sampling method of ConfGen was used for conformational sampling to generate efficiently active conformers. Conformational energies were evaluated using the force field OPLS3 (pH 7.0) and the GB/SA solvation treatment. The conformers with maximum energy window of 10 kcal/mol were used to filter each minimized conformer from a set of conformers. A minimum root mean square deviation (RMSD) of 1.0 Å was used to eliminate the redundant conformers.

2.3. Creation of pharmacophore sites

Pharmacophore hypotheses were generated using the PHASE module implemented in Schrödinger. The available six built-in types of pharmacophore features, including hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic region (H), negative charged group (N), positively charged group (P) and aromatic ring (R) were defined by a set of chemical structural patterns using SMARTS queries. The conformational set used to generate sites for the development of pharmacophore model was included with these six pharmacophore features.

2.4. Finding common pharmacophore and scoring hypotheses

Each pharmacophore is represented by a set of points in 3D space that communicate with different chemical features to facilitate non covalent binding between the ligand and receptor. The terminal box size of 1 Å was used to generate common pharmacophore hypotheses (CPH) after applying default feature definitions to each ligand. Based on the tree-based partitioning algorithm and pharmacophoric features, possible CPH were generated and ranked considering their best correlation with the three dimensional spatial arrangement of chemical features mapping on the imported dataset consist of 23 known active compounds of RyR (acetamido derivatives). CPH were scored by providing an overall maximum RMSD of 1.2 Å and vector score value to 0.5. Further, the best CPH was selected based on the survival score.

2.5. Validation of the pharmacophore model

The selected pharmacophore model was validated using successive Guner-Henry scoring method. This method was used to evaluate the reliability and accuracy of the generated pharmacophore model. For validating the pharmacophore model, an initial ligand decoy set of 1000 drug-like compounds with an average molecular weight of 360 Da was downloaded from Schrödinger website. All the compounds screened based on the fitness score were mixed with 1000 decoy set of molecules to develop the database. A database screening was carried out using "PHASE find matches" option available in PHASE module. A set of statistical parameters, including enrichment factor (EF) and goodness of hit score (GH) were calculated to predict the selectivity of the model and accuracy of screened hits.

2.6. Database screening

Pharmacophore-based virtual screening is used to search for molecules that are similar to the chemical structures of known lead molecules in large libraries of chemical compounds. In the present study, the best pharmacophore model was used as 3D search query for the retrieval of potent molecules from ChemBridge chemical