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Original article

Potential virtual lead identification in the discovery of renin inhibitors: Application of ligand and structure-based pharmacophore modeling approaches

Sundarapandian Thangapandian, Shalini John, Sugunadevi Sakkiah, Keun Woo Lee*

Division of Applied Life Science (BK21 Program), Plant Molecular Biology and Biotechnology Research Center (PMBBRC), Gyeongsang National University (GNU), 900 Gazwa-dong, Jinju 660-701, Republic of Korea

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ABSTRACT

Renin, an enzyme by cleaving angiotensinogen to angiotensin-I, controls the first and rate-limiting step of renin-angiotensin system that is associated with blood pressure. Thus Ligand and structure-based pharmacophore models were developed in this study to identify new potential leads inhibiting this rate-limiting enzyme as an efficient way to treat blood pressure. X-ray predicted binding modes of most active compounds were used in ligand-based approach whereas the 3D structural information of renin was used in structure-based approach. Pharmacophore models were validated using various methods and utilized in database searching to identify potential hits. Drug-like filters and molecular docking studies led us identifying the final hits to be employed in designing new class of future renin inhibitors.

1. Introduction

Renin is one of the members from the family of aspartic proteases that includes other enzymes such as pepsin, cathepsin and chymosin etc [1]. Renin is known to exist in two forms, the inactive proenzyme prorenin and active mature renin. Prorenin is transformed into mature renin by the cleavage of its prosegment, which is made of 43 amino acids. Renin is a monospecific enzyme that displays remarkable specificity to its only known substrate, angiotensinogen and making it an ideal target to block renin-angiotensin system (RAS) [2] and thereby to treat high blood pressure. When blood pressure falls, the kidneys undergo several reactions converting prorenin to renin. Renin controls the first and rate-limiting step of the RAS and cleaves angiotensinogen to inactive decapeptide angiotensin-I, which is subsequently converted to angiotensin-II by the action of angiotensin-converting enzyme (ACE) [3]. This angiotensin-II finally causes the release of aldosterone, which is known for its pressor responses, from the adrenal glands. Unlike renin, ACE is not specific toward the conversion of angiotensin-I to angiotensin-II. It also has effects on a number of other peptides including bradykinin and thus making itself not suitable for specific RAS inhibition. Thus potent inhibitors of renin enzyme could therefore provide a new alternative way to treat high blood pressure without inhibiting other biological substances and thereby with no side effects. Renin enzyme is 406 amino acids long and containing two homologous lobes with an active site at the interface. Two aspartic acid residues, one located in each lobe of the renin molecule, are essential for the proteolytic mechanism of the enzyme. The active site of renin can accommodate seven amino acid units of the substrate, angiotensinogen, and cleaves the peptide bond between Leu10-Val11 within angiotensinogen to generate angiotensin-I [4]. The very early renin inhibition attempts were based on antibodies developed against renin [5,6]. Immunological inhibition of renin reduced blood pressure in volume-depleted normotensive marmosets and provided the proof of concept of renin inhibition [7]. The first synthetic renin inhibitor was pepstatin. First-generation renin inhibitors were peptide analogs of the prosegment of renin or substrate analogs of the amino-terminal sequence of angiotensinogen containing the renin cleavage site [8–10]. These inhibitors were not orally active and had to be given parenterally [10,11]. Further chemical modifications led to the development of compounds with high stability and longer duration of action [12]. In late 1980s, high molecular weight orally active renin inhibitors including enalkiren and remikiren were developed [13,14].

Abbreviations: RAS, renin-angiotensinogen system; ACE, angiotensin-converting enzyme; PDB, protein data bank; DS, discovery studio; Pharm-A, pharmacophore A; HA, hydrogen bond acceptor; HD, hydrogen bond donor; HY, hydrophobic; Pharm-B, pharmacophore B; E, enrichment factor; GOLD, genetic optimization for ligand docking; RMSD, root mean square deviation; PI, positive ionizable; RA, ring aromatic; ADMET, absorption, distribution, metabolism, excretion and toxicity.

* Corresponding author.
E-mail address: kwlee@gnu.ac.kr (K.W. Lee).

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Upon oral administration they have shown the bioavailability of less than 2%, a short half-life and weak anti-hypertensive activity [15]. Very recently, some functional foods and nutraceuticals were used to lower the blood pressure where the mechanism is mediated by inhibition and down-regulation of expression of ACE and renin [16]. As a result of structure-based drug design, aliskiren was discovered by Ciba–Geigy, Novartis, Basel, Switzerland but the synthetic pathway had many steps and was not suitable for industrial manufacture [17]. This difficulty in industrial synthesis of aliskiren was overcome by Speedel AG by designing a cost-effective method of production [18]. Variety of renin inhibitors were designed after the success of aliskiren. These set of inhibitors, for which aliskiren is a prototype, is only the fourth class of drugs to lower the blood pressure by blocking the RAS. Previously existing classes are beta blockers, ACE inhibitors and angiotensin receptor blockers. Further ligand and structure-based studies may bring out new classes of chemical compounds as future and potent renin inhibitors.

Computer-aided drug design methodologies such as ligand and structure-based pharmacophore modeling combined with molecular docking were employed to identify chemical compounds with a potential to block renin enzyme that is involved in the generation of hypertensive peptides from angiotensinogen. Newly identified chemical compounds, in this study, can be utilized in the designing of novel anti-hypertensive agents, eventually.

2. Materials and methods

2.1. Generation of pharmacophore models: common feature based approach

Common feature based pharmacophore modeling is performed with a set of highly active inhibitors of a particular target considering their chemical groups as pharmacologically important components for their activity. Experimentally evaluated highly active renin inhibitors whose binding conformations are known at renin's active site, crystallographically, are used in pharmacophore model generation. Thirty-seven crystal structures were determined, released and available in protein data bank (PDB) for human renin at different resolutions, to date. Among them, 34 crystal structures are complexed with different small molecule inhibitors with different renin inhibitory activity values ranging from 0.16 nM to 6560 nM of IC_{50} values [19,20]. Twelve highly active co-crystallized inhibitors, of these 34 crystal structures, with the IC_{50} values less than 50 nM were chosen based on their chemical structure and activity values to be used as training set compounds. Training set selection is considered most important for the quality of automatically generated pharmacophore models. All the 12 inhibitors were extracted from the active site of renin and their bond orders were corrected in Accelrys Discovery Studio 2.5 (DS) software and represented in Fig. 1. Diverse conformations generation, which is the initial step of any conventional ligand-based pharmacophore modeling methodology in order to cover the possible biological conformation, was not performed in this study since the training set compounds are already at their crystallographically determined biological conformations. *Feature Mapping* protocol as available in DS was used to identify the inherent features present in the training set compounds. *Minimum Inter-feature Distance* value was set to 2 Å from the default value of 2.97 Å, so that the functional groups located close to each other in the distance of 2 Å were also considered during pharmacophore generation. *Principal* and *Max-OmitFeat* values of 2 and 0, respectively, were set to all compounds in the training set as it includes only most active compounds. All other control parameters were kept at their default values. Ten pharmacophore models were generated using *Common Feature*

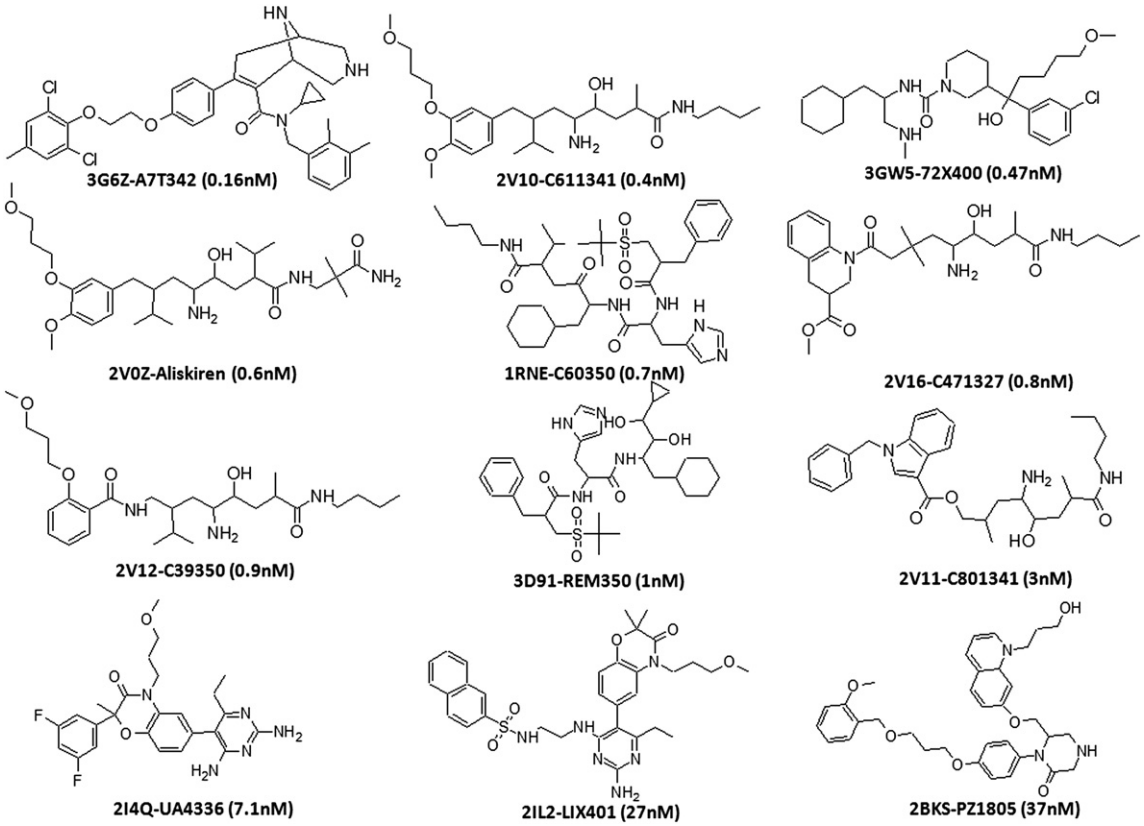

Fig. 1. Training set compounds employed in common feature pharmacophore generation are displayed with their PDB code, inhibitor name and IC_{50} values in parenthesis.