European Journal of Medicinal Chemistry 46 (2011) 2469–2476

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

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

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ARTICLE INFO

Article history:
Received 23 November 2010
Received in revised form
15 March 2011
Accepted 16 March 2011
Available online 25 March 2011

Keywords:
Ligand and structure-based pharmacophore
Renin
Blood pressure
Database screening
Molecular docking

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.

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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.

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doi:10.1016/j.ejmech.2011.03.035

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*

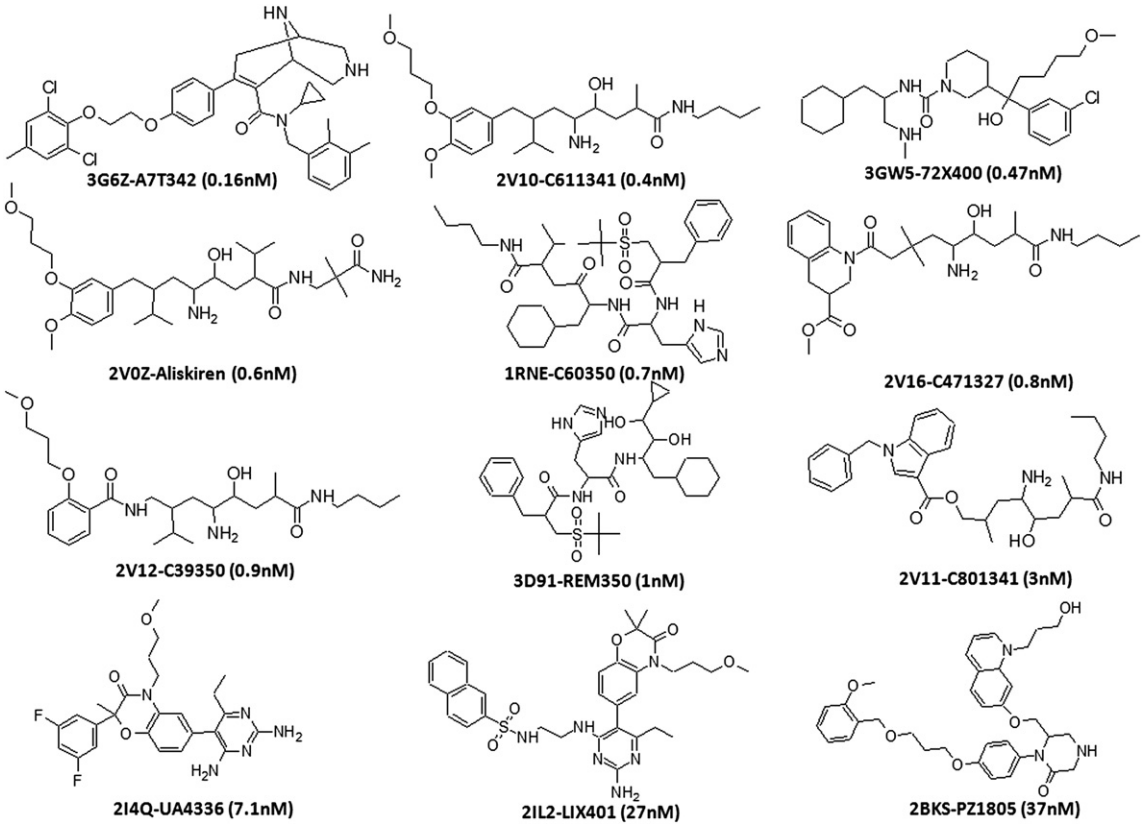

Fig. 1. Training set compounds employed in common feature pharmacophore generation are displayed with their PDB code, inhibitor name and IC_{50} values in parenthesis.

Table 1
Results of the common feature pharmacophore generation calculation.

Pharmacophore Hypotheses	Features[a]	Rank[b]	Direct Hit	Partial Hit
1	HHDA	112.017	111111111111	000000000000
2	HHAA	109.206	111111111111	000000000000
3	HHDA	108.471	111111111111	000000000000
4	HHAA	107.242	111111111111	000000000000
5	HHDA	106.326	111111111111	000000000000
6	HHDA	106.085	111111111111	000000000000
7	HHDA	106.062	111111111111	000000000000
8	HHAA	105.329	111111111111	000000000000
9	HHAA	103.788	111111111111	000000000000
10	HHAA	103.369	111111111111	000000000000

[a] H, hydrophobic; A, hydrogen bond acceptor; D, hydrogen bond donor.
[b] The higher the rank score the less likely that the training set compounds fit the pharmacophore by chance correlation.

Pharmacophore Generation protocol selecting the pharmacophoric features identified by feature mapping study. The best common feature pharmacophore model (Pharm-A) was selected based on the ranking score, best-fit values and the interaction points available at the active site of renin.

2.2. Generation of pharmacophore models: structure-based approach

Structure-based pharmacophore modeling has been handled successfully by various pharmaceutical companies in designing novel drugs with potent biological activity in many therapeutic areas. This strategy can effectively be used where there is insufficient information on ligands (inhibitors or activators) that are experimentally proved to block or induce the activity of a particular therapeutic target. It can also be used to extract more information from the receptor side which can enable a medicinal chemist to have a deeper insight and design new set of ligands that would turn as potent drugs eventually [20]. In this study, a crystal structure of renin complexed with a drug molecule, aliskiren, (PDB code: 2V0Z) crystallized at 2.2 Å resolution [21] was employed to generate structure-based pharmacophore model. This aliskiren-bound renin structure was selected to be used in pharmacophore generation after superimposing two other structures (PDB codes 3G6Z and 3GW5) bound with other inhibitors with potent renin inhibition. The superimposition of the structures has shown that all of the catalytically important residues including two aspartates were spotted at same positions (Fig. S1). It has been reported already that two water molecules present in the active site of renin along with the catalytically important amino acid residues are important for the interaction of this drug molecule, aliskiren [22]. Thus, a sphere comprising two active site water molecules and residues that are within 6 Å distance from the drug molecule, aliskiren, was generated using *Binding Site* tool available in DS. Employing *Interaction Generation* protocol of DS, complimentary pharmacophoric features corresponding to all the possible interaction points available at the active site were generated. This protocol identifies hydrophilic features such as hydrogen bond acceptor (HA) and donor (HD) and lipophilic features such as hydrophobic (HY) features based on the active site residues that are present inside the created sphere. Finally *Edit and Cluster pharmacophores* tool as available in DS was utilized to edit the redundant and pharmacophoric features generated with no catalytic importance. Only the representative pharmacophoric features with catalytic importance were selected. The final structure-based pharmacophore model (Pharm-B) comprises the most important pharmacophoric features that necessarily involve in inhibitor binding.

Table 2
Best-fit values of the training set compounds based on Pharm-A and Pharm-B.

PDB ID	Inhibitor Name	IC_{50} nM	Fit Value	
			Pharm-A	Pharm-B
3G6Z	A7T342	0.16	4.000	3.683
2V10	C611341	0.4	2.801	3.462
3GW5	72X400	0.47	3.206	3.186
2V0Z	Aliskiren	0.6	2.866	3.260
1RNE	C60350	0.7	1.784	3.700
2V16	C471327	0.8	2.883	3.434
2V12	C39350	0.9	2.360	3.352
3D91	REM350	1	2.415	3.301
2V11	C801341	3	1.427	3.213
2I4Q	UA4336	7.1	2.178	1.996
2IL2	LIX401	27	1.899	2.231
2BKS	PZ1805	37	1.129	1.359

2.3. Validation of pharmacophore models

For the validation of generated pharmacophore models, a database containing active and inactive compounds was prepared obtaining compounds from the literature resources such as binding database and patents. The purpose of this validation procedure is to investigate the capability of a pharmacophore model to identify only the active compounds rather than in actives. *Ligand Pharmacophore Mapping* protocol available in DS with *BEST flexible conformation search* option was used in this validation to screen the compounds that fit the features in the pharmacophore models. Enrichment factor (E) value was calculated to determine the validity of the generated pharmacophore models. The E-value is calculated using the following formula $E = [(Ha \times D)/(Ht \times A)]$, where D, A, Ht and Ha represent the total number of compounds of the database, total number of actives, total number compounds screened by a pharmacophore model and total number of active compounds screened, respectively.

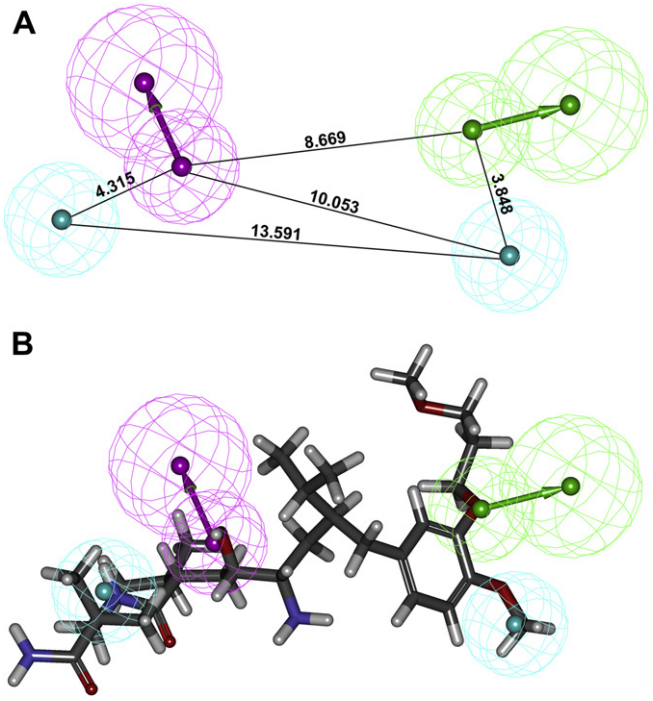

Fig. 2. Pharm-A and its overlay with a training set compound. (A) Chemical features of Pharm-A with their inter-feature distance constraints. (B) Aliskiren, one of the training set compounds, overlaid on Pharm-A hypothesis. Color coding: green - HA; pink - HD; cyan - HY. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

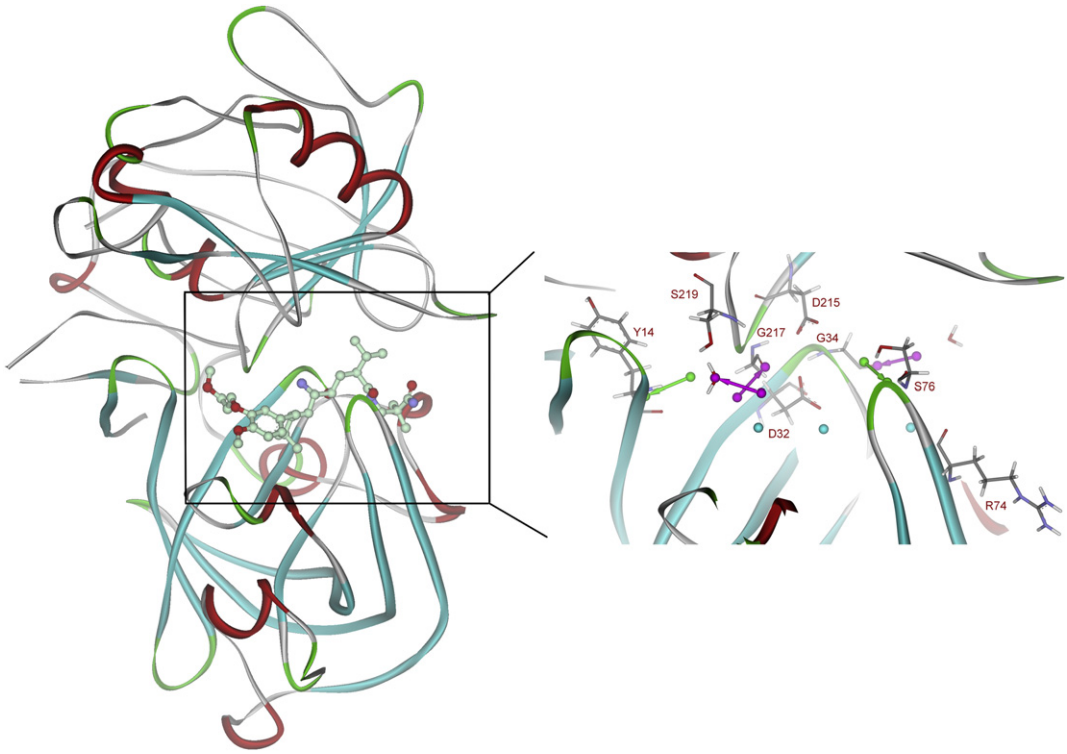

Fig. 3. Full and zoomed view of 3D structure of renin bound with aliskiren (PDB code 2V0Z). Two lobes that make renin enzyme are shown with aliskiren bound at the interface (ball and stick mode). The zoomed view shows the important active site residues (stick form in element color) along with the generated pharmacophoric features (small spheres). Color coding: green, HA; magenta, HD and cyan, HY features. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.4. Database screening

The generated pharmacophore models based on two different methodologies were used as 3D queries to search chemical databases of commercially available chemical compounds. Database screening serves the purpose of finding diverse, potential virtual leads suitable for further optimization and provides the preference of having easily available and/or synthesizable compounds as hits for further steps in drug development. A molecule of the database must map all the pharmacophoric features of a pharmacophore model to be identified as a hit. All the screening processes were performed using *Ligand Pharmacophore mapping* protocol with *Best Flexible conformation Search* method. The identified hits from the databases were subjected to various drug-like screening filters to select only the compounds with drug-like properties rejecting the compounds with non drug-like properties. Compounds identified with drug-like properties through these screenings were considered in molecular docking study.

2.5. Molecular docking

Molecular docking experiments were performed using GOLD (Genetic Optimization for Ligand Docking) program version 4.1. GOLD is an automated docking program that uses genetic algorithm to explore the ligand conformational flexibility with partial flexibility of protein's active site [23]. The algorithm was tested on a data set of over 300 complexes extracted from the PDB. GOLD succeeded in more than 70% cases in reproducing the experimentally bound conformation of the ligand [24]. 2V0Z, a crystal structure of human renin bound with aliskiren, was used as protein molecule. All the water molecules present in the protein except two water molecules that mediate the hydrogen bond interactions with inhibitor, aliskiren, were removed and hydrogen atoms were added to the protein structure. A binding site was created with the radius of 10 Å

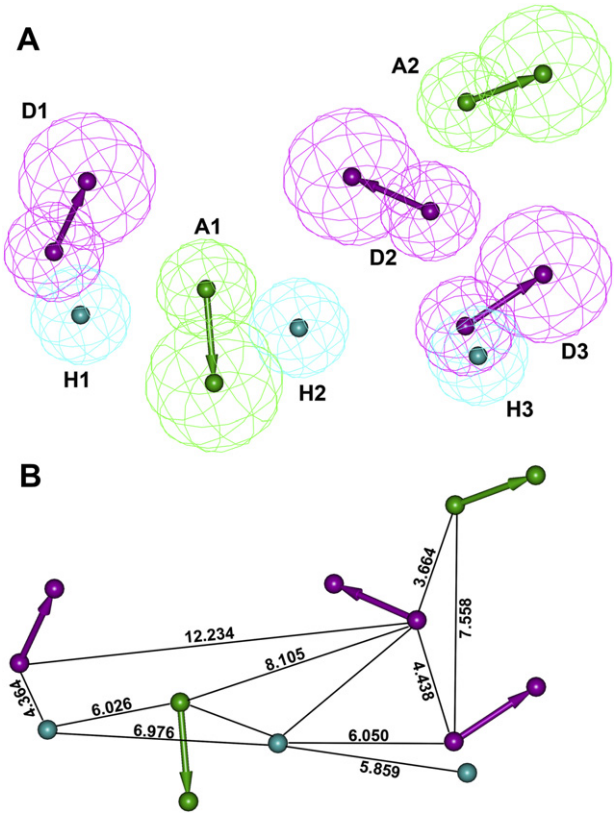

Fig. 4. Structure-based pharmacophore model, Pharm-B. (A) Arrangement of pharmacophoric features of Pharm-B. H, A and D represent HY, HA and HD features, respectively. (B) Pharmacophoric features are shown with inter-feature distance constraints. Feature cages are hidden for clear view.

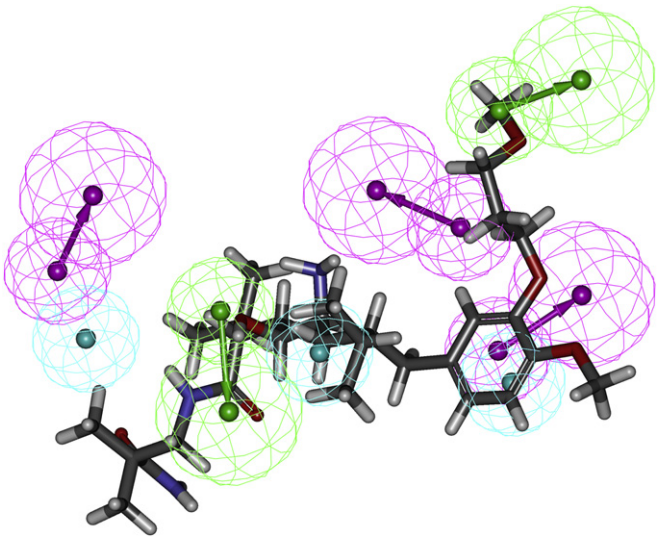

Fig. 5. Pharm-B and aliskiren overlay. Aliskiren mapped four of eight pharmacophoric features of Pharm-B.

around the ligand present in the crystal structure. All hit molecules along with training set were docked into the created binding site. A value for *Maximum save conformations* was set to 10 and if the RMSD between any of the 5 docked conformations is less than 1.5 Å it was set to quit the genetic algorithm or move on to the next compound in the list of compounds given for docking. All other options were kept default during docking experiments. At the end of each run GOLD separates and ranks all the docked conformations based on the fitness score. The fitness score function that is implemented in GOLD consists of H-bonding, complex energy and the ligand internal energy terms. The final hits were selected based on the binding mode and molecular interactions observed at the active site. Finally the novelty of the selected compounds was confirmed using *Scifinder Scholar* [25] and *Pubchem* structure search [26] tools.

3. Results and discussion

3.1. Common feature pharmacophore model

The training set compounds used in pharmacophore generation comprised twelve most active diverse compounds in terms of their chemical structures. The crystallographically determined biological conformation of every compound was used in this study without generating diverse conformers. The features selected for this run were HA, HD, positive ionizable (PI), ring aromatic (RA), and HY. Ten common feature pharmacophore models were generated. All the resulted pharmacophore models were made of four features containing two HY, one or two HA and one or no HD features (Table 1). All the generated pharmacophore models could fit all the training set compounds. The rank scores ranged from 112.017 to 103.369 between the generated pharmacophore models. As given in Table 1, all the generated pharmacophore models had similar pharmacophoric features and ranking scores. In order to choose the best model, fit values of the training set compounds based on the generated pharmacophore hypotheses were compared. In this analysis, first hypothesis has shown highly correlating calculated best-fit values and thus was selected as the best model (Pharm-A) (Table 2). The direct and partial hit values indicate whether or not a molecule in the training set mapped every feature of the pharmacophore hypothesis (Table 1). The best hypothesis Pharm-A possesses two HY and each of HA and HD features (Fig. 2A). Every training set compound has mapped all pharmacophoric features of Pharm-A. For instance, the central hydroxyl group and the long ether moiety attached to the phenyl ring of aliskiren, a well-known drug candidate which is in active development, were mapped on the HD and HA features of Pharm-A whereas the two methyl groups present in the sides mapped over the HY features (Fig. 2B). The importance of the generated pharmacophoric features was analyzed based on the essential active site amino acids comparing with the LigPlot graph obtained from PDBsum for the PDB structure with a code 2V0Z [27]. The HA and HD features of Pharm-A were generated to interact with two important aspartic acid (D32 and D215) and a serine (S219) or tyrosine (Y14) residues in the active

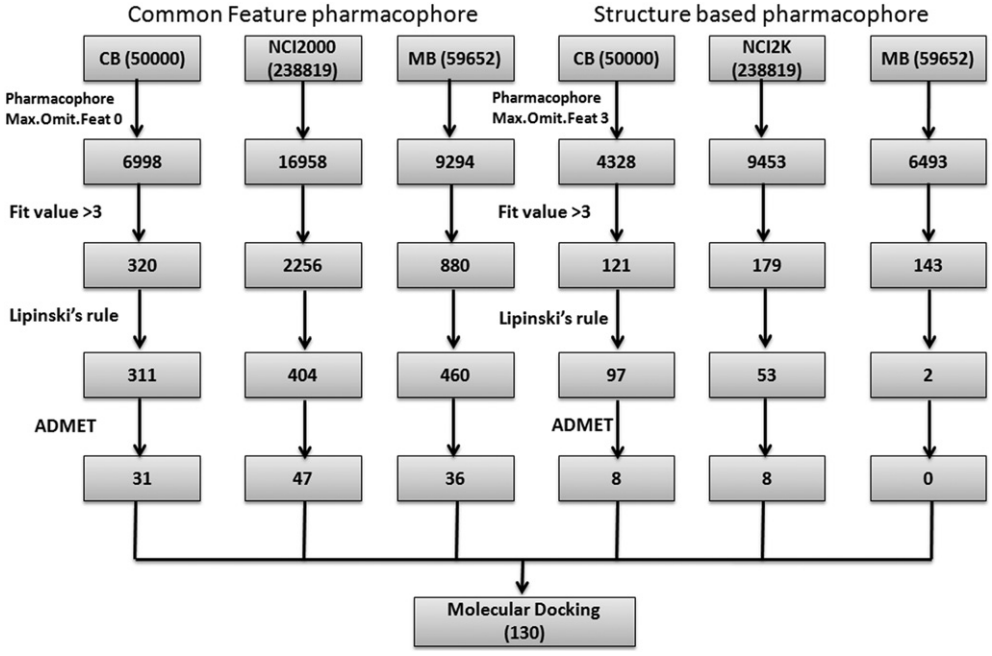

Fig. 6. Database screening of three databases using Pharm-A and Pharm-B models.

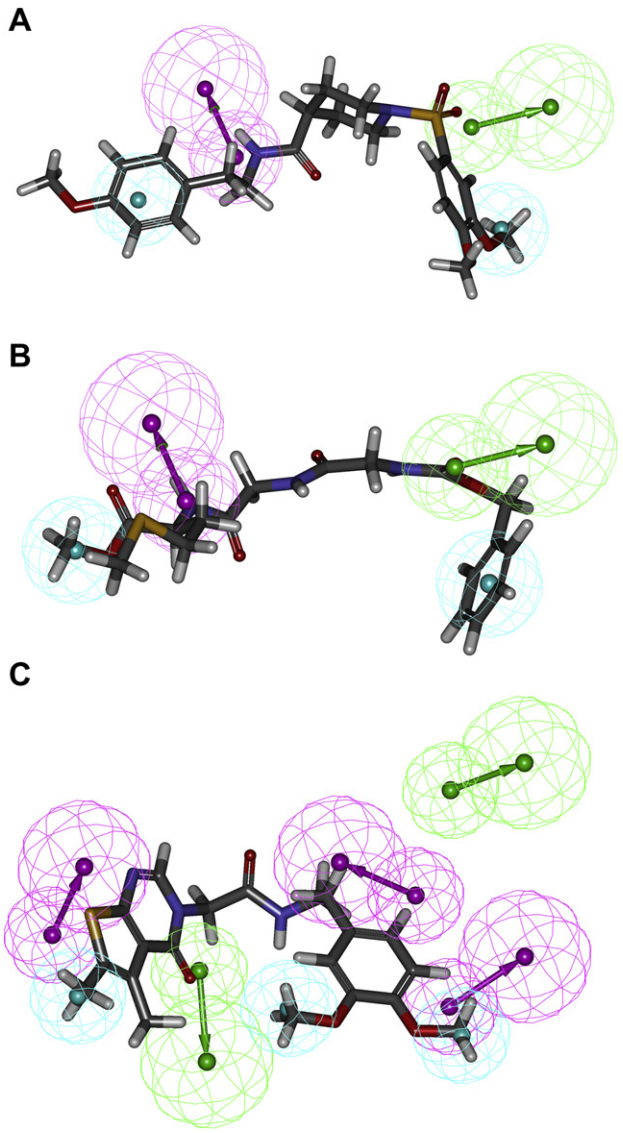

Fig. 7. Pharmacophore hypotheses and database hits overlay. Overlay of (A) compound 20060 and (B) NCI 0169134 upon Pharm-A hypothesis. (C) Overlay of compound 24015 upon Pharm-B hypothesis.

site. Two HY features were closely located to the important F117 and Y75 residues at the active site.

3.2. Structure-based pharmacophore model

This pharmacophore model was generated based on the lipophilic and hydrophilic interaction points available from the active site of renin. The very important interactions were considered during the development of this structure-based pharmacophore model (Fig. 3). Two water molecules interacted from the sides in aliskiren binding were also considered in pharmacophore development. The final structure-based pharmacophore hypothesis (Pharm-B) comprised eight pharmacophoric features generated as complimentary features to Y14, D32, R74, S76, D215, G217, S219 and one of the two active site water molecules (Figs. 3 and 4). The compounds mapping on some of these identified features can have the potential to inhibit the enzyme with high affinity. Twelve training set compounds used in the generation of common feature pharmacophore model were employed to check whether the structure-based pharmacophore model, Pharm-B, possess some of the common chemical features of known active compounds. The fit values were calculated for these compounds and observed to correlate well with biological activities (Table 2). All of the training set compounds have mapped with any four of the eight pharmacophoric features of the structure-based pharmacophore model. Aliskiren, one of the training set compounds, has mapped with two HY (HY2 and HY3) and two HA features of Pharm-B (Fig. 5).

3.3. Validation of pharmacophore models

A set of 140 known renin inhibitors was used in a primary validation to evaluate the presence of common chemical features that are already present in known renin inhibitors. Of 140 compounds, 131 compounds mapped on all the features of Pharm-A with hit rate of 93.57% whereas Pharm-B has achieved a hit rate of 92.14% (129 of 140) mapping any four of its features. In addition to this, a database containing 1386 compounds including 140 renin inhibitors and inactive compounds was used to validate the capability of the pharmacophore models to identify the renin inhibitors among inactive compounds. *Ligand Pharmacophore Mapping* protocol was used to perform this validation procedure. E-value was calculated for both pharmacophore models, Pharm-A and Pharm-B, using this database. Pharm-A has shown an E-value of 8.208 indicating the probability to identify 8 active compounds than an inactive compound whereas Pharm-B has shown 6.941 with the probability to pick around 7 active compounds rather than an inactive compound. These results have proven the ability of the generated pharmacophore models to identify the actives from inactives. Thus, these pharmacophore models were utilized in database screening to retrieve compounds with new chemical scaffolds to use them in future drug designing.

3.4. Database screening

Three chemical databases, namely, Maybridge, Chembridge and NCI2000 containing diverse chemical compounds were screened using both the generated pharmacophore models as 3D queries employing *Ligand Pharmacophore Mapping* protocol available in DS with *Best Flexible* search option. A compound has to map all the pharmacophoric features of Pharm-A to be identified as hits. This rule was relaxed to any five out of eight features for screening with Pharm-B as this pharmacophore contains eight features which are too large for a single small molecule to possess. The compounds retrieved from the database were subjected to various screenings based on fit value, drug-likeness prediction using Lipinski's rule of five and ADMET properties. Fit value filter was fixed based on the fit values of the most active compound in the training set. All screening experiments were performed using the *Ligand Pharmacophore Mapping* protocol with the *Best Flexible* search option as available in DS. The database hits scored a fit value greater than the most active compound were retained. In addition, hit compounds with high fit values were predicted for the drug-likeness using Lipinski's rule of five. A Lipinski-positive compound has i) a molecular weight less than 500; ii) less than 10 hydrogen bond acceptor groups; iii) less than 5 hydrogen bond donor groups and iv) an octanol/water partition co-efficient (LogP) value less than 5. As a second level of drug-like screening, ADMET properties were calculated for every database hit compounds. In this level, the compounds predicted for good absorption, optimal solubility, low blood brain barrier penetration, non-inhibition to CYP2D6 and non-hepatotoxicity were identified and considered further. A total of 130 compounds (114 and 16 compounds from common feature and structure-based pharmacophore models, respectively) satisfying all the drug-likeness screenings were selected for molecular docking

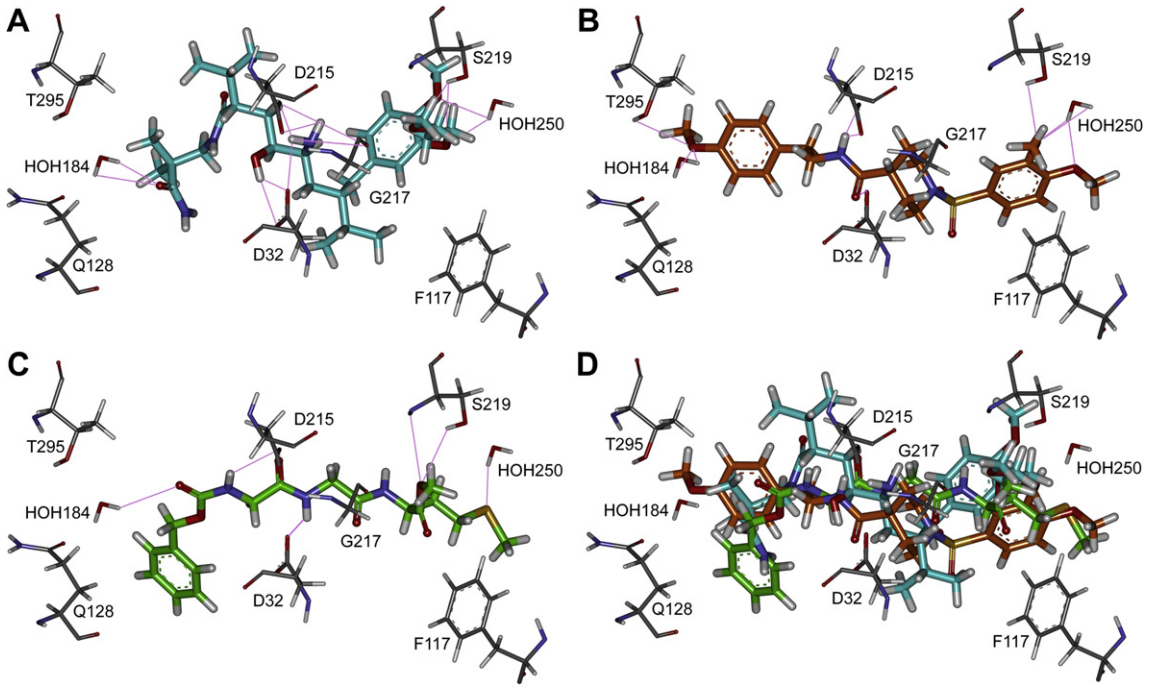

Fig. 8. Molecular docking results. (A) Aliskiren is shown in cyan color while (B) Compound 20060 and (C) NCI 0169134 are shown in orange and green color, respectively. (D) The overlay of all three compounds is shown at lower right. Hydrogen bond interactions are shown in pink lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

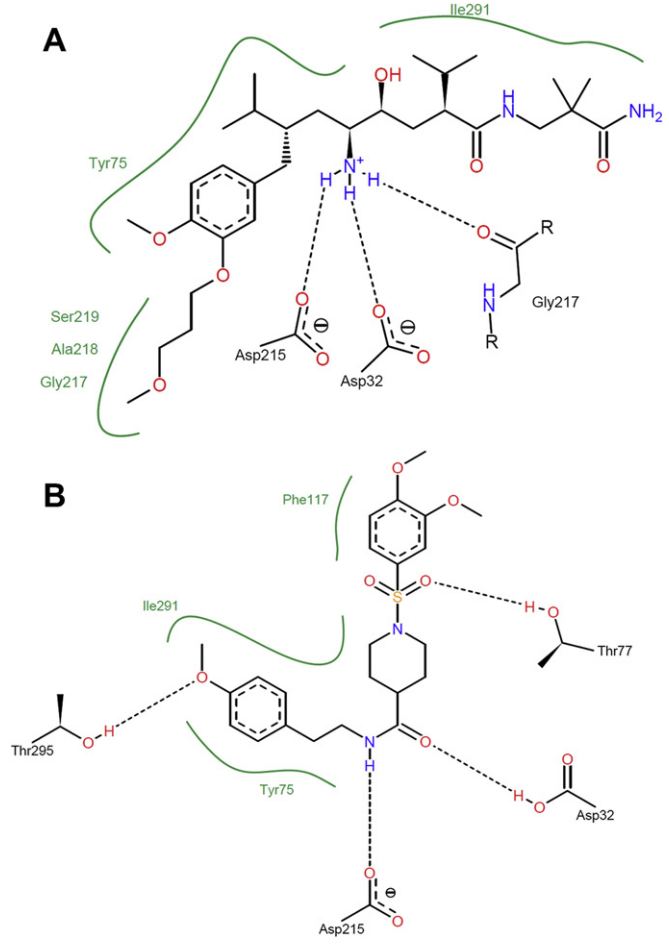

Fig. 9. Molecular interactions observed at the active site for (A) aliskiren and (B) one of the hits, Compound 20060.

study. Database screening using both the pharmacophore models and the steps involved in screening only the drug-like compounds are illustrated in Fig. 6.

3.5. Molecular docking

The 130 drug-like compounds resulted from both the ligand and structure-based pharmacophore modeling along with the training set compounds were subjected to molecular docking employing GOLD program. Binding modes, GOLD fitness scores and molecular interactions of the database hit compounds were compared with the training set compounds. One of the training set compounds and the very successful renin inhibitor, aliskiren, scored a GOLD fitness score of 49.18. This compound has scored the fit values of 3.831 and 3.260 over Pharm-A and Pharm-B, respectively. Thus the compounds that scored the GOLD fitness score greater than 50 and fit value more than 3 were chosen and their interactions with the catalytically important residues were analyzed. A total of 19 compounds have scored better GOLD fitness scores than aliskiren

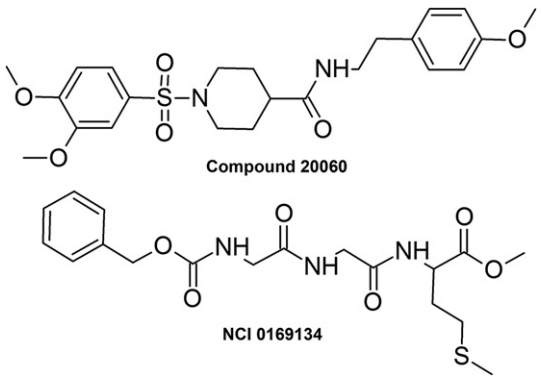

Fig. 10. Two-dimensional chemical structures of identified hits.

along with the better fit values. Based on the molecular interaction at the active site, Compound 20060 and NCI 0169134 from Chembridge and NCI2000 databases, respectively, were chosen as possible virtual leads to be utilized in potent renin inhibitor design. The pharmacophore overlay of these compounds revealed that they fit all the features of the Pharm-A well (Fig. 7A and B). Aliskiren has formed a strong hydrogen bond network at the active site of renin with the amino acids D32, D215, G217, S219 and two water molecules (Fig. 8A). Compound 20060 with the fit value and GOLD fitness score value of 3.673 and 54.63, respectively, has formed a hydrogen bond network with D32, D215, S219, T295 and both water molecules at the active site (Fig. 8B). NCI 0169134 has scored a better fit value (3.890) than aliskiren and also has scored a better GOLD fitness score of 50.02. Its binding has shown hydrogen bond interactions with D32, D215 and S219 as well as the both water molecules in the active site (Fig. 8C). The binding modes of the identified hits were similar to the binding of aliskiren (Fig. 8D). In terms of hydrophobic interactions, aliskiren binds in a position that it can hydrophobically interact with Y75, G217, A218, S219 and I291 residues. In other hand both the hit compounds have also formed hydrophobic interactions with same amino acids (Fig. 9). Noticeably, both of these identified leads are the hits identified by common feature pharmacophore model. From the 19 best scoring compounds identified from molecular docking study only one compound, namely Compound 24015 of Chembridge database (Fig. 7C), that was identified by structure-based pharmacophore model has scored a GOLD fitness score value of greater than 50. This compound with a GOLD fitness score of 50.22 has interacted with the important aspartate residues as well as T295 and one of the two active site water molecules (data not shown). Although all of these hit compounds have shown considerable interactions with the active site components but further optimization based on other interaction points identified in structure-based pharmacophore model can improve their binding. In terms of chemical structures of the hits, Compound 20060 is a derivative of p-methoxyphenylethylamine containing piperidine and dimethoxy phenyl ring in its structure whereas the other hit, NCI 0169134, is a derivative of benzyl alcohol containing tripeptide central moiety with an ester and a thioether substitutions at one end (Fig. 10).

4. Conclusions

Two computational methodologies were employed in this study to develop ligand- (Pharm-A) and structure-based (Pharm-B) pharmacophore models to be utilized in designing novel and potent renin inhibitors as anti-hypertensive agents. Pharm-A composed of four chemical features including two HY, one HA and one HD features but Pharm-B was made of eight features including all the features present in Pharm-A. Both the generated pharmacophore models were validated for its quality to identify new reliable chemical compounds. The validation procedure included various methods based on best-fit value calculation, investigation of required complimentary chemical features to satisfy the active site requirements and enrichment study. All the training set compounds have mapped all the features of Pharm-A but they did not map all the features of Pharm-B. Thus we have selected the compounds mapping all chemical features of Pharm-A and five or more chemical features of Pharm-B from the database screening procedure. Three chemical databases were screened to identify hit compounds to be utilized in potent drug design. The hit compounds mapping the chemical features of the generated pharmacophore models were further analyzed and refined using variety of drug-like filters to select the compounds with favorable drug-like properties. These drug-like hits were subjected to molecular docking study. Binding modes and the molecular interactions of the hits were analyzed for the essential interactions with catalytically important residues at the active site. Finally, two compounds with a great potential to be utilized in future renin inhibitor designing are reported as a final outcome of this study. These final hits are structurally diverse to each other and their novelty was also confirmed using *SciFinder* and *Pubchem* structure search that they were not reported earlier for renin inhibition.

Acknowledgments

This research was supported by Basic Science Research Program (2009-0073267)Pioneer Research Center Program (2009-0081539), and Environmental Biotechnology National Core Research Center program (20090091489) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (MEST)And all students were recipients of fellowship from the BK21 Program of MEST.

Appendix. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2011.03.035.

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