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Modeling the binding modes of Kv1.5 potassium channel and blockers

Qian Yang [a], Lupei Du [a], Xiaojian Wang [a], Minyong Li [b,*], Qidong You [a,**]

[a] Department of Medicinal Chemistry, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China
[b] Department of Chemistry, Georgia State University, Atlanta, GA 30302, USA

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ABSTRACT

The ultra-rapid delayed rectifier potassium current (I_{Kur}), encoded by *Kv1.5* gene, is the critical determinant of Phase I repolarization of action potential duration (APD). The evidences that *Kv1.5* gene expresses more extensively in human atrial myocytes than in ventricle and the I_{Kur} currents has not been recorded in the human ventricle, suggest Kv1.5 potassium channel as a selective target for the treatment of atrial fibrillation (AF). Recent mutagenesis studies have provided us some evidences that are useful in designing Kv1.5 blockers. In order to further evaluate these molecular biological information, the homology model of Kv1.5 potassium channel was established based on the Kv1.2 crystal structure (PDB entry: 2A79) using MODELLER 9v2 program. After the molecular dynamics refinement, the optimized homology model was assessed as a reliable structure by PROCHECK, ERRAT, WHAT-IF, PROSA2003 and DOPE graph. The results of molecular docking studies on different Kv1.5 inhibitors are in agreement with the published mutagenesis data. Based on the docking conformations, a pharmacophore model was developed by HipHop algorithm in order to probe the common features of blockers. By analyzing the results, active site architecture, certain key residues and pharmacophore common-features that are responsible for substrate specificity were identified on the Kv1.5 potassium channel, which would be very helpful in understanding the blockade mechanism of Kv1.5 potassium channel and providing insights into rational design of novel Kv1.5 blockers.

1. Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmias associated with heart failure, stroke and mortality [1]. The therapeutic strategy of atrial fibrillation is to restore normal cardiac rhythm, termed "atrial stabilization". However, existing treatment with class I or class III antiarrhythmic agents such as dofetilide [2] and sotalol [3], could produce negative feedback on the ventricular repolarizations, which is widely considered as a critical risk factor for *Torsades de pointes*. These serious side effects, attributed to the unselective blockade of the potassium currents in both atrial and ventricular myocytes [4], hindered the appropriate usage and fair evaluation of these antiarrhythmic agents in clinical trials. Therefore, targeting the atrial-selective current would be an appealing strategy for atrial fibrillation treatment [5].

Specifically existing in the ventricular, the ultra-rapid delayed rectifier potassium current (I_{Kur}), encoded by *Kv1.5* gene, is a crucial determinant of Phase I repolarization during action potential duration [6,7]. The evidences that *Kv1.5* gene expresses more extensively in human atrial myocytes than in ventricle, and I_{Kur} currents has not been recorded in the human ventricle [6,8], suggest Kv1.5 potassium channel as a potential selective target for the treatment of atrial fibrillation [9].

During recent years, several pharmaceutical companies such as Aventis [10,11], Merck [12,13] and Procter & Gamble [14–18], have engaged in developing new types of selective Kv1.5 blockers, and some clinical trials have yet been launched [19]. The binding modes of Kv1.5 potassium channel with multi-mechanism antiarrhythmic agents like quinidine, bupivacaine, benzocaine, were reported according to the mutation data. The generally identification of the key residues that influence the inhibitory effect include Thr507, Leu510, Val514 of the S6 segment and Thr479 near the selectivity filter [20,21]. These early investigations would prompt the process of detailed mutagenesis studies and binding mode analysis.

In the absence of an experimentally determined crystal structure, homology modeling could provide a rational opportunity to obtain a reasonable 3D model. It is generally recognized that homology modeling of proteins is currently the most accurate method for 3D structure prediction, yielding models suitable for a

* Corresponding author.
** Corresponding author. Tel.: +86 25 8327 1351; fax: +86 25 8327 1351.
E-mail addresses: mli@gsu.edu (M. Li), youqidong@gmail.com, youqd@cpu.edu.cn (Q. You).

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wide spectrum of applications, such as structure-based molecular design and mechanism investigation [22–24]. This approach is able to provide a reasonable structural model for a given membrane protein sequence with related templates sharing more than 25% sequence identity [25,26].

So far there are several reported homology models for Kv1.5 protein based on the crystal structure of KcsA [27,28]. However, the only 36% sequence identity between KcsA and Kv1.5 would have negative effect on the accuracy of homology model. Recently Ander et al. [29] reported a Kv1.5 homology model based on Kv1.2 potassium channel. After automated docking of eight bisaryl scaffold compounds and the binding free energy calculation, they suggested the non-polar binding as an important factor in the channel–ligand interaction. They also supposed that pharmacophore models based only on optimized unbound-type ligand conformations may not necessarily capture the geometric features of ligands bound to the channel cavity. However, this hypothesis could not be concluded due to the deficiency of structure diversity of the blockers and the lack of bound-type ligand conformations [29]. To further study the cases of blockers with different scaffolds, in the current report we developed the Kv1.5 homology model based on Kv1.2 backbone, the binding mode investigations of different Kv1.5 blockers was carried out. Furthermore, a pharmacophore model derived from the docking conformations was established. Such efforts were made along the same pipeline of our research interest in understanding factors contributing to potassium channels and their blockers [30–32].

We acknowledge that a homology model is different from an experimentally determined structure. However, it should still be very useful in helping us understand the binding modes of Kv1.5 and its blockers, and avoid obvious pitfalls in our further design by using the combination of homology modeling and pharmacophore building. It should be noted that in the long history of the development and application of the pharmacophore modeling, exploitation of available experimental protein structures is a new feature [33]; however, several studies already indicate enhanced performance of a protein structure-based approach in pharmacophore modeling [34,35]. Therefore, we believe that our efforts here will represent a promising binding mode by docking and pharmacophore modeling and for further biological testing.

2. Methods

2.1. Homology modeling

In the homology modeling phase, we would like to look for an experimentally determined structure of high sequence identity with the Kv1.5 potassium channel. For this, the crystal structure of the Kv1.2 potassium channel is known at 2.9 Å resolution [36]. The construction of protein models by homology modeling normally proceeds along a series of well-defined and commonly accepted steps: (1) sequence alignment between the target and the template; (2) building an initial model; (3) refining the model; (4) evaluating the quality of the model [23,26].

The amino acid sequence of the human Kv1.5 potassium channel was retrieved from Swiss-Prot database (accession number P22460, entry name KCNA5_HUMAN) and aligned to Kv1.2 potassium channel (PDB entry: 2A79) [36] by T-Coffee multiple alignment program [37] and ESPript program [38]. The secondary structure-based sequence alignment between Kv1.5 and Kv1.2 is 70%, and the S5/H5/S6 domains of Kv1.5 and Kv1.2 are highly conserved, which showed a suitable case for the construction of a credible Kv1.5 potassium channel. Using MODELLER 9v2 program with default parameters [39], a monomer model of the S5/H5/S6 domains of Kv1.5 was then constructed based on Kv1.2 crystal structure. In the next step, the monomer coordinates were then aligned with KcsA tetramer crystal structure (PDB entry: 1BL8) [40] to assemble the tetramer homology model (Fig. 1).

2.2. Refinement of homology model

The rough tetramer model was embedded with POPC lipid bilayer model in VMD program [41] as depicted in literature [42], then was refined with NAMD program [43] using the CHARMM27 force field [44]. In brief, the temperature was maintained at 296 K by means of Langevin dynamics using a collision frequency of 1/ps. A fully flexible cell constrained to orthorhombic symmetry at constant pressure (1 atm) was employed by means of the Nosé-Hoover Langevin Piston algorithm. Initial coordinates were taken from a previously equilibrated MD simulation. The van der Waals interactions were switched smoothly to zero over the region 10–11 Å and electrostatic interactions were included via the smooth particle-mesh Ewald summation. A neighbor list, used for calculating the non-bonded interactions, was kept to 12.5 Å and updated every eight steps. The impulse-based Verlet-I/r-RESPA method was used to perform multiple time-stepping: 4 fs for the long-range electrostatic forces, 2 fs for short-range non-bonded forces, and 1 fs for bonded forces [45]. Data for analysis were taken from 10 ns of the simulation, where the area/lipid and *d*-spacing for the simulation were stable with time.

2.3. Assessment of the homology model

To obtain an accurate homology model, it is very important that appropriate steps are built into the process to assess the quality of the model [46]. Therefore, in the modeling phase, the model quality was assessed by the geometric quality of the backbone conformation, the residue interaction, the residue contact and the energy profile of the structure using different methods, including PROCHECK [47], ERRAT [48], WHAT-IF [49], PROSA2003 [50] and DOPE [51].

2.4. Validation of the model by docking analysis

The ability to predict the correct ligand binding pose is important for a successful homology model, and the only validation is to check whether it is in consistent with published mutation data. In the current study, the binding modes of the three Kv1.5 blockers AVE-0118, S-0100176 and KN-93 (Fig. 2) could be the representative cases attribute to their structure diversity and the availability of mutation data.

In the validation phase, Insight II/Builder was used to construct the molecular files for the three compounds involved in the binding profile analysis. All hydrogen atoms were presented for all of the constructed ligands. The ligands were refined using PM3 method in MOPAC 7 program [52] and assigned with AM1-BCC partial charge [53,54] by QuACPAC program [55]. All partial charges on the atoms of the Kv1.5 model were derived from the AMBER 8 force field parameters. Docking of the ligands into the active site around Ile508 and Val512 that equivalent to Tyr652 and Phe656 of the hERG potassium channel [56] was performed by DOCK 5.4 program [57]. After docking, the ligand–receptor complexes were then optimized using the same protocol as depicted in the refinement phase and analyzed by HBPLUS 3.06 [58], LIGPLOT 4.22 [59] and Pymol [60] programs.

2.5. Docking-based pharmacophore modeling

Based on the homology modeling and molecular docking results, we made an attempt to identify the hypothetical 3D

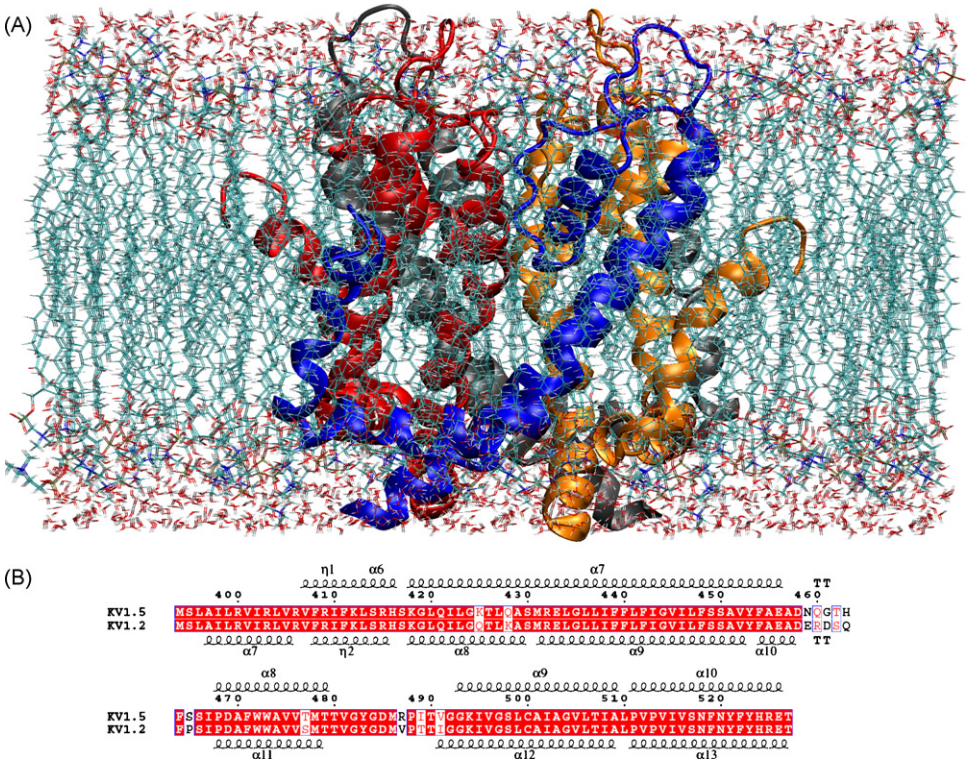

Fig. 1. (A) The homology model of Kv1.5 potassium channel in POPC membrane model; the four different colors represent four monomers. (B) Secondary structure-based sequence alignment between Kv1.2 and Kv1.5 potassium channel.

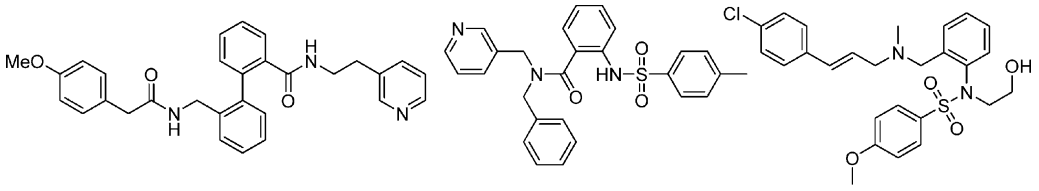

Fig. 2. The Kv1.5 potassium channel blockers involved in the binding profile analysis.

receptor-based pharmacophore model by using common-feature hypothesis generation approach "HipHop" implemented in Catalyst 4.10 package. In particular, HipHop algorithm finds common feature pharmacophore models among a set of highly active compounds thus it carries out a 'qualitative model' without the use of activity data, which represents the essential 3D arrangement of functional groups common to a set of molecules for interacting with a specific biological target [61].

In the hypothesis generation phase, the docking conformations of AVE-0118, S-0100176 and KN-93 were directly used as input without any structural minimization and conformational search. A default uncertainty factor of 3 for each compound was defined, and four chemical features, including hydrogen-bond acceptor (A), hydrogen-bond donor (D), aromatic ring (R) and hydrophobic (H) group, were selected to form the pharmacophore hypothesis using HipHop. A Principal number of 2 and MaxOmitFeat number of 0 was defined for the good mapping of all features of these compounds on a hypothesis model [62].

2.6. Hardware and softwares

The homology modeling (MODELLER 9v2), binding analysis (HBPLUS 3.06 and Ligplot 4.22), visualization of models (PyMOL 0.99) and pharmacophore modeling (Catalyst 4.10) were carried out on a Linux workstation. The molecular mechanics and molecular dynamics simulations (NAMD 2.6) were performed on a Linux-based 40-node cluster. The PROCHECK and ERRAT (http://nihserver.mbi.ucla.edu/SAVS/), and WHAT-IF (http://swift.cmbi.kun.nl/WIWWWI/) validation were executed on-line.

3. Results and discussion

3.1. Characteristics of homology model

During the refinement of homology model by molecular mechanics and molecular dynamics simulation, the root-mean-square deviation (RMSD, Å) of the protein backbone atoms relative to the crystal structure of Kv1.2 potassium channel are plotted as a function of time in Fig. 3. It should be noted that after 3 ns, the RMSD of each system tends to be convergent, indicating the system is stable and the system has been equilibrated.

After structural refinement, the geometric quality of the backbone conformation, the residue interaction, the residue contact and the energy profile of the structure are all well within the restrictions established for reliable structures. The root-mean-square deviation between the backbone atoms of the Kv1.2

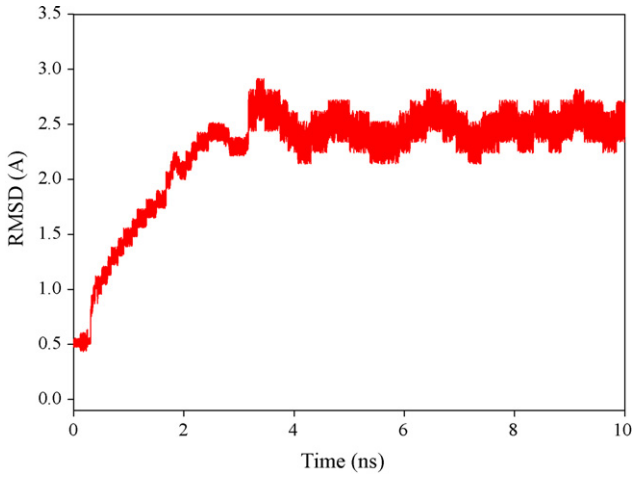

Fig. 3. Time dependence of the RMSDs (Å) from homology model of Kv1.5 potassium channel and the crystal structures of Kv1.2 potassium channel for the Cα atoms in the 10 ns MD simulations.

template and the optimized Kv1.5 homology model was 2.03 Å, again indicating a close homology feature. All evaluations suggest that a model of high quality for the Kv1.5 has been obtained to allow for the examination of protein–substrate interactions.

3.2. Validation of homology model

The first validation was carried out using Ramachandran's plot calculations computed with PROCHECK program by checking the detailed residue-by-residue stereo-chemical quality of a protein structure [47]. The ϕ and φ distributions of the Ramachandran's plot of non-glycine, non-proline residues are summarized in Table 1 and Fig. 4. Altogether, 97.3% of the residues in homology model were in favored and allowed regions. Comparing with the template, the homology model has a similar Ramachandran plot with a relatively low percentage of residues having general torsion angles (2.7%).

ERRAT is a so-called "overall quality factor" for non-bonded atomic interactions, and higher scores mean higher quality [48]. The normally accepted range is >50 for a high quality model [48]. In the current case, the ERRAT score for the model is 80.508, well within the range of a high quality model, in the meantime the ERRAT score for template is 90.984 (Table 1). Thus, the above analysis suggests that the backbone conformation and non-bonded interactions of homology model are all reasonable within a normal range.

WHAT-IF is used to check the normality of the local environment of amino acids [49]. For the WHAT-IF evaluation, the quality of the distribution of atom types is determined around amino fragments. For a reliable structure, the WHAT-IF packing scores should be above −5.0. In this case, none of the scores for each residue in homology model is lower than −5.0 as depicted in Fig. 5. Therefore, the WHAT-IF evaluation also indicates that the homology mode structure is very reasonable.

The interaction energy per residue was also calculated by the PROSA2003 program [50]. In this analysis, the interaction energy of each residue with the remainder of a protein is computed to judge whether or not it fulfills certain energy criteria. The PROSA *Z*-Score indicates overall model quality. For the Kv1.5 homology model, it gains *Z*-Score of −1.43, when compared with *Z*-Score of −1.87 for Kv1.2 template. Fig. 6 displays the PROSA2003 energy profiles calculated for the homology model along with the template. The energy profile of the homology model is in consistent with a

Table 1
Quality of structures checked by PROCHECK and ERRAT

	PROCHECK[a]							ERRAT score
	Ramachandran plot quality (%)				Goodness factor			
	Core	Allowed	General	Disallowed	Dihedrals	Covalent	Overall	
Model	86.6	10.7	2.7	0.0	−0.39	−0.42	−0.37	80.508
Template	89.7	10.3	0.0	0.0	0.18	0.60	0.35	90.984

[a] Ramachandran plot qualities show the percentage (%) of residues belonging to the core, allowed, generally allowed and disallowed region of the plot; goodness factors show the quality of covalent and overall bond/angle distances; these scores should be above −0.5 for a reliable model.

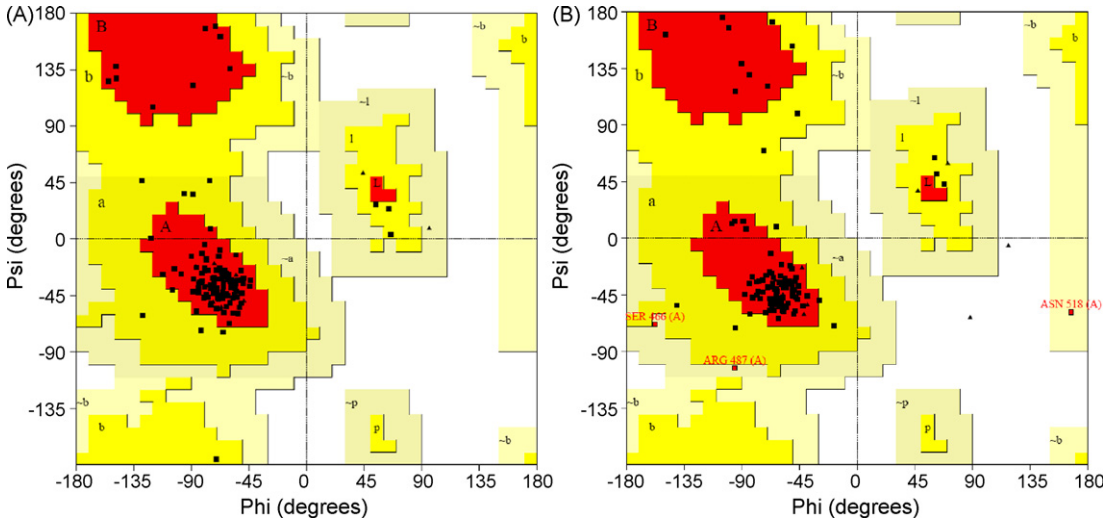

Fig. 4. Ramachandran's map: (A) Kv1.2 template and (B) Kv1.5 homology model.

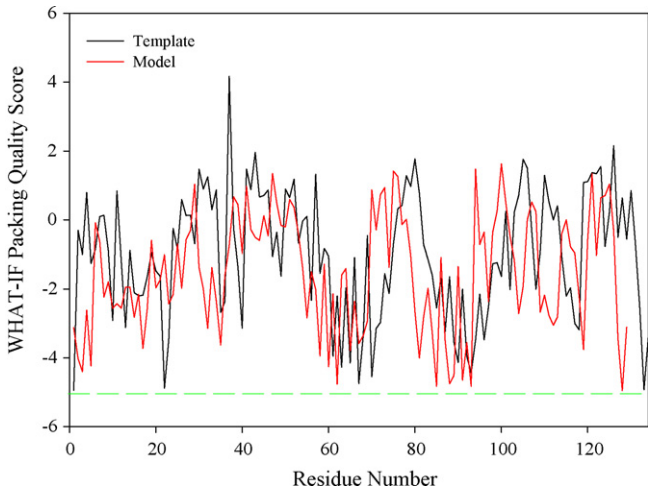

Fig. 5. The WHAT-IF packing quality scores calculated for the template and the homology model. The score should be above −5 for a reliable model.

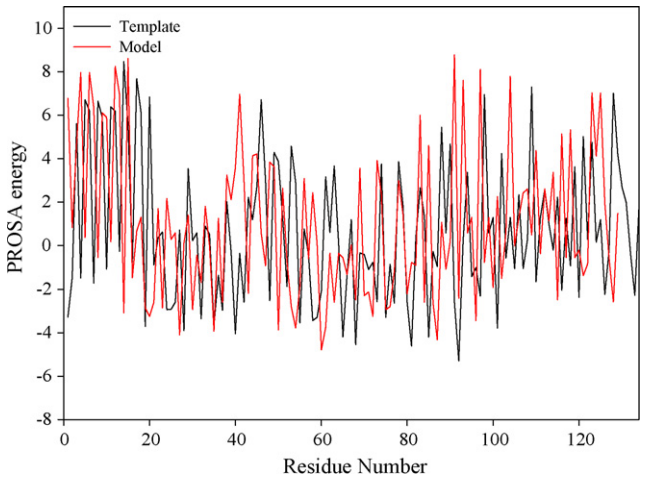

Fig. 6. PROSA2003 energy profiles calculated for the template and the homology model.

reliable conformation based on its similarity with that of the template.

DOPE, or discrete optimized protein energy, is a statistical potential used to assess homology models in protein structure prediction [51]. DOPE confirms again that a reasonable model was obtained with a suitable energy score comparable to that of the template: −12837.4 for the model and −13729.3 for the template, respectively (Fig. 7).

In brief, the geometric quality of the backbone conformation, the residue interaction, the residue contact and the energy profile of the structure are all well within the limits established for reliable structures. All evaluations suggest that a reasonable homology model for Kv1.5 has been obtained to allow for examination of protein–substrate interactions.

3.3. Molecular docking of AVE-0118 into Kv1.5 homology model

The docking conformation and corresponding complex analysis of AVE-0118 are depicted in Fig. 8. AVE-0118 is trapped in a strong hydrophobic cavity, in which the 4-methoxyphenyl-acetamido fringe is involved in hydrophobic interaction with Thr 480 and Ile 508 in the tetramer, meanwhile the biphenyl scaffold of the compound is engaged in the hydrophobic contribution of Ile 508, Pro 513, Val 512 and Val 505. The computational result is in

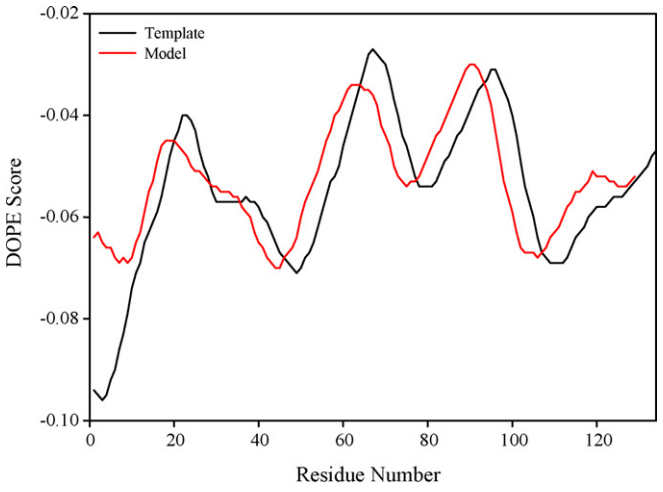

Fig. 7. DOPE energy profiles calculated for the template and the homology model.

consistent with the mutation result reported by Decher and co-workers, which showed that the binding site of AVE-0118 was Thr 479, Thr 480 in the selectivity filter and Ile 502, Val 505, Ile 508, Leu 510, Val 512, Val 516 in the S6 domain [63].

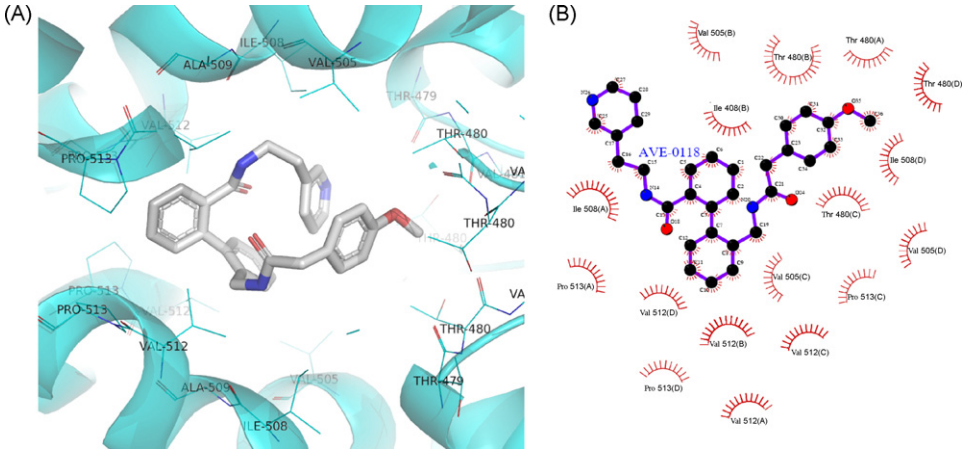

Fig. 8. (A) The docking conformation of AVE-0118 in the active site of Kv1.5 homology model. (B) A schematic illustration of the interactions of AVE-0118 with Kv1.5 homology model.

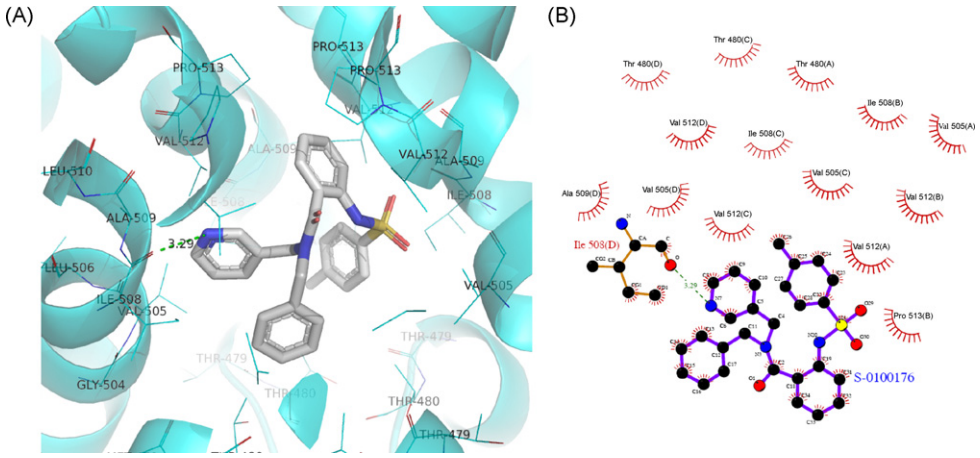

Fig. 9. (A) The docking conformation of S-0100176 in the active site of Kv1.5 homology model. (B) A schematic illustration of the interactions of S-0100176 with Kv1.5 homology model.

3.4. Molecular docking of S-0100176 into Kv1.5 homology model

Fig. 9 represents the docking conformation and corresponding complex analysis of S-0100176. The aromatic framework of S-0100176 is located in a strong hydrophobic environment correlated with the residue Thr 480, Val 505, Ile 508, Ala 509, Val 512 and Pro 513, while the nitrogen atom in the pyridine group is engaged in the hydrogen bond interaction with Ile 508. The computational result is in consistent with the Ala scanning mutagenesis reported by Decher and co workers, which highlighting the IC_{50} reduction by I508A (150-fold), V512A (99-fold), coincidently the same with the presumption that Ile 508 and Val 512 of Kv1.5 are located in the positions equivalent to Tyr 652 and Phe 656 of the hERG potassium channel [56].

3.5. Molecular docking of KN-93 into Kv1.5 homology model

In the case of KN-93, a broad-spectrum Kv family open channel blocker, the computational result showed very similar binding profile when compared with AVE-0118. Fig. 10 represents the predicted conformation and schematic binding plot of KN-93 with Kv1.5 homology model, which was also in consistent with the binding mode reported by Fendida and his collaborators [64].

3.6. Docking-based pharmacophore modeling

To be an ideal homology model for accurate predicting the activity of heterogeneous blockers, the coherence of the homology model with the ligand-based model should be examined. For this reason, a receptor-based pharmacophore model was established using the docking conformations of AVE-0118, S-0100176 and KN-93 by using common-feature hypothesis generation approach HipHop implemented in Catalyst 4.10 package [61]. HipHop produces 10 hypotheses, and Hypo1 is the best significant pharmacophore hypothesis in this study, characterized by the highest ranking score (Table 2). All the 10 hypotheses have the same features of three hydrophobic groups. In Hypo1, it is significantly that the docking conformations of these three compounds could be overlapped into three hydrophobic points, in which the distances between two hydrophobic points are 5.91, 6.71 and 9.75 Å (Fig. 11), respectively. These results were in high consistent with the three-hydrophobic-center pharmacophore model derived by Aventis [10], where the distances between two hydrophobic points are 6.5, 6.6 and 12.6 Å, respectively. These good mapping between docking conformations and pharmacophore model again suggested a reliable homology model.

The mapping of docking conformation of AVE-0118 onto pharmacophore model Hypo1 is represented in Fig. 12. AVE-0118 matches with these three hydrophobic points very well, by

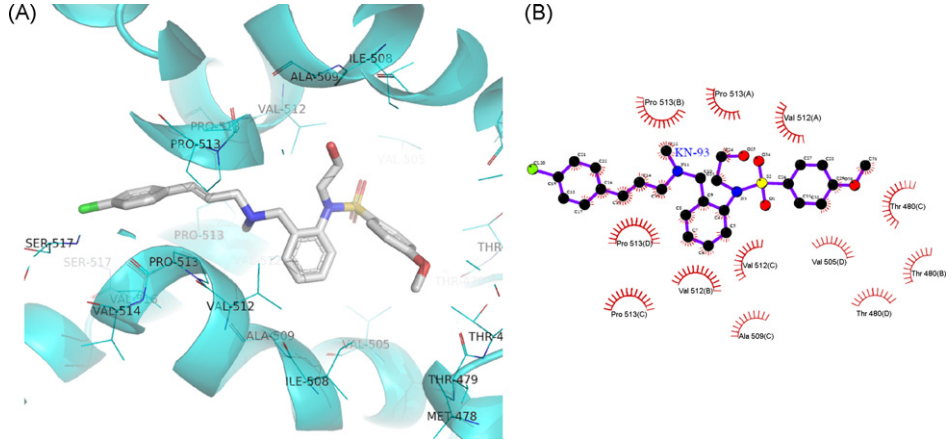

Fig. 10. (A) The docking conformation of KN-93 in the active site of Kv1.5 homology model. (B) A schematic illustration of the interactions of KN-93 with Kv1.5 homology model.

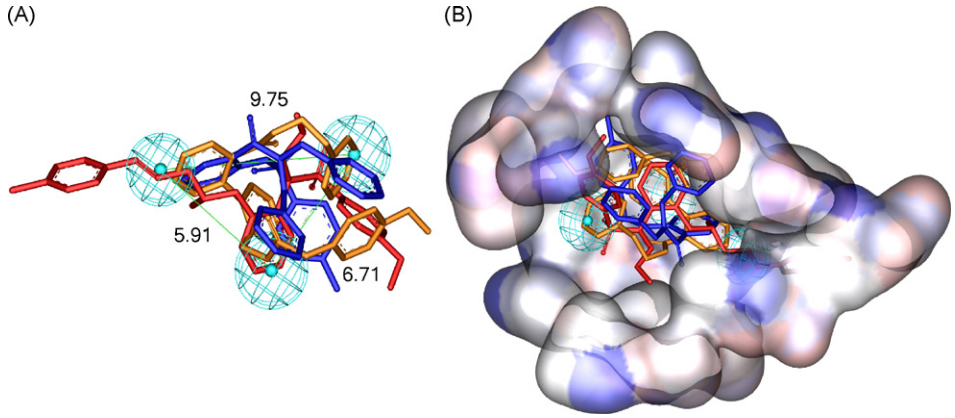

Fig. 11. (A) The Hypo 1 is mapped onto the docking conformations of AVE-0118 (red sticks), S-0100176 (blue sticks) and KN-93 (yellow sticks). Distances between pharmacophore features are reported in angstroms. The hydrophobic pharmacophore feature is coded as blue spheres. (B) The pharmacophore model and docking conformations around the active site surface of Kv1.5 homology model.

Table 2
Results of the common feature hypothesis run[a]

Hypothesis No.	Composition	Ranking score	Direct hit	Partial hit
1	HHH	108.900	111	000
2	HHH	108.799	111	000
3	HHH	108.593	111	000
4	HHH	108.388	111	000
5	HHH	108.365	111	000
6	HHH	108.256	111	000
7	HHH	108.152	111	000
8	HHH	108.126	111	000
9	HHH	108.084	111	000
10	HHH	108.018	111	000

[a] Direct Hit, all the features of the hypothesis are mapped. Direct hit = 1 means yes and direct hit = 0 is no. Partial hit, partial mapping of the hypothesis. Partial hit = 1 means yes and partial hit = 0 means no.

locating at pyridyl ring, 4-methoxyphenyl ring and biphenyl moiety, respectively. The docking results suggest that AVE-0118 could be captured in hydrophobic networks, in which the 4-methoxyphenyl moiety is involved in hydrophobic interaction with Thr 480 and Ile 508, the pyridyl ring has hydrophobic contact with Val 505, Ile 508 and Pro 513, the biphenyl scaffold is engaged in the hydrophobic binding with Ile 508, Pro 513, Val 512 and Val 505. Obviously, this mapping model has a good fit with the docking environment. In the case of S-0100176, three phenyl rings could also be mapped with all three hydrophobic features, respectively, by contacting with Thr 480, Val 505, Ile 508, Ala 509, Val 512 and Pro 513 as suggested by molecular docking (Fig. 13). It should be noted such a binding mode has been suggested by mutagenesis study [56]. KN-93 can also be fitted with all features of Hypo1 (Fig. 14): three hydrophobic features have good maps with the methoxyphenyl ring (in interaction with Thr 480 and Val 505), the

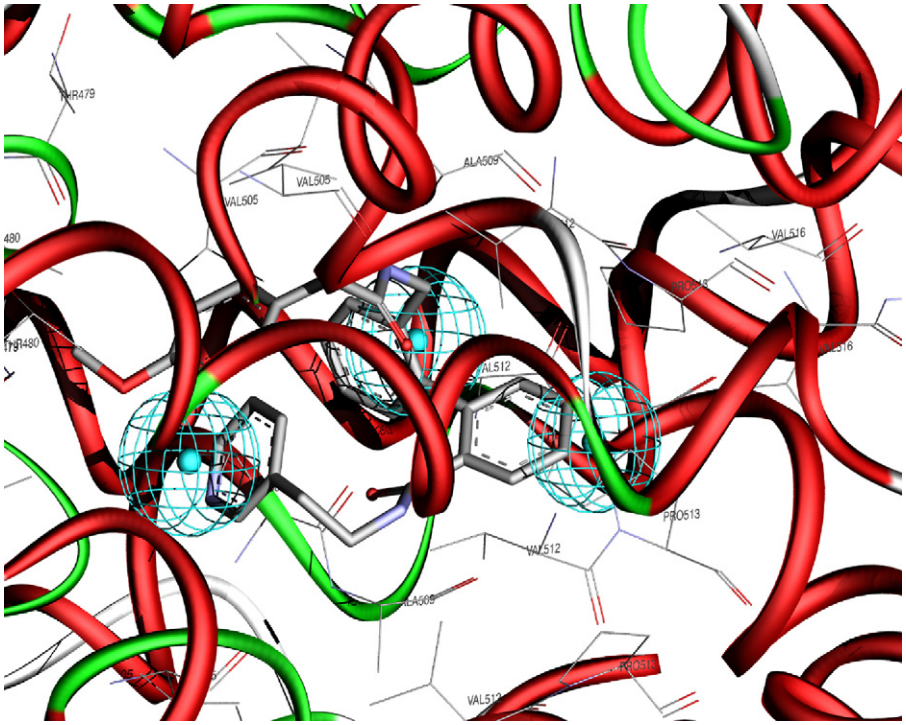

Fig. 12. Pharmacophore Hypo1 mapped with AVE-0118 and the key residues around the active site of KV1.5 homology model. The residues are represented as lines and ribbons, and AVE-0118 is shown in sticks. The hydrophobic pharmacophore feature is coded as blue spheres.

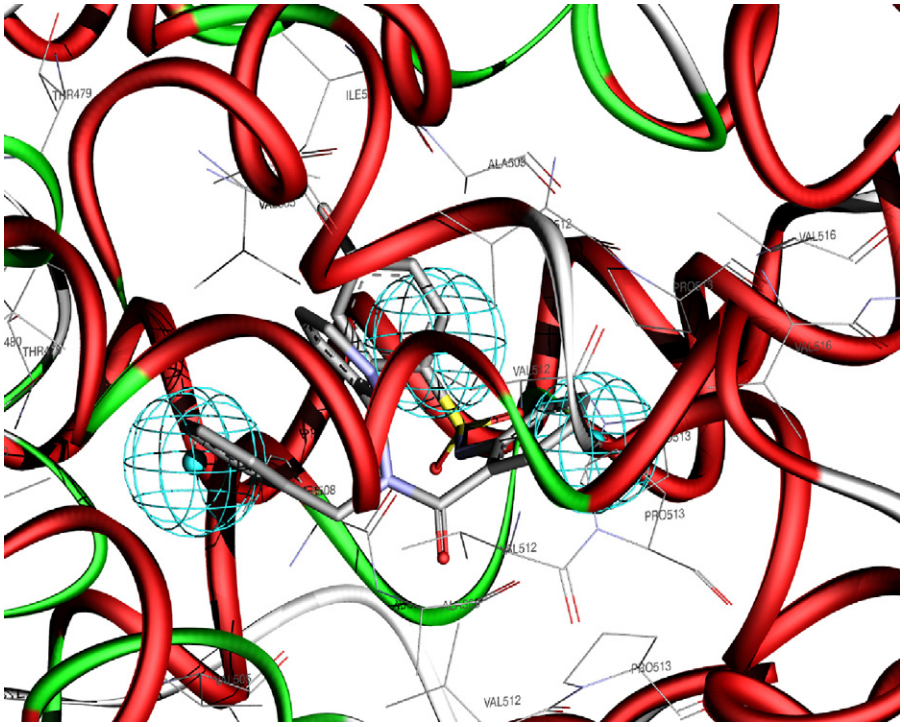

Fig. 13. Pharmacophore Hypo1 mapped with S-0100176 and the key residues around the active site of KV1.5 homology model. The residues are represented as lines and ribbons, and S-0100176 is shown in sticks. The hydrophobic pharmacophore feature is coded as blue spheres.

middle phenyl moiety (in interaction with Ala 509 and Val 512), and the 4-chlorophenyl aryl group (in interaction with Val 513 and Pro 513). In summary, the overlapping of pharmacophore features and molecules around the active site can provide strong confidence on both the pharmacophore Hypo1 and homology model. Therefore, this structure-based pharmacophore model will provide more detailed information and accuracy in its description of ligand binding with Kv1.5 channel. We think such an application of pharmacophore models can therefore be of great useful in the detection of new leads.

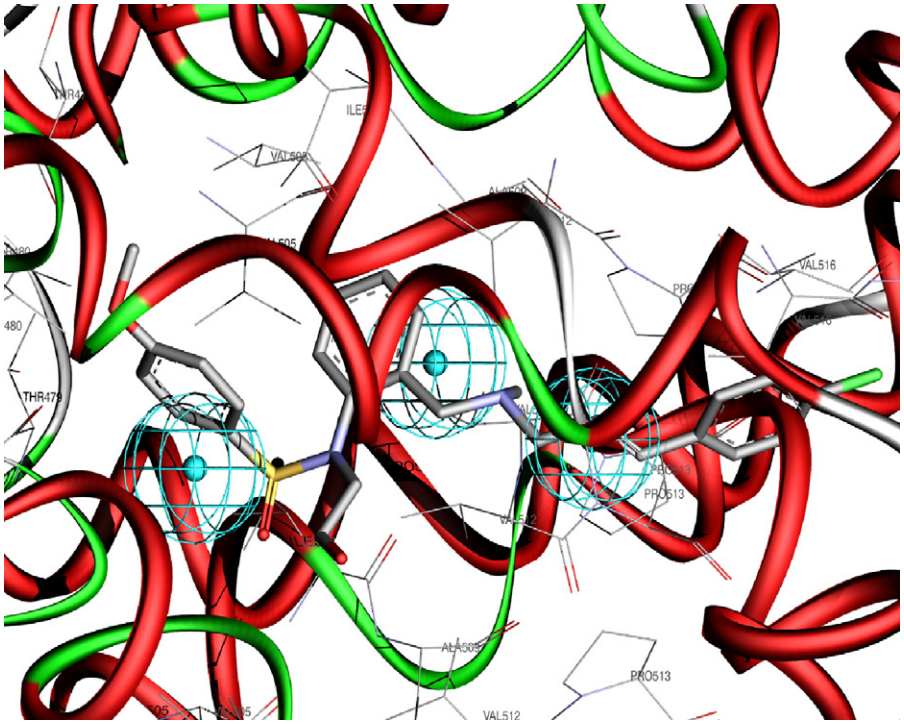

Fig. 14. Pharmacophore Hypo1 mapped with KN-93 and the key residues around the active site of KV1.5 homology model. The residues are represented as lines and ribbons, and KN-93 is shown in sticks. The hydrophobic pharmacophore feature is coded as blue spheres.

4. Conclusion

In this paper, we have developed high accurate homology model of Kv1.5 potassium channel using Kv1.2 crystal structure. The quality of a homology model depends on the level of sequence identity between the template of known 3D structure and the protein to be modeled. In this case, the level of sequence identity between the target and the template (70%) is far exceeding the threshold of 30% sequence identity. This model has been qualified using several validation methods, including PROCHECK, ERRAT, WHAT-IF, PROSA2003 and DOPE. All evidences suggested that the geometric quality of the backbone conformation, the residue interaction, the residue contact and the energy profile of the structure are all well within the limits established for reliable structures. The docking studies of this homology model were also in consistent with the mutagenesis studies and previous modeling studies. Furthermore, based on the docking conformations, a pharmacophore model was built using HipHop algorithm. This pharmacophore hypothesis was in accordance with both published pharmacophore model and mutation data, which indicated a doubled hit rate by applying the homology model in virtual screening protocol. Different from classical homology modeling, the combination of homology modeling and pharmacophore building will still be very useful in helping us understand the binding modes of Kv1.5 and its blockers, and avoid obvious pitfalls in our further design. In conclusion, we believe that these results may help in the understanding of mechanism of action of Kv1.5 potassium channel and the design of selective Kv1.5 blockers for the treatment of AF.

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