Exploring blocker binding to a homology model of the open hERG K^+ channel using docking and molecular dynamics methods

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Received 1 March 2005; revised 7 April 2005; accepted 15 April 2005

Available online 29 April 2005

Edited by Gianni Cesareni

Abstract Binding of blockers to the human voltage-gated hERG potassium channel is studied using a combination of homology modelling, automated docking calculations and molecular dynamics simulations, where binding affinities are evaluated using the linear interaction energy method. A homology model was constructed based on the available crystal structure of the bacterial KvAP channel and the affinities of a series of sertindole analogues predicted using this model. The calculations reproduce the relative binding affinities of these compounds very well and indicate that both polar interactions near the intracellular opening of the selectivity filter as well as hydrophobic complementarity in the region around F656 are important for blocker binding. These results are consistent with recent alanine scanning mutation experiments on the blocking of the hERG channel by other compounds.

Keywords: Ion channels; hERG; Blocker; Docking; Molecular dynamics; Linear interaction energy method

1. Introduction

The human ether á go-go related gene (hERG) potassium channel is of great interest in drug design because of its interactions with a large variety of drugs [1]. A high concentration of hERG channels is found in the heart muscle tissue and blocking of these channels can lead to cardiac arrhythmias associated with prolongation of the QT wave, the period of ventricular repolarization. Prolongation of the QT interval increases the risk of torsades de pointes, an arrhythmia which can cause ventricular fibrillation, syncope and sudden death. QT prolongation can be induced either by drugs or by genetic mutations in the ion channel expressing genes, and in the latter case often occurs in relation to exercise or emotional stress [2–4]. Due to hERG blocking and serious cardiac side effects a number of drugs have been removed from the market [5]. The interactions of pharmaceutical agents with hERG are thus generally highly unwanted as they can lead to severe complications, and a molecular understanding of these interactions is desirable in the design of new drugs. As there is no 3D structure available (particularly for the inner part) of the hERG channel, computational studies of binding interactions have to rely on homology modelling based on the bacterial K^+ channel structures that have been solved [6].

The voltage-dependent hERG potassium (Kv) channel has a 50% sequence identity to the *Drosophila melanogaster* ether á go-go (Eag) gene [7] and has also six membrane spanning segments per each of its four subunits. Segments S1–S4 form the voltage sensor part where the S4 helix is thought to be the most important of the four with its several charged arginines. The sequences spanning through S5–P–S6 form the central channel cavity (Fig. 1) where blockers bind. Between the two helices P and S6 one finds the highly conserved selectivity filter (SF) where ion discrimination occurs (name convention adopted from [6]).

The first crystal structure of a voltage-dependent potassium channel in its open state was reported for the KvAP channel from the thermophilic archaebacteria *Aeropyrum pernix* by Jiang et al. [6]. This channel has been shown to possess several conserved structural features with eukaryotic Kv channels [8]. With this in mind the KvAP channel makes a good template for homology modelling of hERG. One part of the sequence that, however, differs significantly between hERG and KvAP is the section between helix S5 and P (Fig. 1). In hERG this part consists of 43 amino acids while KvAP comprises only 11 residues [6,9]. On the other hand, the focus of the present work is not on the position or function of the S5-P loop but caution, nevertheless, has to be exercised in the sequence alignment.

The voltage-sensing segments contain charged residues in order to be able to respond to changes in the membrane potential and the protein mechanics causes the mostly hydrophobic inner helices to undergo conformational changes, thereby being able to close or open the channel pore. In the voltage-gated KvAP and Ca^{2+}-activated *Methanobacterium thermoautotrophicum* (MthK) potassium channels, the conserved G648 residue of the S6 helix has been shown to act as a hinge point in the bending of the helix, thus effecting opening (and closure) of the channel [6,10]. This event occurs when the membrane depolarizes, i.e. when the voltage of the intracellular relative to the extracellular side rises, resulting in an outwards conduction of potassium ions. In the cardiac action potential, a number of different channel types contribute, and the hERG channel specifically carries the delayed rectifier current denoted I_{Kr}. The binding of compounds that cause blockade of hERG occurs in the open state [5,11–13]. Besides the hinge function, another important feature of S6 is the two residues Y652 and F656, positioned close to the selectivity filter, which are reported to interact with several hERG blockers [14]. The residues T623, S624 and V625,

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0014-5793/$30.00
doi:10.1016/j.febslet.2005.04.039

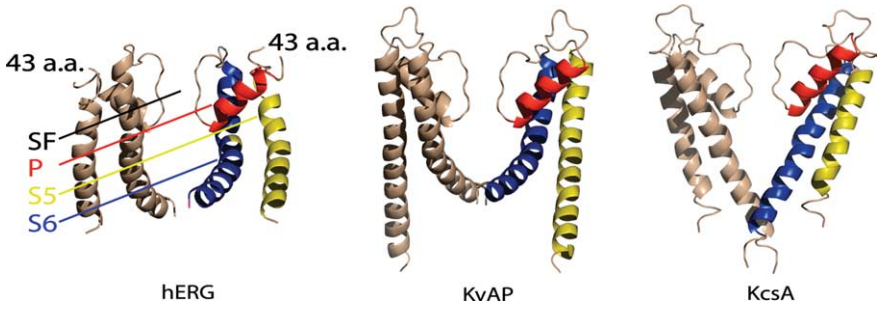

Fig. 1. Homology model of the open hERG channel, crystal structure of the open KvAP channel [6] and of the closed KcsA channel [38] (only two out of four subunits are shown in each channel for clarity and the extracellular side is at the top). The 43 amino acid S5-P loop (not explicitly modelled) of hERG channel is cut out from the picture. The G648 hinge point in hERG's S6 helix is indicated in yellow (coordinates of the model are available upon request).

the last residue of P and the first two of SF, respectively, have also been shown to be important for ligand binding [13,14].

Sertindole, an indolylpiperidine compound, is a strong blocker of the hERG channel and this withdrawn antipsychotic drug can lead to QT wave prolongation [15]. The compound and some of its molecular analogues (Fig. 2) constitute a good starting set of molecules to study because they span the spectrum from strong blockers to weak ones [16] and they also differ in their net charge (+1 or 0). The compounds all have a basic nitrogen on the piperidino moiety as well as aromatic groups. Here, we use a homology-derived model of the open-state hERG pore for automated docking of sertindole analogues. The best docking solutions are then refined by molecular dynamics (MD) simulations and the linear interaction (LIE) method [17,18] is used to obtain relative binding affinities of the ligands from these calculations. This type of procedure has earlier proven efficient for predicting binding affinities and structures for new protein-ligand complexes [19].

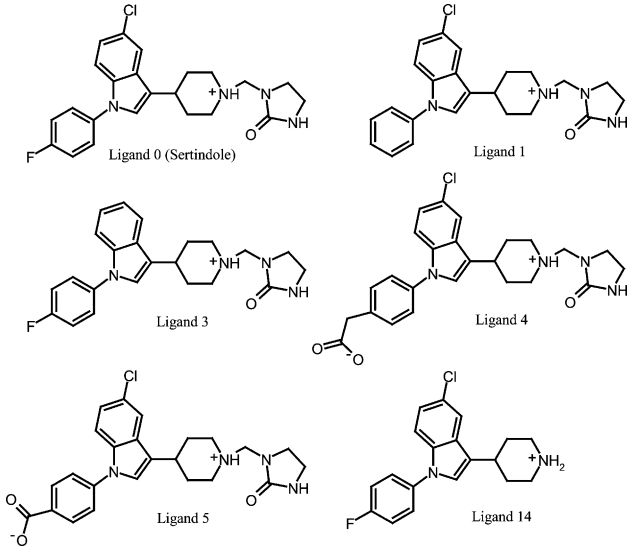

Fig. 2. Sertindole (ligand 0) is a QT-wave prolonging antipsychotic drug with strong binding to the hERG channel. The sertindole analogues shown have a wide spectrum of binding energies, ranging from good to weak binders (IC_{50} values of 3–75 000 nM). The numbering of the ligands is adopted from Pearlstein et al. [16].

2. Methods

2.1. Alignment and homology modelling

The amino acid sequence of hERG was taken from the ExPASy server [20] and the KvAP sequence with a 3.2 Å resolution structure from the PDB entry 1ORQ [6]. In hERG we have used the sequence from S5 to S6 and in KvAP a somewhat longer sequence, 10 residues N-terminal of S5 and 10 residues C-terminal of S6. These two sequences were aligned with the program T-Coffee (Tree-based consistency objective function for alignment evaluation) [21]. The method of T-Coffee uses a mixture of local and global pair-wise alignment, Lalign and ClustalW, respectively, and was found to yield better results, pertaining to more sensible gaps, matching of conserved and other important residues etc., than any of these individual tools. The two S6 helices have a sequence identity of 39% which should be enough for a reliable model. Helix S5 has a somewhat lower identity and the P helices show slightly better homology than S5.

The SWISS-MODEL software [22] was then used for straightforward homology modelling. The program uses a combination of the provided template structure, constraint programming, scoring functions and fragment libraries to predict the position of all the residues in the homology model. The aligned sequences where submitted in the alignment mode. The result was replicated into four identical subunits that were assembled to a full tetrameric channel. This molecular structure was then relaxed and checked, with respect to its bond lengths, angles and atomic clashes, using the molecular dynamics package Q [23].

2.2. Docking

The AutoDock 3.0 software [24] was used to obtain unbiased binding positions for the selected blockers in the hERG channel. This automated docking program uses a grid based method for energy calculation of the flexible ligand in complex with a rigid protein. Points on the 3D grid are placed so that they cover the entire inner cavity of hERG, and are probed with the atoms that constitute the blocker. These points make up lookup tables with interaction energies between the atoms in the blocker and the channel using the Amber type charges employed in AutoDock [24,25]. The program uses an empirical scoring function which includes the five energy terms in Eq. (1) [24]. The first three are standard molecular mechanics terms, whereas ΔG_{tor} handles internal torsions as well as the global rotation and translation contributions and ΔG_{sol} represents the desolvation and hydrophobic binding contributions.

$$\Delta G_{dock} = \Delta G_{vdw} + \Delta G_{hbond} + \Delta G_{elec} + \Delta G_{tor} + \Delta G_{sol} \quad (1)$$

In searching the potential surface of the complex the docking procedure uses a Lamarckian genetic algorithm with local search (LGA). In our setup we used a starting population of 250 ligand conformations with a stopping criterion of a maximum of ten million energy evaluations. The number of dockings was set to 150 to get good statistics of the docked complex and the resulting poses were clustered in groups on the basis of an RMSD value of 2 Å relative to the initial position of the starting ligand. These numbers together with AutoDock's default parameters has been shown to be a good setting for blind docking [26]. In the docking calculations we used the loading

state 0000 [27], i.e. just water in the selectivity filter. This due to the fact that the actual loading state of the filter was found not to affect the docking results. All flexible torsion angles were free to rotate. Docked poses with the lowest energy in the two to three highest populated clusters were selected for MD refinement. This was done for each of the ligands. Some of these were subsequently discarded on the basis of bad interactions and binding energetics with the protein. We also discarded poses where the ligands were docked with their urea group pointing away from the selectivity filter in accordance with experimental results [16].

2.3. MD/LIE simulations

Partial atomic charges for all ligands were obtained using the restrained electrostatic potential (RESP) fitting procedure at the HF/6-31G* level [28], as was done earlier for quaternary ammonium blockers in the KcsA channel [29]. These charges were used together with the Amber95 force field [30].

All MD simulations were carried out with the program Q [23]. Spherical simulation systems of radius 28 Å were used and calculations were done for each ligand free in water and bound inside the hERG pore. A 28 Å thick model membrane of non-polar atoms surrounds the channel [31] and counter ions are present in the solvent so that the same net charge in bound and free states is attained [32]. Long-range electrostatic interactions not involving the blockers were treated by a multipole expansion beyond 10 Å using the local reaction field method [33]. The water surface was subjected to radial and polarization restraints according to our version of the SCAAS model [23,34]. In the channel simulations all atoms outside the solvent sphere were kept fixed by a harmonic restraint of 100 kcal mol^{-1} Å^{-2}. The lipid atoms were restrained to a 3D grid with 2.5 kcal mol^{-1} Å^{-2} force constants and the four ions/waters in the SF were restrained to their respective planes perpendicular to the pore axis with 50 kcal mol^{-1} Å^{-2} force constants in order to prevent possible changes in the filter loading state [27]. Each simulation started with stepwise heating from 0 to 300 K followed by an equilibration of the system for up to 250 ps, after which potential energies were sufficiently stable. Data collection was then run for 250 ps using a time-step of 1 fs, except in case of ligand 14 where 500 ps of data collection was used.

Relative binding free energies, $\Delta\Delta G_{\text{bind}}$, were estimated from the MD simulations with the LIE approach [18], from the scaled differences of the average ligand intermolecular electrostatic and van der Waals energies in the complex and in aqueous solution, according to

$$\Delta G_{\text{bind}} = \alpha\left(\langle V^{\text{vdw}}_{l-s}\rangle_{\text{bound}} - \langle V^{\text{vdw}}_{l-s}\rangle_{\text{free}}\right) + \beta\left(\langle V^{\text{el}}_{l-s}\rangle_{\text{bound}} - \langle V^{\text{el}}_{l-s}\rangle_{\text{free}}\right) \quad (2)$$

Here our earlier parameterization with $\alpha = 0.18$, $\beta = 0.5$ for compounds with net charge +1 and $\beta = 0.43$ for neutral ones [35] is used. The two terms in Eq. (2) that are scaled by α and β, respectively, denote non-polar and polar binding free energy contributions. It has previously been shown that an additional constant term, γ, reflecting the hydrophobicity of the protein binding site, may be needed to reproduce absolute binding affinities [36]. However, since we are mainly concerned with relative affinities here and since there are no experimental absolute binding constants available (only IC_{50} values), such a constant is not considered here although its optimal value can be seen below to be about −10 kcal/mol in this case.

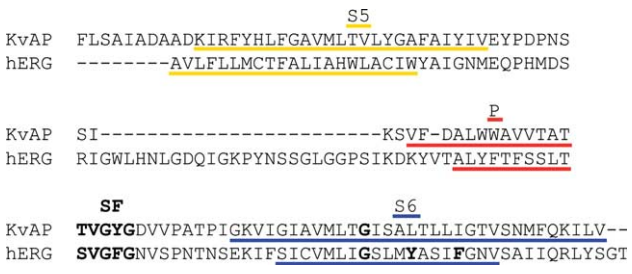

Fig. 3. The sequence 137–240 of KvAP and sequence 548–670 of hERG aligned with T-Coffee. The bold faced letters indicate the well conserved SF (S624-G628), the glycine hinge (G648) and the two aromatic residues important for ligand binding (Y652 and F656). The same color code as in Fig. 1 is used.

3. Results

The result of the alignment for the two sequences shows that we are able to get a good match between the amino acids in the three different parts: P, SF and S6 (Fig. 3), and this is also what is expected from the level of sequence identity. Regarding the conserved and structurally well defined parts of the selectivity filter and the glycine hinge they all result in an unambiguous alignment. The S5 part is more difficult to align in a good way due to its low sequence identity with KvAP so here we get a more uncertain result from T-Coffee. Homology modelling with this alignment resulted in a seemingly reasonable structure showing some features that were expected and required. Since blocker binding most likely involves interactions with the walls of the internal water cavity, the orientation of backbone and side chain groups lining the cavity will be important. Here, we encouragingly find that the side chains of both Y652 and F656 point into the cavity and thereby allow interactions with channel blockers.

The calculations with AutoDock resulted in a set of different ligand conformations ranging from clearly poor poses to apparently favorable conformations in each of the six ligand cases. For almost all of the ligands we obtained only a few highly populated clusters indicating that these are the ones with the most likely pose. As expected, the binding conformations are also fourfold redundant due to the symmetry of the channel. The estimated binding free energies that AutoDock produced for the best ranked poses of each ligand are almost equal, i.e. the program is not able to discriminate between good and weak binders to the ion channel. Fig. 4 shows the clustering result from the docking of ligand 0 and ligand 5, the two ligands with the best and worst IC_{50} values, respectively. We have from all ligand dockings chosen the pose with the lowest docked energy, from the best two or three clusters of each ligand, for the subsequent MD calculations. Ligand 14's docking poses were all found in the very vicinity of the inner

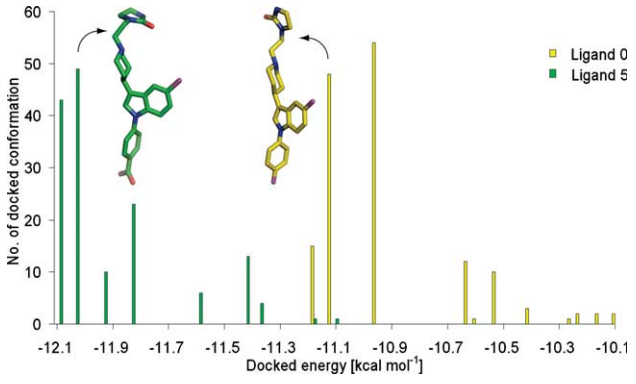

Fig. 4. Two cluster distributions with an RMSD criterion of 2 Å are shown. These represent ligand 0 (yellow carbons) and ligand 5 (green carbons) which are the ligands with the lowest and highest IC_{50} values, respectively. The poses showing the best binding affinities in the MD/LIE simulations originate from the indicated clusters.

part of the SF, binding orthogonal to the pore axis. This pose is not in agreement with the prediction that the ligands hydrophobic parts would interact with Y652 and F656 [16], and the resulting binding affinity from the LIE calculations showed a too positive value. We therefore also examined an alternative pose of ligand 14 that was built from the corresponding best docking pose of ligand 0, and thus maintains the same basic orientation of the common scaffold.

Quantum mechanical calculations yielded rather similar RESP atomic charges for the ligands, although there are obviously variations following the differences in the atomic composition of the blockers. Moreover, the conformations resulting from gas-phase geometry optimization generally coincide well with those obtained from the docking and MD simulations, which indicates that the atomic charges for the blockers are reasonable.

The 1010 and 0101 loading states of the selectivity filter are the ones involved in ion conduction by the KcsA channel [27,37] and in blocker binding to the modelled Kv1.5 channel a preference for 1010 state has been predicted for the local anesthetic bupivacaine [19]. This suggests that one of these states would also be the most likely for blocker binding to hERG and the binding free energies for hERG are again found to be lowest with the 1010 filter loading state and rise with a few of kcal mol^{-1} in the 0101 state, which reflects an electrostatic repulsion of the filter ions with the positively charged quaternary nitrogen of the blockers (data not shown). Table 1 shows the energetics obtained from the MD/LIE simulations and their comparison to experimental IC_{50} values. By plotting the relative LIE free energies versus the experimental ones, obtained as $\Delta\Delta G^{expt}_{bind} = RT \ln(IC_{50}/IC^{ligand\,1}_{50})$, one can directly see the good correlation between calculated and experimental numbers (Fig. 5). The root mean square positional deviations for heavy atoms between the initial and final structures for all complexes range between 1.2 and 1.7 Å, and the structural changes are mainly found in the C-terminal part of the S6 helices while the rest has smaller movements.

Ligands 0, 1 and 3 are predicted to have very similar binding conformations and positions in the channel, where ligand 0 is the compound that both from the calculations and experiment is found to have the highest affinity and its detailed interactions with the channel pore are shown in Fig. 6. The conformation of ligand 1 is slightly more extended than for the other compounds which have their cyclic urea more bent towards the basic nitrogen. It is noteworthy that the charged nitrogen of ligand 0 is situated almost in perfect agreement with the crystallographic position of the cavity potassium ion in KvAP (Fig. 6). Ligands 1 and 3 are found to be slightly offset to the side (by <1 Å) from the pore axis, while for ligands 4 and 5 this offset is about 2 Å and is accompanied by an upwards shift along the pore axis of about 2 Å. Ligand 14 also has its basic nitrogen approximately 2 Å up along the pore axis as compared to ligand 0. The MD structures show hydrogen bonding between the carbonyl oxygen in compounds 0, 1, 3 and 5 and the water molecule in the fourth position of the selectivity filter (Fig. 6).

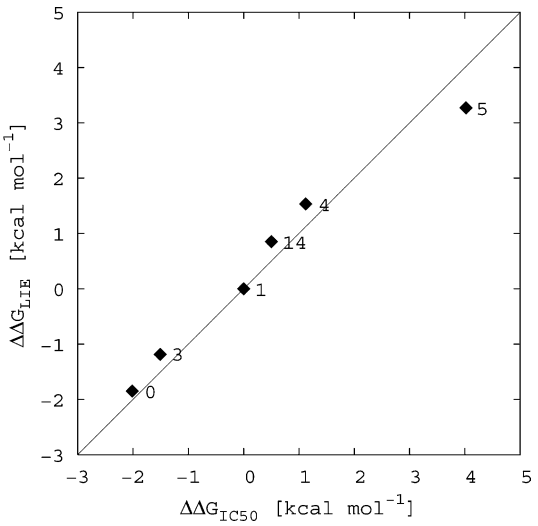

Fig. 5. Calculated relative binding free energies from the MD/LIE simulations vs. experimental IC_{50} values [16]. The value for ligand 1 is used as (an arbitrary) reference for the relative binding free energies.

Favorable hydrogen bonding interactions are further seen with the residues at the inner mouth of the selectivity filter. Ligand 0 shows very good interactions with the carbonyl oxygen of T623 as well as the hydroxyl of S624 (Fig. 6). The carbonyl oxygen of L622 also provides a favorable interaction with the cyclic urea NH group of ligand 4 and, in this case, compensates for the lack of H-bonding to filter water molecule in the fourth position that is found for the other ligands. The non-polar residues Y652, A653 and F656 clearly provide favorable hydrophobic interactions for all the ligands, although these contacts are less optimal for compound 4, 5 and 14 that are shifted slightly upwards relative to ligand 0 (Fig. 6). As a result of their lower binding position along the axis and more extended conformations ligands 0, 1 and 3 make hydrophobic contact with the C_α atom of G657. The main reason for the lower affinities of ligands 4 and 5 appears to be that their carboxylate groups are placed so as to make contact with

Table 1
Calculated binding energetics (kcal/mol) and experimental IC_{50} values (nM) [16] for the selected hERG blockers[a]

	$\Delta G_{AutoDock}$	$\langle V^{vdw}_{l-s}\rangle_w$	$\langle V^{el}_{l-s}\rangle_w$	$\langle V^{vdw}_{l-s}\rangle_p$	$\langle V^{el}_{l-s}\rangle_p$	ΔG^{el}_{corr}	ΔG_{LIE}	IC_{50}
Ligand 0	−11.1	−39.1 ± 0.1	−119.1 ± 1.6	−57.1 ± 0.5	−118.4 ± 1.0	2.4	−0.6 ± 0.4	3
Ligand 1	−10.5	−38.0 ± 0.2	−116.9 ± 1.2	−60.3 ± 0.6	−111.0 ± 0.3	2.4	1.3 ± 0.9	88
Ligand 3	−7.2	−36.6 ± 0.3	−116.0 ± 0.8	−56.8 ± 0.2	−113.2 ± 0.1	2.4	0.1 ± 0.4	7
Ligand 4	−12.4	−35.7 ± 0.0	−201.1 ± 0.3	−60.0 ± 1.1	−184.0 ± 0.5	−0.1	2.9 ± 0.5	579
Ligand 5	−12.0	−34.3 ± 0.5	−191.2 ± 0.9	−58.7 ± 1.1	−170.2 ± 2.5	−0.1	4.6 ± 1.8	75000
Ligand 14	–[b]	−29.2 ± 0.1	−110.1 ± 0.0	−43.0 ± 1.5	−105.6 ± 5.0	2.4	2.1 ± 2.8	204

[a]The tabulated ΔG_{LIE} values are corrected with the long-range electrostatic contribution ΔG^{el}_{corr} [23]. Errors are calculated by taking the difference between the average of the first and the second half of a full trajectory.
[b]Structure built from the ligand 0 docking pose. The selected docked ligand from AutoDock yielded too weak binding when refined by MD/LIE calculations.

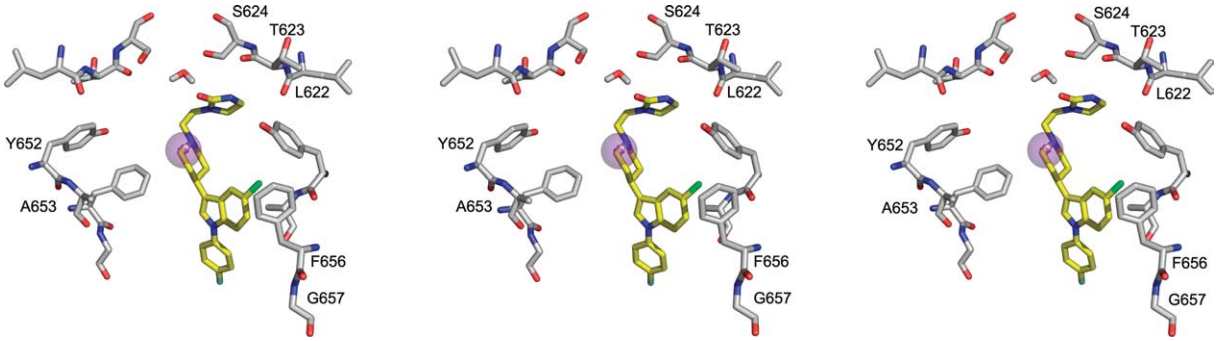

Fig. 6. Stereo picture (cross-eyed and wall-eyed) showing ligand 0 in its position after MD simulation (representative snapshot) together with hERG residues with important polar and non-polar binding contributions for the different ligands. The water in position 4 is shown and the position of the fifth crystallographic K^+ from 1ORQ [6] (transparent sphere) is indicated for comparison.

the solvent and also attain favorable interactions with the backbone NH group of G657 and the sidechain NH of Q664. The latter interactions are, however, weaker and not within the range of H-bonding. The overall effect of this type of binding mode is that the polar binding contribution for these compounds is less favorable than for the other ones and especially so for ligand 5.

4. Discussion

In this work, we have reported modelling of the hERG channel based on the open-state structure of the voltage-gated KvAP potassium channel. Automated docking of channel blockers with this 3D model of hERG was performed and binding affinities were obtained by MD/LIE simulations of the docked complexes. The results of these calculations yield a quantitatively correct ranking of the set of sertindole analogues with a binding affinity spanning 6 kcal mol^{-1} and thus provide support for the present model. Even though the resolution of KvAP is rather low (3.2 Å), the backbone is well defined, which together with the 39% sequence identity of the S6 helices should provide a reasonable template. In particular, since the hERG blockers are known to bind in the open state, it is evident that the KvAP structure should provide a more relevant template than either the KcsA (closed and not voltage-gated) or MthK (open, but not voltage-gated) structures.

The residues F656 seem to be particularly important for binding of the examined blockers to hERG, since they define a rather narrow ring and make hydrophobic contacts with the ligands. This is in agreement with results reported by Mitcheson et al. [14] and Pearlstein et al. [16]. All of the ligands have good contacts in this region, and even ligand 14 that lacks the polar cyclic urea group attains a substantial binding energy due to interactions in this region of the channel. Also, the Y652 residues interact with the ligands as described earlier [14,16] and we also find significant van der Waals interactions with A653 and G657 for the present ligands. Even if these residues are rotated slightly about the S6 helix they can still be of importance for binding ligands. The hinge residue G648 does not appear to play a direct role in blocker binding although its importance is obvious if it acts as a hinge in opening and closing the channel and this probably explains its sensitivity to mutations [14].

An apparent trend can also be observed between the position of the basic nitrogen and the inhibition strength of the ligands. For ligands that position their quaternary ammonium nitrogen close to the crystallographic cavity ion our calculations predict a stronger affinity and this interaction weakens as the ligands' basic nitrogen is shifted up towards the selectivity filter. This could be indicative of an effect from the pore helices that are pointing to the cavity ion. Close to the filter the channel presents a rather polar environment with several potentially favorable interactions. The water in the fourth position provides hydrogen bonding possibilities and we also find that L622, T623 and S624 contribute with favorable binding energy to the ligands examined here. Hence, the present model of hERG essentially predicts that polar complementarity in the upper part of the cavity and non-polar interactions in the hydrophobic region around F656 are the major determinants of blocker affinity.

Acknowledgements: Support from AstraZeneca and the Swedish Research Council (VR) is gratefully acknowledged.

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