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Drug Block of the hERG Potassium Channel: Insight From Modeling

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ABSTRACT **Many commonly used, structurally diverse, drugs block the human ether-a-go-go-related gene (hERG) K^+ channel to cause acquired long QT syndrome, which can lead to sudden death via lethal cardiac arrhythmias. This undesirable side effect is a major hurdle in the development of safe drugs. To gain insight about the structure of hERG and the nature of drug block we have produced structural models of the channel pore domain, into each of which we have docked a set of 20 hERG blockers. In the absence of an experimentally determined three-dimensional structure of hERG, each of the models was validated against site-directed mutagenesis data. First, hERG models were produced of the open and closed channel states, based on homology with the prokaryotic K^+ channel crystal structures. The modeled complexes were in partial agreement with the mutagenesis data. To improve agreement with mutagenesis data, a KcsA-based model was refined by rotating the four copies of the S6 transmembrane helix half a residue position toward the C-terminus, so as to place all residues known to be involved in drug binding in positions lining the central cavity. This model produces complexes that are consistent with mutagenesis data for smaller, but not larger, ligands. Larger ligands could be accommodated following refinement of this model by enlarging the cavity using the inherent flexibility about the glycine hinge (Gly648) in S6, to produce results consistent with the experimental data for the majority of ligands tested. Proteins 2007;68:568–580.** **© 2007 Wiley-Liss, Inc.**

Key words: hERG; molecular modeling; potassium channel; drug block; gating; helix motion

INTRODUCTION

The voltage-dependant human ether-a-go-go-related gene (hERG) potassium (K^+) channel is expressed in cardiac cells and neurons. hERG mediates the rapidly activating delayed rectifier K^+ current (I_{Kr}),[1] one of several K^+ current responsible for the repolarization of the cell membrane during a cardiac action potential.[2,3] Block of hERG by a wide range of medications is linked to acquired long QT syndrome (LQTS), resulting in an increased risk of sudden death via ventricular arrhythmias.[4] The potential for a drug to cause QT prolongation has been the cited reason for withdrawal of several medications from the pharmaceutical market. Consequently, time consuming and expensive screening to detect inhibition of hERG currents has become an essential part of compound development.[5,6] The development and improved safety profile of new medications would therefore result from a more detailed understanding of the molecular basis of drug binding to hERG.[7]

Analogous to the Kv family of potassium channels, hERG comprises a tetramer, with each subunit containing six transmembrane helices[8] [Fig. 1(A)]. The first four of these α-helices (S1–S4) comprise the voltage-sensor domain and the remaining two (S5–S6) form the pore domain of the channel. Movements of the voltage-sensor domain enable the pore domain to open and close in response to changes in membrane potential. The pore domain contains the highly conserved K^+ channel features—the pore helix and selectivity filter—which permit selective passage of K^+ ions. hERG also contains a lengthy S5-P linker (termed the turret), which contains an amphipathic helix (the turret helix).[9,10] The N-terminal domain—containing the Per-Arnt-Sim (PAS) domain[11]—and the C-terminal domain—which has a cyclic nucleotide binding domain—are both located on the intracellular side of the membrane. The drug binding site is contained within the central pore cavity[12,13] of the pore domain, located below the selectivity filter and flanked by the four S6 helices [Fig. 1(B)] of the tetrameric channel. Scanning alanine mutagenesis studies of this region have identified a number of residues as being involved in drug binding—residues Thr623, Ser624, and Val625[14–18] at the base of the selectivity filter, immediately preceding the G(Y/F)G K^+ channel signature sequence,

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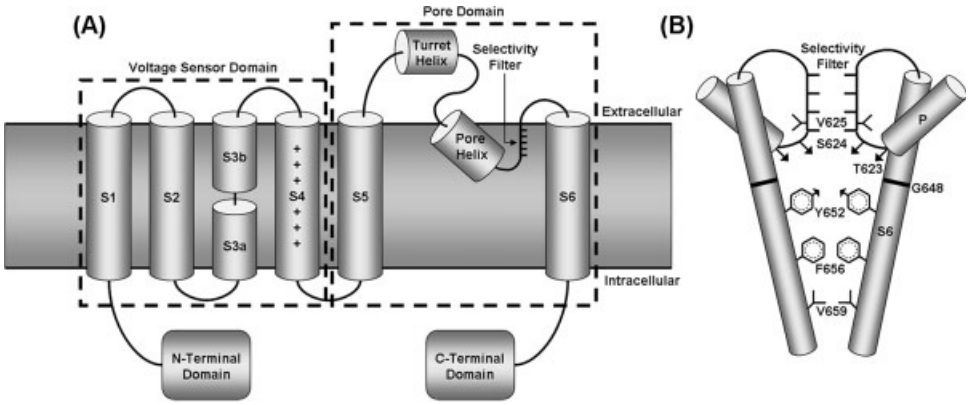

Fig. 1. (**A**) Schematic diagram of the transmembrane topology of a hERG subunit. The transmembrane portion of the hERG channel contains the voltage-sensor and pore domains. A complete hERG channel is comprised of a tetramer of subunits, which fashion a membrane pore. (**B**) Schematic illustrating positions—suggested by scanning alanine mutagenesis—of residues involved in drug binding. The pore helix is denoted P.

and residues Gly648,[14–17] Tyr652,[14–21] Phe656,[14–21] and Val659,[14–17] all of which lie on the same face of S6 [Fig. 1(B)].

A pharmacophore derived from hERG channel blockers[22] gives insight into the molecular determinants of drug molecules that lead to hERG channel binding. Additionally, understanding into the structural determinants of drug binding in hERG has been provided by analogy with the pore domain (S5–S6) of crystal structures for other K^+ channels, with which the amino acids in the pore helix and selectivity filter of hERG show a high degree of amino acid sequence similarity. The first such structure to be determined, that of the bacterial K^+ channel KcsA from *Streptomyces lividans*,[23] was used as the basis (structural template) for a number of homology models of hERG.[16,17,20,24–26] This crystal structure of KcsA is in the closed form. However, block of hERG channels by most drugs requires opening of the activation gate.[12,13] Therefore, once the crystal structure of a K^+ channel in the open state had been determined—that of the bacterial channel MthK from *Methanothermobacter thermautotrophicus*[27]—a number of open state homology models of hERG were produced based on this structural template.[19,26,28] Subsequently, the bacterial voltage-dependent K^+ channel, KvAP from *Aeropyrum pernix*, was also crystallized in the open state[29] and has been used as the structural template for two homology models of hERG.[30,31] The structures of the open and closed states of K^+ channels differ primarily in the S6 helices. In the closed state, the S6 helices are relatively straight and cross over one another toward their C-terminal end to create a point of constriction below the central cavity. This constriction is alleviated in the open state by a kink and swivel of the S6 helices about a glycine hinge—the result of this conformational change is to splay apart the C-terminal ends of the helices and thereby allow access to the central cavity and selectivity filter. A recent study[32] has produced a KcsA[33]-based model as the starting point, and then applied different degrees of "kink" about the glycine hinge[34,35] in S6 to produce both a "partially open state" model and a "fully open state" model.

The presence of the two aromatic amino acids, corresponding to Tyr652 and Phe656 in S6 of hERG—residues critical for binding most hERG channel blockers[14–21]—is unique to the EAG family of potassium channels, suggesting that block is specific. However, most blockers show a far greater affinity for hERG than its relative, EAG, with which it shares 60% sequence identity from the N-terminal end of the pore helix (Tyr610) to the C-terminal end of S6 (Ser668). The principle difference between ERG and EAG is that EAG channels do not inactivate, which may be explained by differences in binding site structure or by changes in K^+ coordination that lead to a reduced electrostatic repulsion between the drug and ion in the inactivated state. One study[36] suggested that residues in the S6 helix of hERG channels and EAG channels do not occupy structurally equivalent positions, as an increase in binding affinity was produced by displacing the key aromatic amino acids in EAG (corresponding to Tyr652 and Phe656 in hERG) one residue toward the C-terminus of S6. This suggests that rotation about, and perhaps displacement along, the helix axis may be required to position the residues in S6 of hERG correctly.

In this study we use a molecular modeling approach—combining homology modeling, molecular docking, normal mode analysis and "morphing"—with a set of 20 hERG blockers to validate structural models of the pore region of hERG against the available body of mutagenesis data. This study suggests that a rotation followed by a slight kink (hinge bending) of the (pore lining) S6 helix should be considered when producing homology models of the hERG K^+ channel.

METHODS

Homology Modeling

Secondary structure and transmembrane helix topology predictions for hERG were performed using PSIPRED v2.4 (Ref. 37) and MEMSAT2 (Ref. 38), respectively, via the PSIPRED server.[39] Proteins of known three-dimensional structure homologous to hERG (i.e. suitable structural

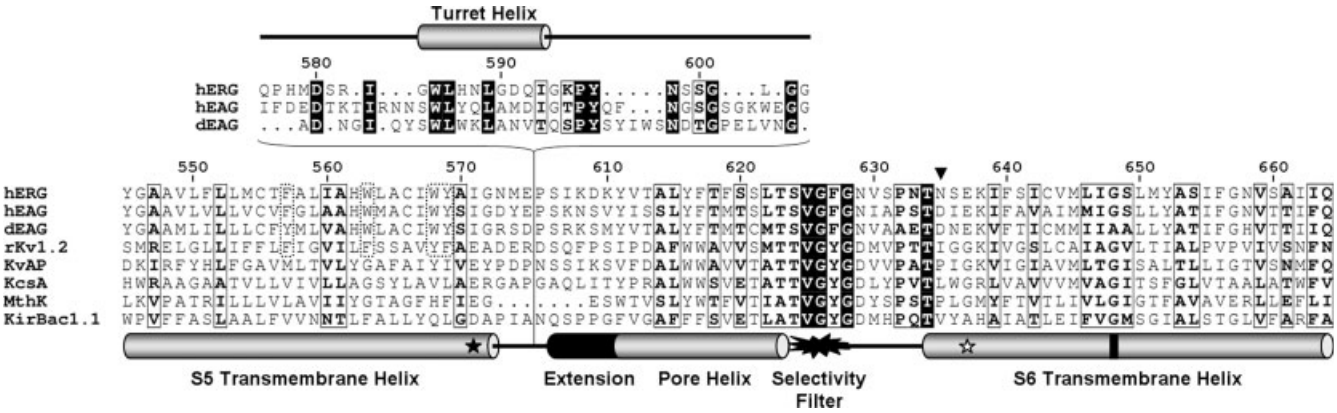

Fig. 2. Sequence alignment used to generate the homology models. This includes hERG (SwissProt accession number: Q12809), hEAG (O95259), dEAG (Q02280), rKv1.2 (PDB code: 2a79), KvAP (1orq), KcsA (1k4c), MthK (1lnq), and KirBac1.1 (1p7b). The secondary structure of hERG, based on predictions and the aligned topology of the crystal structures, is shown beside the alignment. The residues involved in the selectivity filter are shown by a serrated oval shape, the helical regions are marked by grey cylinders, and the loop regions are identified by lines. The predicted extension to the pore helix is shown by the black cylinder. The Turret region of hERG, which is excluded from the homology models, is shown above the alignment, and is aligned with the linkers of hEAG and dEAG. The crystal structures of the K^+ channels possess an acidic amino acid at the C-terminal end of the S5 transmembrane helix, marked by a black star, which permits structural alignment of all five structures, including Kv1.2. The acidic residue that is predicted to form equivalent interactions in the EAG-family of channels is marked by a white star (S6). The EAG-family of sequences was aligned with other voltage-sensitive K^+ channels, exemplified here by rKv1.2, based on the positions of a number of bulky aromatic residues (surrounded by a dashed box). The proposed glycine-hinge is marked by a black box on the S6 helix. To produce the initial rotated model, the sequence of the S6 transmembrane helix of hERG was displaced one residue position toward the C-terminus—as indicated by the inverted triangle above the sequence.

templates) were identified by scanning part of the amino acid sequence of hERG (SwissProt[40] accession number Q12809), corresponding to the region extending from the beginning of the pore helix to the C-terminal end of S6, against the sequences of those structures in the Protein Data Bank[41] using PSI-Blast.[42] This identified the K^+ channels MthK[27] (PDB[41] accession code 1lnq), KvAP[29] (1orq), KirBac1.1[43] (1p7b), KcsA[23,33,44] (1bl8, 1k4c and 1k4d), and Kv1.2[45] (2a79) as suitable template structures. The sequence of hERG was aligned with other eukaryotic voltage-sensitive channels and the sequences of the crystal structures, using ClustalW[46] (Fig. 2). The figure for the sequence alignment was enhanced using ESPRIPT.[47]

The final alignment was then used to generate a set of 10 homology models of hERG based on each of these crystal structures, using Modeller 6v2 and 7v7 (Ref. 48), applying four fold symmetry restraints in all cases. Based on the secondary structure prediction, an additional set of 10 models, for both the open and closed forms, was produced, in which the pore helix was extended by four residues at its N-terminal end in the hERG models by applying α-helical restraints. The full Turret of hERG was omitted from all models and replaced by forming a peptide bond between Glu575 and Pro605, making this region the same length as in the templates (Fig. 2). This loop was refined using Modloop.[49] In each case, the model with the best modeller energy was selected.

Rotation of S6 Helix

In an attempt to improve the correlation between the modeled complexes and available mutagenesis data, the impact of rotating the S6 helix about its axis in the closed state models was investigated. To generate the rotation, two homology models—in which, for simplicity, the pore helix is not extended—were created using KcsA (1bl8) as a template. The two resulting models differ in sequence alignment by one residue position only within the S6 helix (Fig. 2)—these were processed by the Morph server[43] to produce a series of 30 intermediate structures.

Generating Kink in S6 Helix

In addition to the open state homology models (based on KvAP and MthK), normal mode analysis (using the elNémo server[50]) was used to simulate the opening of hERG. This approach was applied to one subunit of the KcsA-based model which had been rotated about the helix axis (structure 16 from the Morph server). elNémo generates a set of 11 structures, of which six describe the transition from the closed state model to a structure comparable to the open state. For each transition step, four copies of the elNémo[50]-generated monomers were assembled into a tetrameric channel based on the position of the corresponding subunits in the structure of KcsA (1bl8).[23] This produced a set of five further tetrameric channels, in which no bad steric clashes were observed. To reduce the deviations from the initial model, the first intermediate (still in the closed state) was selected for further investigation. This hERG model is referred to as the "rotated–hinged model".

Drug Docking

A range of drugs (Table I) known to bind to hERG with varying degrees of affinity were docked into the homology models using GOLD v3.0 (Ref. 63), with the fitness function ChemScore[64,65] to generate 10 solutions

TABLE I. Drugs Known to Bind hERG

Compound	IC_{50} (nM)	Cell type	Mutagenesis	ChemScore values (kJ/mol) Rotated	KcsA	KvAP	References
Astemizole	0.9	HEK293	—	52.3	45.3	31.4	21
Cisapride	6.5	HEK293	**Y652A, F656A**	40.5	33.8	26.8	15,18,51
E-4031	7.7	HEK293	**T623A**, **S624A**, V625A, **G648A, Y652A, F656A, F656V, V659A**	49.6	41.0	26.5	52
Dofetilide	9.5–15	HEK293	S620T, S631A, **T623A**, **S624A**, V625A, **G648A, Y652A, F656A, F656V, V659A**	44.3	37.3	25.0	52–55
Sertindole	14	HEK293	—	45.3	44.3	31.6	21,56
Ibutilide	28	Oocyte	**T623A, S624A**, V625A, **G648A, Y652A, F656A, V659A**	48.5	38.1	29.5	16,17
MK-499	34	Oocyte	**T623A**, V625A, **G648A, Y652A, F656A, V659A**	39.6	33.6	29.3	15,18
Terfenadine	56–204	HEK293	**Y652A, F656A**	54.0	46.0	37.5	15,18
Dronedarone	59	HEK293	**Y652A, F656A** (partial attenuation)	48.7	42.7	35.4	57
Amiodarone	70	HEK293	**Y652A, F656A** (partial attenuation)	38.3	39.5	33.1	57
Loratadine	173	HEK293	—	46.6	43.6	30.8	58
Propafenone	2,160	Oocyte	**F656A**	40.8	43.0	31.9	59
Chloroquine	2,500	HEK293	G628C/S631C, **Y652A, F656A**	40.3	42.2	32.7	19
Fluvoxamine	~3,000	HEK293	S631A, **Y652A, F656A** (partial attenuation)	31.7	38.4	24.3	60,61
Quinidine	8,000	Oocyte	V625A, **Y652A, F656A, F656V**	39.5	42.0	32.7	20,53
Vesnarinone	17,700	Oocyte	**T623A, S624A**, V625A, **G648A, F656A, V659A**	33.8	41.4	29.6	14
Fexofenadine	21,570	HEK293	—	50.2	45.6	30.8	21
Cetirizine	>30,000	Oocyte	—	38.9	45.2	27.8	62
Mosapride	—	COS-7	—	37.1	42.5	30.5	51
Clofilium	uncertain	Oocyte	**T623A, S624A**, V625A, **G648A, Y652A, F656A, V659A**	53.6	47.3	30.5	16,17

Mutations in bold suggest a direct interaction between the sidechain of the residue and the bound compound. Compounds without mutagenesis data have either been used in pharmacophore generation, are unprotonated at physiological pH, or contain a carboxylate group.

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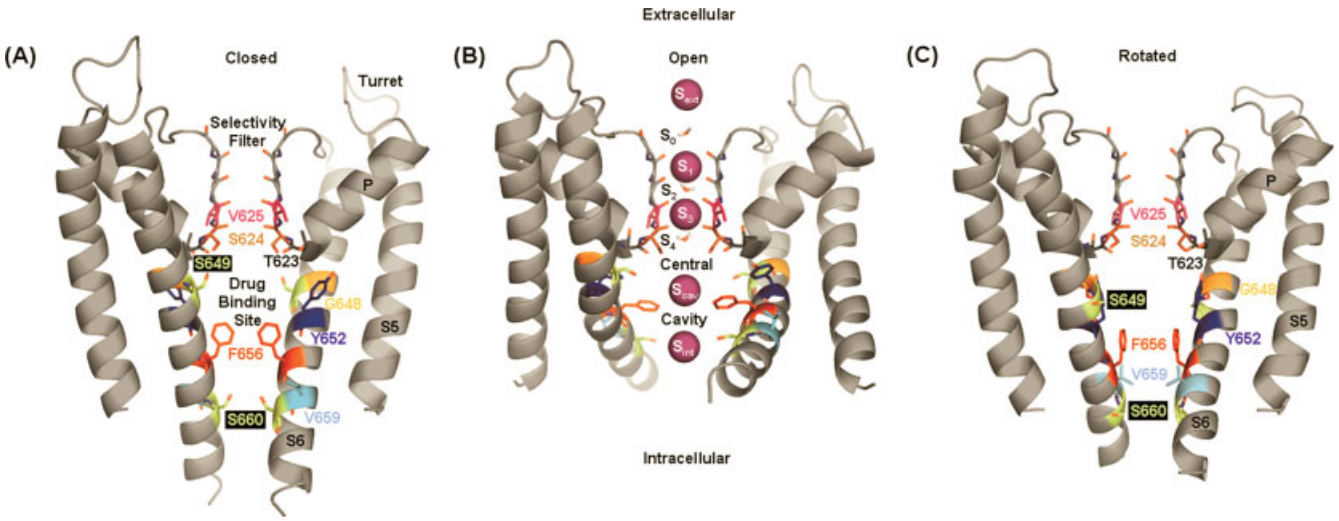

Fig. 3. Models of the pore domain of hERG used for docking studies. (**A**) KcsA-based model (closed state); (**B**) KvAP-based model (open state); (**C**) rotated–hinged hERG model (closed state). These are viewed as a cross-section through the membrane, with only two of the four subunits displayed, describing the outer (S5), pore (P), and inner (S6) helices of hERG. Note that most of the Turret has been omitted (see text). The residues suggested by alanine mutagenesis suggests to be involved in drug binding are Thr623 (dark grey), Ser624 (brown), Val625 (violet), Gly648 (orange), Tyr652 (blue), Phe656 (red), and Val659 (cyan). The hydroxyl sidechains of the Thr623 and Ser624 residues are shown in different conformations in the models—in the closed state and rotated–hinged model, the residues are shown in the predicted optimal conformation for drug binding, while in the open state they are orientated to permit favorable ion coordination. For the dockings performed in the KvAP-based model, both residues were orientated as in the closed and rotated–hinged models. The highly conserved Gly648 is thought to confer a point of flexibility (the "glycine hinge") to the inner helix and to be a pivot point for channel opening. Two serine residues—Ser649 and Ser660—which are not implicated in the binding of all drugs tested are shown in yellow. In the open state model, K^+ ions are shown in purple at K^+ ion sites S_{ext}, S_1, S_3, S_{cav}, and S_{int}, interspersed by water molecules that are found at sites S_0, S_2, and S_4.

for each ligand. The dockings were ranked according to the value of the ChemScore fitness function—which includes terms for hydrogen bonding, hydrophobic interactions, steric overlap between the protein and ligand, and the internal energy of the ligand—and only the best ranked solution for each ligand was included in further analysis. All dockings were performed in the central cavity of the channel using a sphere of 20 Å radius, centered on the coordinates of the S_{cav} K^+ ion, which encompasses all the residues identified by scanning alanine mutagenesis.[16] Binding poses were visualized, for analysis, using Pymol.[66]

RESULTS AND DISCUSSION

Evaluation of Homology Models

Before discussing the results of the drug binding study, it is important to first assess the structure of the homology models into which the ligands are docked. There are three factors to be considered when generating the homology models: (i) the appropriateness of the conformation of the template structure; (ii) the similarity of the sequence of hERG to those of the templates—in particular, the level of conservation of the amino acids of interest; and (iii) the accuracy of the sequence alignment. Inherent in the modeling process is that the model adopts a similar backbone conformation to its template(s). Thus, the KirBac1.1-[43] and KcsA[23,33]-based hERG models are in the closed state, while those based on KvAP,[29] MthK,[27,35] and Kv1.2[45] are in the open state (Fig. 3). Since the rotated–hinged model was also based on KcsA, and despite the slight opening induced by the elNémo method, it is also in the closed state. The change in state is primarily brought about by the S6 helices. In the closed state, the S6 helices are relatively straight and cross over one another to cause a point of constriction below the central cavity. This constriction is alleviated in the open state by a kink and swivel of the S6 helices about a glycine hinge[34,35]—splaying the helices apart to allow access to the central cavity and selectivity filter. As the central cavity is inaccessible in the closed state, the channel must therefore be in the open conformation to allow the drugs to enter and initially bind. In most cases, the drug is likely to stay within the cavity upon channel closure—remaining "trapped" until the channel reactivates. Thus, it is important to consider both the closed and open states of the channel when modeling drug binding. However, since the drug docking complexes in our open state model were less consistent with the experimental data, as discussed later, the manuscript focuses for the most part on a model of the closed state.

S5 Transmembrane Helix

Although the S5 helix is not implicated in drug binding, its packing and interactions with the pore and S6 helices are of significance. Interestingly, the S5 helix is found in different conformations within the crystal structures. The structures of KvAP,[29] KirBac1.1,[43] and more recently Kv1.2[45] channels have displayed equivalent, slanted, outer (S5) helices, while the outer helix of

KcsA[23,33] is bowed, seemingly around Gly49 (equivalent to Trp563 in hERG, based on the sequence alignment). The absence of an equivalent glycine in hERG suggests that the S5 helix is likely to adopt a slanted outer helix.

While it is possible to predict the location of the S5 helix, alignment (i.e. correct register) of the hERG sequence is not trivial, primarily because of the long S5-P linker in hERG and limited amino acid conservation. Analysis of the crystal structures identifies a negatively charged residue at the top (i.e. C-terminal end) of every outer helix (equivalent to S5 in hERG), which—in all but KirBac1.1[43]—is a glutamate and appears to interact with backbone amide nitrogens and a conserved threonine hydroxyl sidechain (hERG Thr634), in the post-selectivity filter loop. This negatively charged residue is located in a structurally conserved position in the five crystal structures. A corresponding negative residue is also found in the majority of voltage-gated potassium channels and is implicated in keeping the post-selectivity filter loop taut, to regulate C-type inactivation.[67–69]

PSIPRED[39] predicts that the S5 helix of hERG terminates at Glu575—quite likely because the algorithm comprises this feature—suggesting that this residue aligns with the other acidic residues. However, since E575C behaves like wild-type,[9] this glutamate does not appear to play an important role in hERG. Interestingly, a glutamate residue is highly conserved in the EAG family, at the top of the inner S6 transmembrane helix—corresponding to Glu637 in hERG. Mutations to this residue result in poor channel expression.[9,70] This residue may be orientated in the models, so that the carboxylate group forms equivalent interactions with the postselectivity filter loop, as observed with the outer helix glutamate in the crystal structures. The similarity of the interactions proposes that an acidic residue is not required at the top of the S5 transmembrane helix in the EAG family of potassium channels, with the glutamate from S6 fulfilling this role.

In addition to Glu575,[30] previous studies have also aligned Tyr569,[8] Ile571,[9] and Pro577,[31] with the conserved negative residue of the crystal structures. While the quality of any of the possible alignments of S5 are relatively poor, alignment of Ile571 with the conserved acidic residues appears most suitable, primarily as this aligns the bulky aromatic residues of hERG with those in other eukaryotic voltage-gated channels, and thus this model has reduced steric clashes at the C-terminal end of S5 (Fig. 2). This alignment positions tyrosine and tryptophan residues at roughly the top and bottom of the S5 helix, where they can interact with the phosphate head groups of the lipid bilayer to anchor the channel in the membrane[52,71] (Fig. 4).

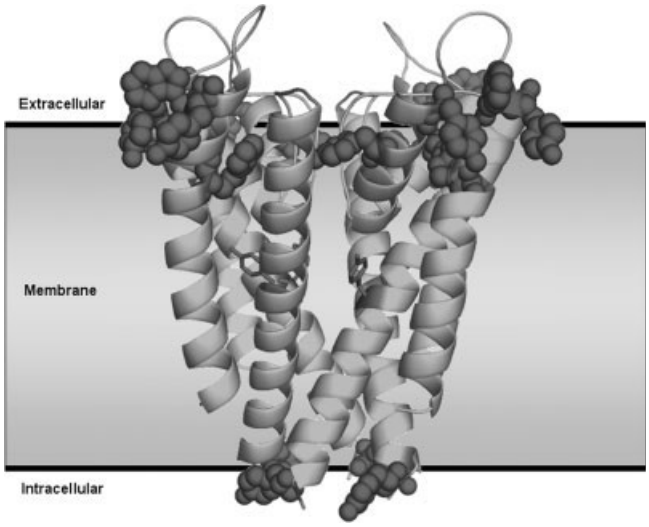

Fig. 4. Likely location of the cell membrane (light grey) relative to tryptophan and tyrosine residues (dark grey spheres) in hERG, illustrated using a tetrameric KcsA-based model of the hERG pore domain. To avoid confusion, Tyr652—which lines the aqueous central cavity of the channel—is shown in stick representation.

Pore Helix and Selectivity Filter

The sequence alignment for the pore helix and selectivity filter is produced with a higher level of confidence than that for S5—with no gaps in the alignment and good residue conservation. As the protein structure is kept rigid during our docking study,[63] it is important that the key residues are positioned in their optimal binding conformation. Thus, it is imperative to evaluate the residues lining the inner cavity, in particular those residues known to be critical for binding. Residues Thr623, Ser624, and Val625, of the pore helix and selectivity filter, shall be discussed first. In the homology models, it is apparent that Thr623 and Ser624 may adopt a rotameric conformation to expose their hydroxyl sidechains to the central cavity, while the sidechain of Val625 is shielded behind the selectivity filter and is unlikely to interact directly with a bound drug. Val625 is highly conserved—as either a valine or isoleucine—in all K^+ channels, and thus is likely required as hydrophobic support for the selectivity filter.[53] Consequently, mutation of Val625 to (the smaller) alanine is liable to have an allosteric effect upon the neighboring residues, and thus influence the shape of the central cavity. Interestingly, slight differences are observed for the corresponding residue in KcsA, depending on whether the structure was crystallized in a high (1k4c) or low (1k4d) concentration of potassium[33]—the structures describe the conducting and proposed inactivated states of the selectivity filter, respectively—although these differences seem to have no impact on the shape of the central cavity. Inactivation is critical for the binding of many drugs to hERG—the observation that the V625A mutant does not inactivate[16] may also account for the reduced drug binding affinity. It is interesting to note that mutation of Val625, to any other residue but alanine, is not tolerated.[18]

Ser624 is represented in all templates by an analogous threonine. The backbone orientation of Ser624 means that its sidechain always faces into the inner cavity, implying that the residue is able to directly interact with the bound drug. The primary role of this residue is to

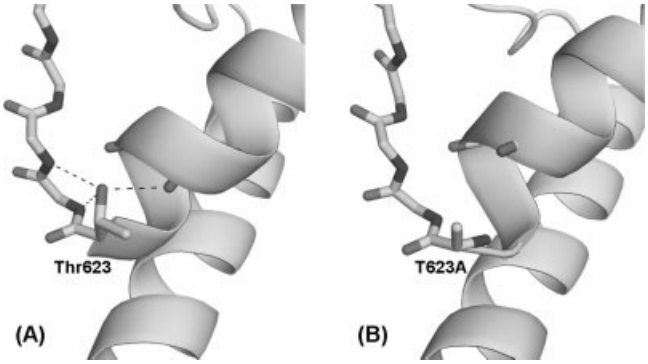

Fig. 5. Comparison of the pore helices of (**A**) KcsA- and (**B**) KirBac1.1-based hERG models. Hydrogen bonds are shown as dashed lines. Substitution of the threonine sidechain to alanine at the hERG 623 position appears to affect the position of the carbonyl oxygen of Phe619, when comparing KcsA- and KirBac1.1-based models.

aid passage of potassium through the selectivity filter, by aiding coordination of ions within the central cavity—forming the S_4 ion coordination site in the filter.[33,57] However, if a potassium ion is found at S_4 it would have an antagonistic effect upon drug binding, as it would compete with the bound compound for the hydroxyl sidechain of Ser624 and also have an electrostatic repulsion effect on the basic molecules. Thus for high affinity binding, it seems unlikely that a potassium ion would be found in this site.

In the Kirbac1.1-based hERG model, the pore helix appears to unravel at its C-terminal end to affect mainly not only the position of Thr623, but also Ser624. More dramatically, this influences the sidechain rotamer adopted by Tyr652—preventing it from pointing toward the pore helix, because of clashes between the sidechain hydroxyl of Tyr652 and the mainchain of the pore helix. This may also be due to slight changes to the position of the inner helix. Thr623 is conserved across a number of voltage-gated K^+ channels—this sidechain appears structurally important in the crystal structures where it is present, interacting with the pore helix mainchain to cap the end of the helix [Fig. 5(A)]. Interestingly, while Thr623 is retained in KcsA and KvAP, it is replaced by an alanine in KirBac1.1—the absence of this helix capping hydrogen bond appears to induce the change at the C-terminal end of the pore helix, in particular, the position of the carbonyl oxygen of Phe619 [Fig. 5(B)]. Thus, the loss of the polar sidechain may affect drug binding through unwinding of the pore helices rather than the loss of a hydrogen bond partner. This could either cause an occlusion of the top corner of the central cavity, or—perhaps less likely—reduce the interaction of the helix dipole with the charged moiety of the drugs within the central cavity.

In most cases, if a compound is influenced by S624A, T623A is also likely to have an impact on drug binding. In addition, a T623A:S624A double mutant had a more pronounced reduction on the binding of a set of clofilium analogues than the single mutations alone.[18] This suggests that one residue may be able to partially compensate for the loss of the other. It is possible to orientate both Thr623 and Ser624, so that both their sidechain hydroxyl groups point toward the top corner of the cavity. In this scenario, Ser624 would not be able to coordinate a potassium ion at S_4 and Thr623 would no longer cap the C-terminal end of the pore helix [Fig. 5(A)]. Such motions could potentially be a component of the inactivation process. Both sidechains were thus orientated in this manner in all models.

S6 Transmembrane Helix

It is possible to align the S6 transmembrane helix (inner helix) using the position of the glycine-hinge residue, Gly648, which is conserved in all templates and is an equal number of residues from the selectivity filter in all cases.[35] The intrinsic flexibility of this residue is believed to permit the movements that open the channel.[34,35] Based on the crystal structures, the drug binding site of hERG differs quite dramatically between the closed and open states. This is caused by the kink, swivel, and slight rise of the S6 helix upon opening.[29,35] In addition to the gross structural changes of the helix backbone, there is a secondary effect brought about by the proximity of the Tyr652 sidechain to the pore helix. This constrains Tyr652 to point toward the cytoplasmic entrance, and thus compels Phe656 to do the same in the open state.

These three residues, Gly648, Tyr652, and Phe656, appear important in the binding of most drugs to hERG. In addition, Val659 is also implicated in drug binding, though in all models, based directly on the crystal structures, this residue does not face into the conduction axis. Interestingly, two residues that mutagenesis studies suggest are not involved in drug binding[14–17]—Ser649 and Ser660—are located in suitable positions to interact with the bound drug. This is particularly the case in the KcsA-based model, where the sidechain of Ser660 forms the constriction point below the central cavity, independent of rotamer orientation, and Ser649 partially shields Tyr652 from the cavity, with the hydroxyl group of Ser660 able to focus on the same region as Thr623 and Ser624 (Fig. 3). This discrepancy between the modeling results and experiment—i.e. residues that line the pore but are apparently not involved in drug binding—is suggestive of a shortcoming in the modeling. Thus despite the merits of the crystal structure-based homology models, it is apparent that further modifications are required to improve the agreement between the models and the available mutagenesis data for drug binding.

S6 Transmembrane Helix Rotation

To overcome this apparent problem with the modeling, possible improvements afforded by rotation of the S6 helix were investigated. Rotation of the S6 transmembrane helices in the KcsA-based model one residue toward the C-terminal end of the helix, as inferred by Chen et al.,[36] dramatically changes the positions of the aromatic residues. This results in the four Tyr652 phenyl rings being

packed into the central cavity, while in contrast Phe656 is inaccessible. This would appear inappropriate as Phe656 is critical for high affinity drug binding. In this scenario, Val659 forms the hydrophobic gate of the channel. To reduce the impact of the rotation, a set of 30 intermediate structures, which represent differing amounts of rotation of the S6 helix, were created. These models were evaluated against the criterion that all residues implicated in binding from the mutagenesis studies are retained within the binding site, while Ser649 and Ser660 are displaced from it. The model that best fulfilled this criterion was intermediate 16. However, in this model the Phe656 residues were tightly packed together across the cavity, and consequently hindered the binding of the larger ligands in preliminary docking trials. Therefore, normal mode analysis, which investigates the intrinsic flexibility of the helix, was used to simulate channel opening and to relieve the constriction. We were encouraged by our validation of this approach, in which we created a model of KvAP based on the KcsA structure and then simulated its opening—the resultant open state model was closely similar to the KvAP crystal structure. This produced a set of five further models, which describe a gradual channel opening. To minimize the deviation from the initial structure, the first intermediate structure—the rotated–hinged model—was selected.

Key Residues in the Rotated–Hinged S6 Helix

In this closed state model, the four residues of the S6 helix, which are predicted to be involved in drug binding, now all face into the conduction pore, while the two serine residues are rotated away. This also removes the partial shielding of Tyr652 by Ser649 as observed in the KcsA-based model. This model positions Tyr652 and Phe656 so that they point up toward the selectivity filter. The roles of two aromatic residues—Tyr652 and Phe656—in drug binding have been well characterized in a recent study,[19] which suggested that while an aromatic group is required at the position of Tyr652 to participate in either π-stacking or cation-π interactions, a hydrophobic residue is required at the position of Phe656 to retain high affinity drug binding. On the other hand, the roles of two other residues Gly648 and Val659 are less certain. Surprisingly, mutation of Gly648 to alanine does not significantly alter activation gating in hERG, which is interesting since a reduction in S6 flexibility would be expected.[16] However, a change in inactivation gating of G648A is observed.[16] The rotated–hinged model suggests that this may be caused by the additional methyl group of the alanine interacting sterically with the pore helix—particularly Leu622—and impacting on the structure of the selectivity filter. Such a structural role for Leu622 is supported by the observation that the L622A channel does not express.[16] Thus, since Gly648 is found in close proximity to the bottom of the pore helix, its role in hERG may be to allow tight packing between the pore and S6 helices. This region of the cavity is also close to the focal point of Thr623 and Ser624 sidechain hydroxyls, which is amplified by the rotation, providing a possible explanation as to why introduction of the methyl group in G648A channels influences drug binding.[14–17] Additionally, the alanine mutant may also displace other residues, most likely Tyr652.[16]

Of the four residues, the V659A mutant has the lowest effect upon drug binding. Considering its hydrophobic nature and its position, one turn of the helix from Phe656, it seems likely that Val659 also forms hydrophobic interactions with drugs, albeit to a lesser extent than Phe656. It is interesting to note that the V659A mutation dramatically slows the rate of channel deactivation,[16] perhaps because its sidechain forms part of the activation gate and thus is required for complete channel closure.

Evaluating the Drug Binding Modes

The structural changes that occur in the binding site between the open and closed states are reflected in the results of the ligand docking study, where a state-dependent change in binding mode is observed. In the open state, the majority of drugs bind within the cavity in a "curled" conformation, roughly perpendicular to the pore axis [Fig. 6(A)]. This is comparable to that observed in a recent docking study within a KvAP-based model.[31] Conversely, binding to the closed state is dependent on the positions of Phe656 in the four subunits. If these are close enough to one another to constrict the pore then the drugs tend to bind in a similar "curled" fashion to the open state. Since the curled binding mode is adopted in both states, it is tempting to suggest that this is the typical mode for binding to hERG, though this binding mode does not compare favorably with available experimental data. On the other hand, if the phenylalanine residues are far enough apart, then a fragment of the drug molecule is observed to lie in the resulting narrow aperture, with the remainder of the drug lying within the central cavity [Fig. 6(B)]. This is observed in our docking studies involving both the KcsA-based and rotated–hinged models. However, Ser649 and Ser660 appear to influence the dockings in the nonrotated model; in some cases, this simply means that these residues may interact with the ligand, and in other instances the ligand binds upturned, as shown for E-4031 [Fig. 6(B)]. The rotated–hinged model reduces these inconsistencies, and most of the drugs investigated bind in a manner that correlates well with available mutagenesis[16,19] and *in silico* studies,[22,28] thus all the dockings discussed will be those performed with the rotated–hinged model of hERG.

Placement of the hERG Pharmacophore Within the Drug Binding Site of the Rotated–Hinged Model

Is it possible to combine the physiochemical properties of the binding site[19] with the features of the pharmacophore created by Cavalli et al.[22] The suggested require-

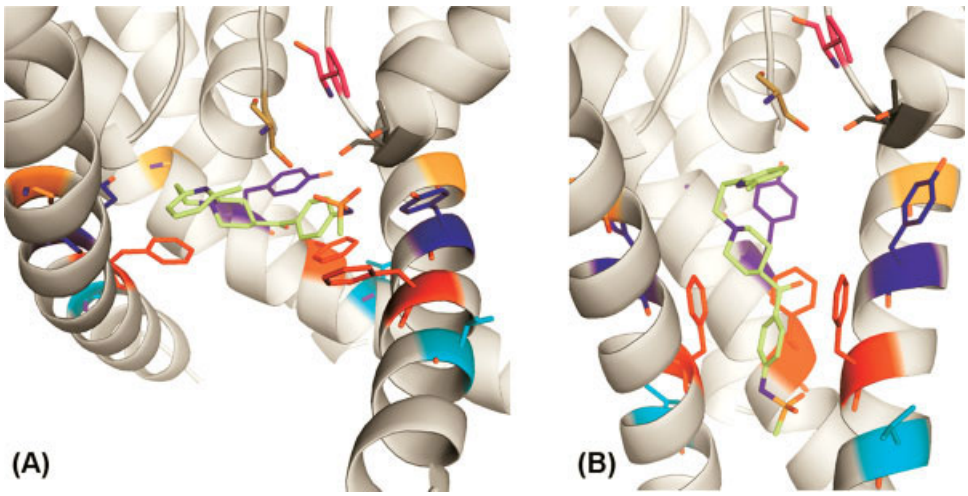

Fig. 6. Dockings of E-4031 to (**A**) KvAP (open state; "curled" ligand pose) and (**B**) KcsA-based (closed state; "upturned" ligand pose) models of hERG to exemplify the different binding modes that are encountered in these homology models. These binding modes show poorer agreement to the mutagenesis data—primarily the residues of the pore helix and selectivity filter—than those for the rotated-hinged hERG model.

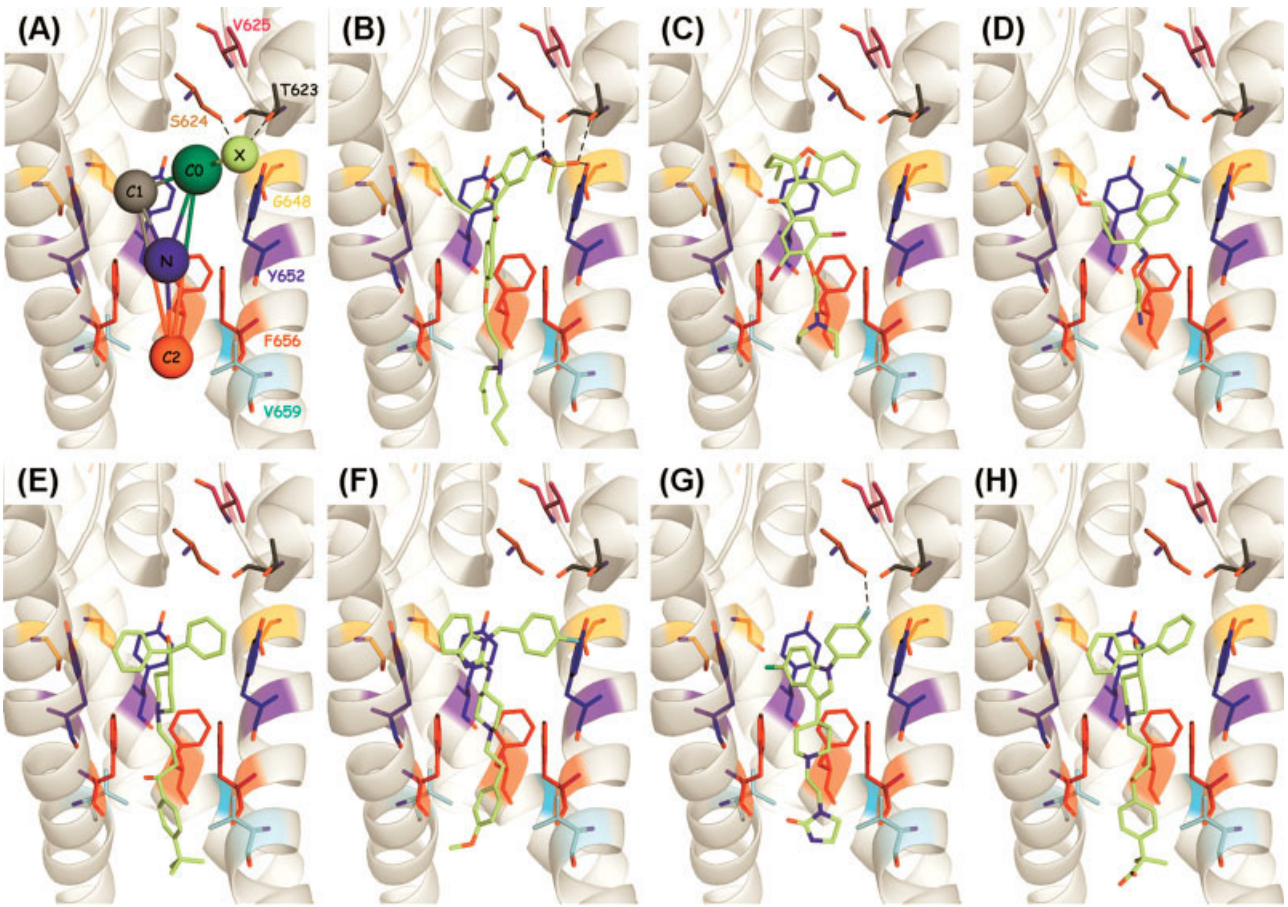

Fig. 7. Dockings within the rotated–hinged hERG model. (**A**) The hERG pharmacophore docked into the central cavity of the rotated–hinged hERG model. Here a hydrogen bond acceptor feature has been added to the pharmacophore (yellow, denoted X) and is generally connected at the para-position of the C0 phenyl ring (green). Thr623 (black sticks) and Ser624 (brown) may hydrogen bond to X in a pocket at the top corner of the cavity, close to the mainchain of Gly648 (orange). The position of this group permits the C0 phenyl ring feature to π-stack against Tyr652 (blue), while C1 (dark grey) may also interact with this residue through either hydrophobic or aromatic contacts. The charged nitrogen feature, N (blue), is placed above Phe656 (red), central to the cavity, and may be coordinated by the combined effects of the pore helix dipole and cooperative cation-π interactions with Tyr652. The region of drug below the nitrogen is usually hydrophobic in nature and relates to the C2 (red) position, permitting hydrophobic contacts with Phe656 and Val659 (cyan). Val625 (magenta) lies away from the binding site and is unlikely to directly interact with a drug. (**B**) Dronedarone, (**C**) Amiodarone, (**D**) Fluvoxamine, (**E**) Terfenadine, (**F**) Astemizole, (**G**) Sertindole, and (**H**) Fexofenadine.

ment for an aromatic residue at the position of Tyr652 in hERG, coupled with the essential C0 phenyl ring in the pharmacophore, suggest a π-stacking interaction between drug and receptor at this site [Fig. 7(A)]. It seems sensible that the N feature of the pharmacophore would bind in a position equivalent to that of a K^+ ion. This best coincides with the S_{cav} K^+ site, which was resolved in a number of K^+ channel crystal structures (Fig. 3). At this site, the nitrogen would be coordinated by the pore helix dipole and by the Tyr652 through cation-π interactions. As both the C2 feature and Phe656 are predicted to primarily form hydrophobic contacts, it is reasonable to position the C2 feature between the Phe656 residues. This then allows the remaining C1 feature to interact with the Tyr652, through either π-stacking or hydrophobic contacts. In this orientation of the pharmacophore, it is feasible that a substituent attached to the C0 phenyl ring, which in many compounds that bind with a high affinity is either a methanesulfonamide or electronegative halogen, may interact with Ser624 or Thr623 or both.[18] Based on this, a hydrogen bond acceptor feature could be added to the pharmacophore at the top corner of the cavity, close to the mainchain of Gly648 [Fig. 7(A)]. It should be noted that the pharmacophore can also be placed into models of the open state of hERG. However, in this orientation, the C2 region of the pharmacophore forms limited contacts with the rest of the protein. The pharmacophore also forms poor contacts with Tyr652 and Phe656, because of the kink and slight rise of the S6 transmembrane helix in the KvAP-based models. If the pharmacophore is positioned perpendicular to the pore axis—in a curled conformation—the couplings between (i) the aromatic C0 and Tyr652, and (ii) the hydrophobic C2 and Phe656 become ambiguous.

Comparison Between the Mutagenesis and Rotated–Hinged Model Dockings

There are now mutagenesis data for many drugs that block the hERG channel. For the majority of these compounds, the docking pose with the highest score fits well with both the mutagenesis and position of the pharmacophore within the channel. The dockings of the methanesulfonanilide compounds, including E-4031,[15] dofetilide,[60] ibutilide,[17,18] and dronedarone[61] [Fig. 7(B)], suggest that the two oxygen atoms of the methanesulfonamide group act as hydrogen bond acceptors, interacting with the hydroxyl groups on the sidechains of Thr623 and Ser624, with a phenyl ring π-stacking against Tyr652 and the remainder of the compound lying vertically along the conduction axis. A fifth methanesulphonamide compound, MK-499,[16,19] is docked in a similar manner, though is not shown to form interactions with either Thr623 or Ser624, perhaps due to structural restraints imposed by its ring system. Of the other dockings, dronedarone is especially interesting, as its protonated nitrogen binds well below the central cavity, at a position roughly the same as the S_{int} K^+ site, which was identified in the crystal structure of KvAP[29] (Fig. 3). A close analogue, amiodarone,[61] docks in a very similar manner to dronedarone, despite lacking the methanesulfonamide group [Fig. 7(C)]. Surprisingly, despite apparent strong involvement in the dockings, the binding of both molecules are only partially attenuated by the Tyr652 and Phe656 mutations.[61] With both aromatic residues forming the major structural components of the cavity, it is difficult to understand how a compound could bind within this site without interacting with either residue. However, it is possible that one aromatic residue compensates for the loss of the other.[61] Although hypothetical, it is tempting to suggest that the position of the central nitrogen within the docking is significant to the attenuation being only partial. It would also be useful to have additional experimental data on the importance of Thr623 and Ser624 in the binding of these compounds. Interestingly, a further compound fluvoxamine,[59] which is shown to be only mildly influenced by the Y652A and F656A mutations, is docked into the channel, so that its protonated nitrogen binds below the Phe656 residues and outside the central cavity. This permits the trifluoromethyl group of the compound to interact with what has been referred to as the "non-aromatic" binding site formed by Ser624 and Thr623[72] [Fig. 7(D)].

The bindings of two other compounds, propafenone[26] and vesnarinone,[14] are not affected by the Y652A mutation, while in these cases, F656A does reduce the drug affinity. In the dockings, both compounds appear to form π-stacking interactions with Tyr652. Again, the nature and position of the charged nitrogen of the compounds appear to be important. In the case of propafenone, the nitrogen is predicted to lie between the Phe656 residues, potentially forming cation-π interactions with this residue, and not within the cavity itself. On the other hand, the nitrogen of vesnarinone is not predicted to be protonated, which reduces the impact of Tyr652, and has been cited as the principle reason why this compound binds with a relatively low affinity.[14]

Three other high affinity compounds for which mutagenesis data are known are terfenadine,[16,19] cisapride,[16,19] and clofilium.[17,18] While terfenadine binds in a manner predicted by the mutagenesis [Fig. 7(E)] and the pharmacophore placement within the channel, clofilium binds with its C0 feature below the Phe656 residues and cisapride docks entirely along the conduction axis, with its C0 encroaching into the selectivity filter, rather than making the expected contacts with Tyr652. In both cases, if GoldScore is used rather than ChemScore, π-stacking with Tyr652 is observed for the binding mode with the highest score, while the attached polar groups may interact with Thr623 and Ser624. In the case of clofilium, this seems a more likely docking mode, given the compound's structural similarity with ibutilide, in addition to the mutagenesis data.[18]

Finally, two further structurally similar compounds for which mutagenesis data are known, investigated in this study, are quinidine[60] and chloroquine.[20] In both cases, the nitrogen sits just above Phe656, with the ring systems of the compounds π-stacked against Tyr652. In

these two dockings, Phe656 seems more important for providing hydrophobic packing to the central cavity, and thus stabilizing the binding mode.

In addition to displaying a better agreement with the results of the mutagenesis, the rotated–hinged model also has an improved correlation between experimental and computationally predicted affinities for compounds binding to hERG. The docking scores obtained from our nonrotated closed and open state homology models (Table I) show no correlation with the affinity (pIC_{50}s) for this set of 20 compounds (Table I)—producing an r^2 value of almost zero. The improvement in correlation when docking into the rotated–hinged model is encouraging and suggests that the modifications made to the original hERG model have merit.

Comparison With Drug Pharmacophore

The placement of the pharmacophore within the central cavity provides a further tool to predict and assess the binding of further compounds within the binding site. The compound with the highest affinity for hERG within this set is astemizole.[22] This compound contains all four features of the pharmacophore, with a fluorine atom attached at the para-position of the C0 phenyl ring. The docking with the highest score agrees with the pharmacophore placement, though in this case the fluorine atom is not within hydrogen bonding distance of the two pore helix residues [Fig. 7(F)]. A similar docking is observed for sertindole[22,28] [Fig. 7(G)]. The C2 "tail" of sertindole is more hydrophilic than usually found in blockers of hERG, which may explain why previous studies have predicted and used an upturned binding mode for this compound.[28,30] However, the similarity of the binding mode of sertindole with that found for the structurally comparable astemizole and terfenadine—which have a hydrophobic tail—suggests that this is the optimum mode of binding of sertindole in hERG.

Influence of Acidic Moieties and Nitrogen Deprotonation

Generally, the addition of an acidic group or absence of a protonated or charged nitrogen within a compound reduces its affinity for hERG. Thus four compounds of this nature, which bear close structural similarity to hERG blockers, were selected to test the predictivity of the docking method. Two compounds, fexofenadine[62] and cetirizine,[58] contain an acidic moiety within their C2 tail. While another two, loratadine[51,73] and mosapride,[74] are not predicted to be protonated at physiological pH. In addition, mosapride is an analogue of cisapride,[74] while fexofenadine is a metabolite of terfenadine.[75] Interestingly, fexofenadine, cetirizine, and loratadine are three widely used antihistamines.[75] The ChemScore values for the docking of cetirizine and mosapride are both low, at 38.9 and 37.1 kJ/mol, respectively, despite binding in a manner that agrees with the pharmacophore. On the other hand, the ChemScore value for fexofenadine was high (50.2 kJ/mol), and the docking mode was identical to that of terfenadine [Fig. 7(H)]. Since the docking suggests that the carboxylate group does not directly interfere with hERG binding, it may be that the acidic group hinders the access of the compounds to the binding site, perhaps by interacting with a site distant from the cavity. A homology model of the C-terminal domain of hERG contains numerous, pore lining, positively charged amino acids that may impede the access of a compound containing an acidic group to the central cavity (Stansfeld and Sutcliffe, unpublished data). Alternatively, as suggested by Pearlstein et al.,[28] the acidic group may cause the molecule to adopt another conformation, conceivably by forming a salt bridge with the protonated nitrogen, hence interfering with drug binding. However, since fexofenadine may still induce pharmacological rescue of trafficking-deficient mutants by binding to the central cavity, it is likely that some of the drug molecules are still able to bind at this site.[54] The final compound, loratadine, binds in an unusual fashion, perpendicular to the pore axis, which may be due to the lack of a basic center or the steric restraints imposed by its ring system. Surprisingly, this binding mode scores highly, but it seems unlikely that this is a suitable reflection of the binding of loratadine.

CONCLUSIONS

With a number of K^+ channel crystal structures now available and aided by the generally good level of conservation of amino acids in the pore helix, selectivity filter, and inner (S6) transmembrane helix, it is possible to use standard homology modeling techniques to generate a variety of homology models representing the pore structure of a K^+ channel in both its open and closed state. In this study, we have created structural models of the hERG pore domain to construct a drug binding site within this channel. The homology models produced by the traditional method position the majority of the amino acids, deemed by mutagenesis to be critical for direct drug binding, so that they line the central cavity. However, in these models—in contrast to mutagenesis studies—Ser649 and Ser660 are also in suitable positions to influence drug binding. A slight rotation of the S6 helix of hERG removes the hydroxyl sidechains of these serine residues from the binding site, whilst retaining the critical aromatic amino acids in the binding site. This also positions the sidechain of Val659 to point toward the pore axis. Qualitatively the method is very efficient, and comparison of the docked poses for the hERG blockers with the known mutagenesis, pharmacophore, and the physicochemistry of the binding site agree well.

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