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The hERG Potassium Channel and Drug Trapping: Insight from Docking Studies with Propafenone Derivatives

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The inner cavity of the hERG potassium ion channel can accommodate large, structurally diverse compounds that can be trapped in the channel by closure of the activation gate. A small set of propafenone derivatives was synthesized, and both use-dependency and recovery from block were tested in order to gain insight into the behavior of these compounds with respect to trapping and non-trapping. Ligand–protein docking into homology models of the closed and open state of the hERG channel provides the first evidence for the molecular basis of drug trapping.

Introduction

Acquired long QT syndrome causes severe cardiac side effects and is a significant problem in clinical studies of drug candidates.[1–3] One of the causes behind the development of arrhythmias related to long QT syndrome is inhibition of the human ether-à-go-go-related gene (hERG) potassium channel.[4–6] Hence there is increasing interest in computational models that can predict affinity for hERG binding in the early phases of drug discovery and development.[7–12] The inner cavity of the hERG channel is composed of a promiscuous binding site and is capable of trapping diverse compounds in the closed-channel state.[13–18]

The majority of drugs interact with hERG within the central cavity of the channel, thus blocking K^+ ion conduction. Based on the data from alanine scanning and site-directed mutagenesis experiments, the drug binding site seems to involve three residues at the base of the selectivity filter (Thr 623, Ser 624, and Val 625) and four on the S6 transmembrane helix (Gly 648, Tyr 652, Phe 656 and Val 659). In the tetrameric channel protein, two concentric rings formed by the symmetric tetrad of Phe 656 groups located closer to the mouth of the channel, and by the four Tyr 652 residues situated closer to the pore helix, thereby facing the interior of the conduction pathway, contribute to multiple and compound-specific interactions. In contrast to other potassium channels, the inner cavity of the hERG K^+ ion channel is sufficiently large to physically accommodate structurally diverse compounds that can be trapped in the channel upon closure of the activation gate.[17,18] This phenomenon of drug trapping was observed from many high- and low-affinity compounds, which only recover from the block relatively slowly.[14–18,19]

We recently investigated the kinetics of hERG channel inhibition and recovery from block by eight blockers at different frequencies.[15] The results indicated that apparently 'trapped' drugs (bepridil, domperidone, E-4031, and terfenadine) do not dissociate from the closed (resting) channel state, whereas amiodarone, cisapride, droperidol, and haloperidol do dissociate. All eight compounds dissociated from the open channels.

In this study, a small set of propafenone derivatives were synthesized and tested for hERG activity. Docking into protein homology models of the open and closed states of the hERG channel provide the first insight into the structural basis of trapping phenomena.

Results and Discussion

Chemistry of propafenone and its derivatives

The preparation of the tertiary amines (Table 1) was carried out analogously to the synthesis of propafenone (Scheme 1).[20] Thus, an appropriate *o*-hydroxyphenone **1** was allowed to react with epichlorohydrine to give the epoxides **2**. Subsequent treatment with an amine yielded the corresponding propanolamines **3**, which were converted into their respective

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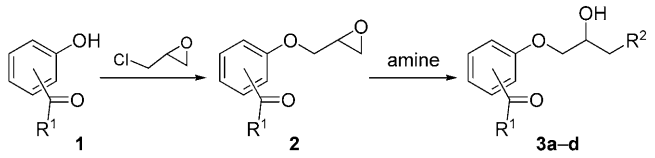


Scheme 1. Synthesis of propafenone derivatives **3a–d**.

hydrochlorides by using standard procedures. Compounds **3a** and **3d** were prepared as previously described.[20–22]

hERG K^+ channel homology models

The open-state homology model of the hERG channel was obtained with the crystal structure of K_vAP as previously described.[23] The four Tyr652 and four Phe656 residues make concentric rings, as mentioned above. The first ring is formed by the four Phe656 groups and is located at the cytoplasmic side of the hERG channel. The planes of the four aromatic residues are parallel to the axis of the channel. The second ring is made by the four Tyr652 groups, which face the inner cavity like Phe656, but their aromatic residues are neither parallel nor perpendicular to the channel axis. The amino acids Thr623, Ser624, and Val625 are placed under the selectivity filter and delimit the top of the inner cavity. The hydroxy groups of Thr623 and Ser624 face the inner cavity, and they can be involved in hydrogen bonding with potent hERG blockers.[14,24]

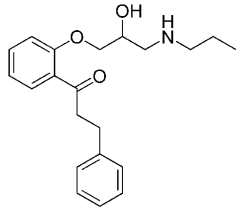
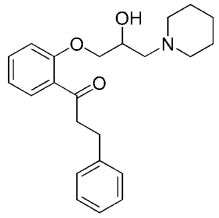
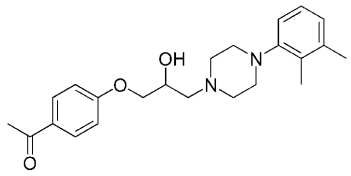
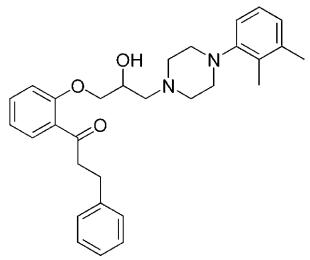
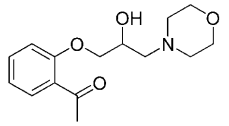
The closed-state homology model of the hERG channel was obtained with the same procedures as for the open conformation. An initial model was generated by using the crystal structure of KcsA (PDB ID: 1K4C[25]). The alignment used for generating the resting-state model is given in the Supporting Information. Details for modeling the S5 P-linker can be found in the report by Tseng et al.[23]

In transition from the open to the closed state, the movement of residues Thr623, Ser624, and Val625 is very small, and so these amino acids are in the same positions in both the open- and closed-state homology models. In contrast to the top of the open-state model, the closed-state model exhibits a significant movement of the S5 and S6 domains, which close the inner pore. In the closed state, the aromatic ring plane of Tyr652 is oriented perpendicular to the axis of the channel with the hydroxy group directed away from the hERG channel, whereas Phe656 has the same orientation as in the open state. From the open state to the closed state, the distances between the C^α atoms of Phe656 and Tyr652 residues from opposite subunits decrease from 15.6 to 9.4 Å and from 19.3 to 14.3 Å, respectively. This also results in a $>30\%$ decrease in the volume of the inner cavity, from 1642.2 $Å^3$ in the open state to 1048.2 $Å^3$ in the closed state.

Trapping of propafenone derivatives

In this study, a systematic analysis of use-dependency of channel block and recovery from the block was performed for a set of five propafenone derivatives (Table 1). Four out of five derivatives tested exhibit only small differences in IC_{50} values, but show different trapping behaviors. The *n*-propylaminopropafenone and the piperidine analogue **3a** are trapped, the two xylylpiperazine analogues **3b** and **3c** are non-trapped. The highly hydrophilic morpholine **3d** is inactive. Half-maximal inhibition of hERG channels was estimated as peak tail current inhibition (Figure 1A,B). Classification of drug trapping was mainly set by assessing recovery from block at rest. For this purpose, channel block was induced by applying 1 Hz conditioning trains until steady state was reached. hERG currents were subsequently measured after a rest period of 330 s at −80 mV resting potential. hERG channels recovered from block to a substantial extent ($>65\%$) with compounds **3b** and **3c** (Figure 1D), whereas negligible recovery ($<5\%$) was observed in the presence of propafenone and compound **3a** (Figure 1C). Recovery from block indicates whether a compound dissociates from the closed channel state during the rest period, or if it is trapped at channel closure.

Table 1. hERG assay results for propafenone derivatives.

Compound	Structure	IC_{50} [μM][a]	Trapped
Propafenone		3.95 ± 2.05	Yes
3a		1.17 ± 0.29	Yes
3b		5.41 ± 1.01	No
3c		2.09 ± 0.55	No
3d		Inactive	ND[b]

[a] Values are the mean of three experiments and were measured in heterologous hERG expressed in *X. laevis* oocytes.[15,19,44] [b] Not determined.

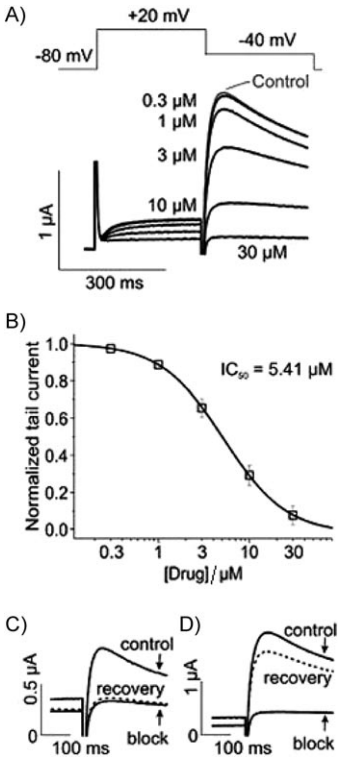


Figure 1. A) Current traces recorded in the absence (control) and presence of increasing concentrations of compound **3b**. The voltage protocol is shown as inset at the top. B) Concentration–response relationship for block of hERG tail current by compound **3b.** C) and D) Recovery of hERG channels from block. Channel block was induced by 1 Hz conditioning pulse trains to reach steady-state. Superimposed current traces are sourced from 1) drug-free control conditions (control), 2) the last pulse of the conditioning train (block), and 3) a single test pulse after a rest period of 330 seconds at −80 mV (recovery, dotted line). Recovery from block was measured in the continued presence of drug. Lack of recovery from block is shown in C) for compound **3a** (4 μM) and recovery from block is illustrated in D) for compound **3c** (6 μM).

Docking studies of propafenone derivatives on hERG

The parent compound propafenone was successfully docked into homology models of the open and closed states of hERG channels. For the closed state, the aromatic rings, particularly the phenyl ring, show contacts with two Tyr652 residues (Figure 2A,B). Furthermore, six hydrogen bonds are predicted between propafenone and the hERG potassium channel; these include two between the hydroxy group of propafenone and Thr623, two between H of the basic nitrogen and Thr623/Ser624, one between the basic nitrogen and Ser624 (not shown in the 2D interaction map), and one between the carbonyl oxygen atom and Ser624. In docking propafenone into the hERG open state, it was observed that the basic nitrogen moves downward to the cytoplasm, and the nitrogen atom no longer shows interaction with Thr623 and Ser624 (Figure 2C,D). Thus, during channel closure the basic nitrogen of propafenone seems to move "up" toward the selectivity filter. This movement may be facilitated by the negative field proposed by Farid et al.,[26] which is located within the pore. Similar docking results were obtained for **3a** (hERG $IC_{50} = 1.17 \pm 0.29$ μM, trapped within the hERG channel). An alignment of hERG open state docked conformations of propafenone and **3a** is given in Figure 4A below, and shows a good overlap of both structures.

Compound **3c** (hERG $IC_{50} = 2.09 \pm 0.55$ μM, non-trapped) is unable to dock into the fully closed pocket of the hERG channel. This molecule bears a 2,3-dimethylphenyl-1-piperazinyl ring instead of propylamine (propafenone) and piperidine **3a**. This moiety leads to an increase in molecular volume; this increase in steric bulk may render the compound too large to fit into the closed state of the channel. However, when docked into the central cavity of the open state, **3c** showed a positioning similar to that of propafenone (Figure 4B) and **3a** (Figure 4C), with the 2,3-dimethylphenyl ring placed at the bottom of the hERG channel cavity (Figure 3A,B). The docking pose of the des-benzyl analogue **3b** (hERG $IC_{50} = 5.41 \pm 1.01$ μM, non-trapped) also places the 2,3-dimethylphenyl ring near the cytoplasmic side (Figure 3C and Figure 4D). Although **3b** lacks one phenyl ring, the hERG non-trapping property of this compound is maintained despite this decrease in molecular volume. These docking results indicate that electrostatic interactions play an important role in drug trapping, because, together with the movement of S6 helices upon channel closure, the protonated nitrogen atoms tend to move up near the base of the K^+ selectivity filter (Figure 5).

Several studies have already shown that the sodium channel blocker propafenone also interacts with the hERG potassium channel.[16,27–31] Witchel et al. performed detailed alanine scanning studies, and docking of propafenone into the open and closed state of the channel provided initial evidence that the drug is trapped within the inner cavity.[16] Their data suggest that the drug–channel interaction is strongly dependent on residue Phe656, but is only weakly sensitive to mutation of Tyr652, Thr623, Ser624, Val625, Gly648, or Val659.[16] The main poses we obtained are in agreement with the results of Witchel et al., in the sense of the important interaction between propafenone and Phe656 (which may involve π-stacking interactions with two or more Phe656 side chains) in the open channel model. However, our docking studies further indicate that propafenone binding to the hERG open state may also involve other amino acids, such as Thr623 and Ser624. Our results also indicate that the protonated nitrogen atom not only interacts via cation–π interactions with Phe656, as commonly postulated, but also shows interactions with Thr623 and/or Ser624, as proposed by Farid et al.[26] and Choe et al.,[32] for example.

In comparing the structures of the four ligands that bind to the hERG channel, the two that are non-trapped both have a large substituent at the nitrogen atom. Although the data set is by far too small to derive any hypotheses that link drug trapping with molecular features, this may be an initial hint that the size of the substituent at the positively charged nitrogen atom plays a role for trapping/non-trapping.

Following the commonly accepted hypothesis of channel blocking, compounds cross the plasma membrane and contact the hERG channel from the cytoplasmic side.[18] Upon opening of the channel, compounds gain access to the binding site in

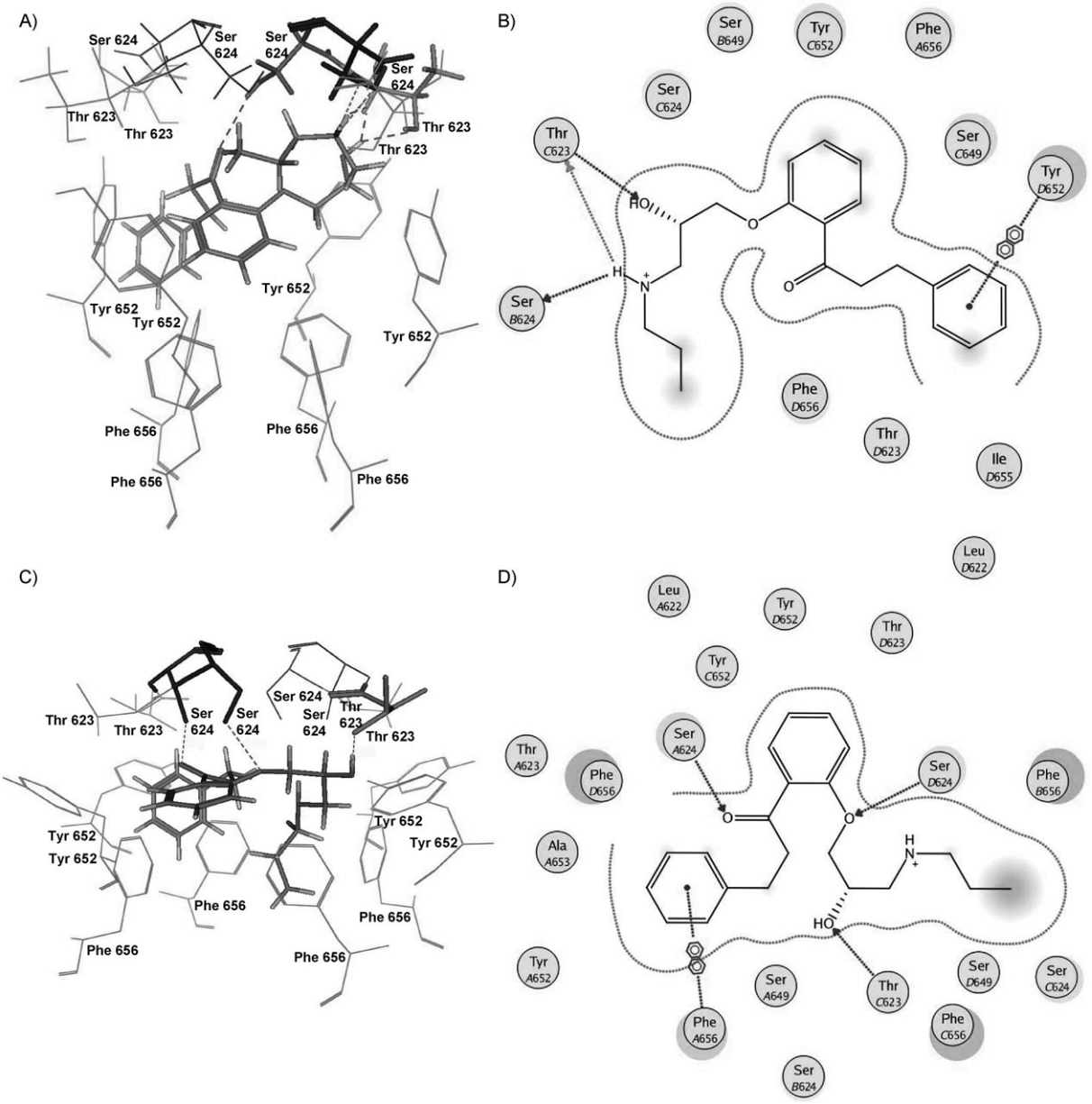


Figure 2. Binding pose of propafenone in the central cavity of hERG homology models in A) closed and C) open states, and 2D ligand interactions of propafenone for B) closed and D) open states. The amino acid residues that form hydrogen bonds within the binding site are represented as sticks, and the ligands are rendered in capped stick. Dotted lines represent hydrogen bonding interactions.

the hERG central cavity. When the channel closes, major rearrangements of the binding site occur. Compounds that are small enough (propafenone, **3a**) may alter their conformation and interaction pattern to fit into the closed pocket. In the case of compounds with large substituents at the nitrogen atom, such as **3b** and **3c**, these substituents may prevent the channel from complete closure and thus allow the dissociation of the compounds from the "closed" state. This type of "foot in the door" mechanism has been postulated by Decher et al. for AVE0118 on the $K_v1.5$ channel,[33] and seems to be valid for propafenones as well.

The primary goal of docking studies is the modeling of small molecules within the protein binding site and the prediction of binding affinities between ligands and target proteins. For the hERG channel, several docking studies have been published[12,16,26,34–40] that clearly indicate that small and large hydrophobic compounds bind to the hERG channel at various regions of the central cavity. This definitely complicates the proper prediction of both binding pose and affinity. Furthermore, the dynamics of the channel must be considered, and docking experiments with different channel states have to be performed. Thus, in order to elucidate the molecular basis of ligand–channel interaction and to shed more light on the phenomenon of drug trapping, it is necessary to combine ligand- and target-based methods with the full armory of biochemical tools, such as cysteine scanning and site-directed mutagenesis.

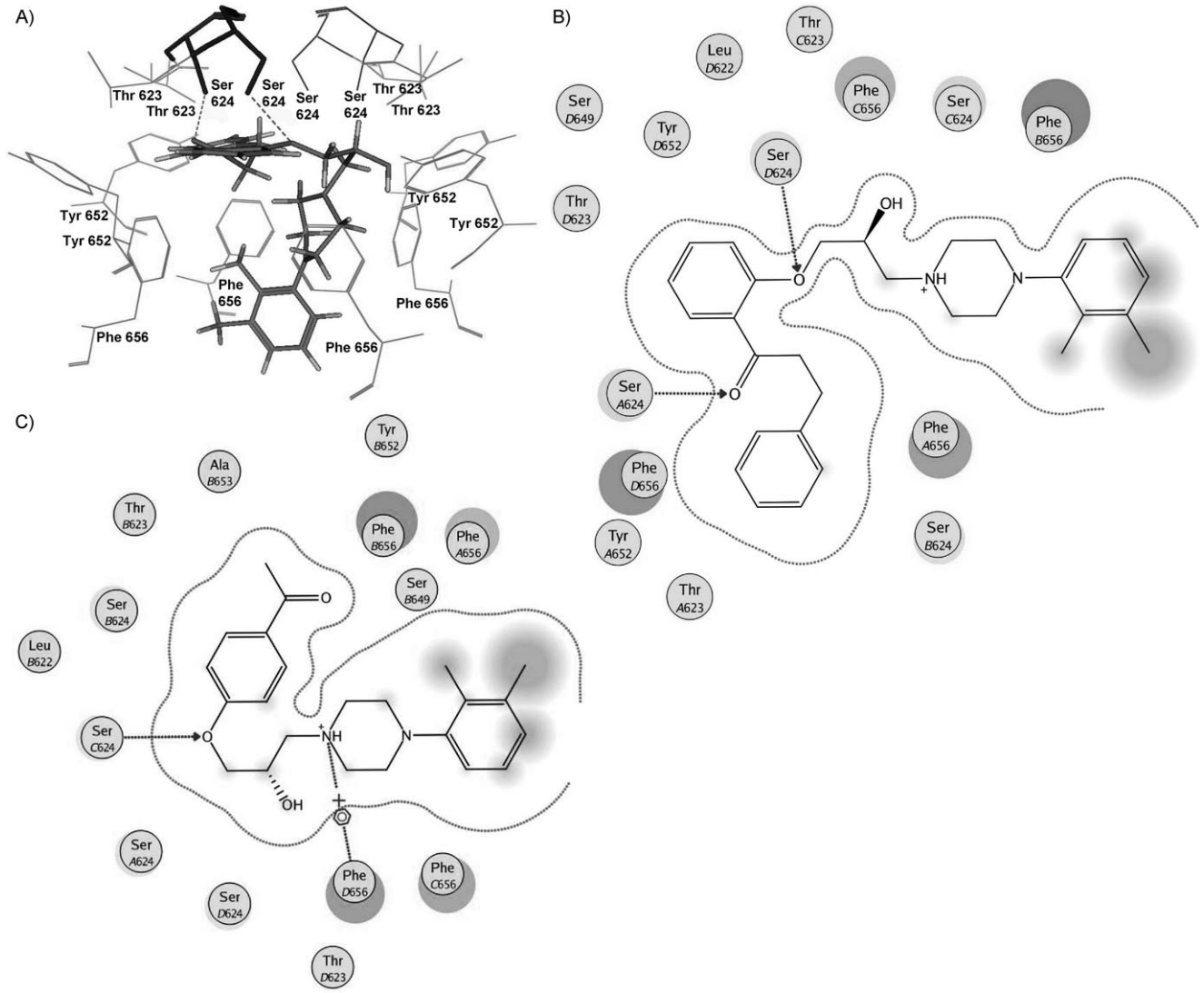


Figure 3. Binding pose of **3c** in the central cavity of the hERG homology model in the A) open state and B) its 2D ligand interaction pattern. C) 2D ligand interaction of **3b** with the hERG open state as generated by MOE. The amino acid residues that form hydrogen bonds within the binding site are represented in stick form, and the ligands are rendered in capped stick. Dotted lines represent hydrogen bonding interactions.

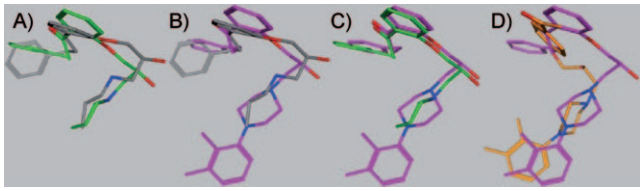


Figure 4. Superposition of docked conformations into the hERG open state. A) Propafenone (gray carbon) and **3a** (green carbon); B) propafenone and **3c** (magenta carbon); C) **3a** and **3c**; D) **3c** and **3b** (orange carbon). Colors of other atoms are red for oxygen and blue for nitrogen.

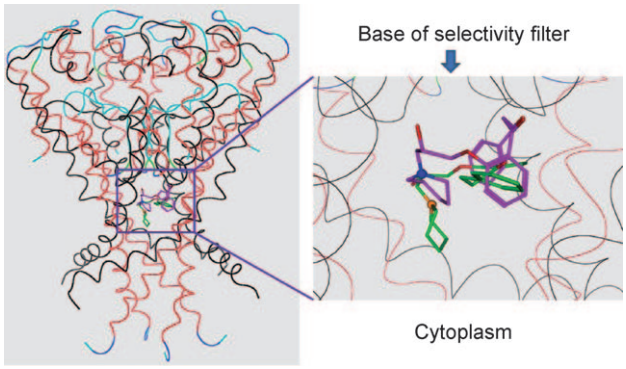


Figure 5. Relative position of the nitrogen atom of **3a** in the closed and open state. Complexes hERG–**3a** of open state (in black line) and closed state (in secondary structural color line) were aligned. Orange ball: nitrogen of **3a** in the hERG open state (green carbon); blue ball: nitrogen of **3a** in the hERG closed state (magenta carbon).

Combining data from all these experiments will improve our knowledge about the drug–channel interaction and enable the development of predictive classification and regression models.[7,41,42]

Conclusions

In this work, a small set of propafenone derivatives were synthesized and biologically tested for hERG activity with special emphasis on use-dependency of the channel block and recovery thereof. Ligand–protein docking into homology models of the closed and open state of the hERG channel provides the first evidence for the molecular basis of drug trapping. Notably, docking into protein homology models, especially into models of promiscuous proteins such as hERG and P-glycoprotein, is a rather uncertain process and needs careful validation. However, in our case the docking results are so far consistent with the experimental findings obtained. This information may provide potential strategies for improving the performance of in silico models for prediction of hERG activity, and should facilitate our understanding of the molecular basis of drug–channel interactions.

Experimental Section

Chemistry

Melting points were determined on a Reichert–Kofler hot-stage microscope and are uncorrected. Elemental analyses were performed by the Microanalytical Laboratory, Institute of Physical Chemistry, University of Vienna. NMR spectra were recorded on Bruker Spectospin (200 MHz). Column chromatographic separations were performed with Merck Kieselgel 60 (70–230 mesh). Yields given below are not optimized and refer to analytically pure material.

General procedure for the synthesis of the tertiary amines 3b and 3c: Epoxide **2** (17.7 mmol),[20–22] and the respective amine (20 mmol) were dissolved in CH_3OH and held at reflux for 6 h. The mixture was evaporated to dryness, and the oily residue was purified by column chromatography (silica gel, CH_2Cl_2/CH_3OH/concd NH_4OH, 200:10:1). The formation of hydrochlorides was carried out by dissolving the amine in dry Et_2O and adding a 1 M solution of HCl in Et_2O. The hydrochloride was filtered off and purified by crystallization.

1-[4-{3-[4-(2,3-dimethylphenyl)piperazin-1-yl]-2-hydroxypropoxy}-phenyl)ethanone (3b): yield 52.8%; mp (HCl): 224–225 °C; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 2.23$ (s, 3H), 2.29 (s, 3H), 2.49 (s, 3H), 2.53–2.91 (m, 10H), 3.47–3.70 (br, 1H, OH), 3.94–4.18 (m, 3H), 6.77–7.02 (m, 5H), 7.95 ppm (d, $J = 9.0$ Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta =$ 14.3, 21.0, 26.7, 52.6, 54.2, 60.7, 65.7, 70.8, 114.6, 117.0, 125.5, 126.2, 131.0, 138.4, 151.7, 163.0 ppm; IR (KBr): $\tilde{\nu} = 3440$, 2941, 2817, 2360, 1675, 1600, 1509, 1473, 1455 cm^{-1}; MS (EI, 70 eV): *m/z* (%): 382 (3) [M^+], 203 (52), 189 (100), 146 (23), 118 (29), 91 (15), 70 (56); Anal. calcd for $C_{23}H_{30}N_2O_3$: C 72.22, H 7.91, N 7.32, found: C 71.96, H 7.96, N 7.57.

1-[2-{3-[4-(2,3-dimethylphenyl)-1-piperazinyl]-2-hydroxypropoxy}-phenyl]-3-phenylpropan-1-one (3c): yield 63.4%; mp (HCl): 165–166 °C; ^{1}H NMR ($CDCl_3$): $\delta = 2.21$ (s, 3H), 2.27 (s, 3H), 2.38–2.85 (m, 10H), 2.97 (t, $J = 7.5$ Hz, 2H), 3.35 (t, $J = 7.5$ Hz, 2H), 3.05–3.85 (br, 1H, OH), 3.89–4.05 (m, 3H), 6.82–7.31 (m, 10H), 7.39 (dt, $J = 1.5$, 7.8 Hz, 1H), 7.63 ppm (dd, $J = 1.5$, 7.8 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 14.3$, 21.0, 30.7, 46.1, 52.6, 54.0, 61.3, 65.6, 71.2, 113.0, 117.0, 121.4, 125.5, 126.2, 126.3, 128.6, 128.8, 130.9, 131.6, 133.9, 138.4, 142.1, 151.7, 158.2, 201.6 ppm; IR (KBr): $\tilde{\nu} = 3460$, 2818, 2350, 1668, 1597, 1473, 1450 cm^{-1}; MS (EI, 70 eV): *m/z* (%): 472 (2) [M^+], 203 (100), 189 (28), 160 (17), 132 (13), 91 (17), 70 (65); Anal. calcd for $C_{30}H_{36}N_2O_3$: C 76.24, H 7.68, N 5.93, found: C 75.81, H 7.68, N 5.76.

Propafenone, **3a**, and **3d** were prepared according to published protocols.[20–22]

hERG assays

Molecular biology: Preparation of stage V–VI oocytes from *Xenopus laevis* (NASCO, Fort Atkinson, WI, USA), synthesis of capped runoff complementary RNA (cRNA) transcripts from linearized complementary DNA (cDNA) templates and injection of cRNA were performed as described previously.[15] cDNAs of hERG (accession number NP_000229) were kindly provided by Dr. Sanguinetti (University of Utah, Salt Lake City, USA).

Voltage clamp analysis: Currents through hERG channels were studied 1–4 days after microinjection of the cRNA using the two-microelectrode voltage clamp technique. The bath solution contained 96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, 1.8 mM $CaCl_2$ (pH 7.5, titrated with NaOH).

Voltage-recording and current-injecting microelectrodes were filled with 3 M KCl and had resistance values between 0.3 and 2 MΩ. Endogenous currents (estimated in oocytes injected with water) did not exceed 0.1 μA. Currents >3 μA were discarded to minimize voltage clamp errors. A precondition for all measurements was the achievement of stable peak current amplitudes over periods of 15 min after an initial run-up. All drugs were applied by means of a new perfusion system enabling solution exchange within ~100 ms.[43]

The pClamp software package version 10.1 (Axon Instruments Inc., Union City, CA, USA) was used for data acquisition. Microcal Origin 7.0 was employed for analysis and curve fitting.

Voltage protocol: The voltage protocol (see inset in Figure 1A) was designed to simulate voltage changes during a cardiac action potential with a 300 ms depolarization to +20 mV (analogous to plateau phase), a repolarization for 300 ms to −40 mV (inducing a tail current) and a final step to the holding potential. The +20 mV depolarization rapidly inactivates hERG channels, thereby limiting the amount of outward current. During the repolarization to −40 mV, the previously activated channels open due to rapid recovery from inactivation. The decreases in the resulting tail current amplitudes were taken as a measure of block development during a pulse train.

Estimation of half-maximal inhibition of hERG channels: hERG channel block was estimated as peak tail current inhibition (Figure 1A,B). The concentration–inhibition curves were fitted using the Hill equation:

$$\frac{I_{hERG,drug}}{I_{hERG,control}} = \frac{100 - A}{1 + \left(\frac{C}{IC_{50}}\right)^{n_H}} + A$$

in which IC_{50} is the concentration at which hERG inhibition is half-maximal, C is the applied drug concentration, A is the fraction of hERG current that is not blocked, and n_H is the Hill coefficient.[15,19,44]

Molecular modeling and docking studies

Preparation of molecular structures: The structures of propafenone and its derivatives were built in MOL2 format using the sketcher module of Sybyl, and Gasteiger–Hückel charges were as-

signed to the ligand atoms. The structures of the molecules were optimized by energy minimization and molecular dynamics using the simulated annealing method.[45] The lowest-energy conformer of each molecule was selected, and a formal charge of +1 was assigned to the basic nitrogen atom.

Preparation of target protein structure and flexible docking: Docking studies were performed on two homology models of the hERG potassium channel: one in an open conformation and the other in a resting state conformation.[23] The active site was defined by all the amino acid residues within a sphere of radius 6.5 Å centered by several residues known to have an important role for drug binding. The docking and subsequent scoring were performed with the default parameters of the FlexX programs implemented in the Sybyl 7.0 software package.[45] Final scores for all FlexX solutions were calculated by the standard scoring function and used for database ranking; 2D ligand interactions were generated with the MOE software package.[46]

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Keywords: docking · drug trapping · hERG · ion channels · propafenone

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