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*Perspective*

# Refining insights into high affinity drug binding to the hERG potassium channel

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**Running title:** Insights into drug binding by the hERG $K^+$ channel

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**Non-standard abbreviations**

FDA, United States' Food and Drug Administration; hERG, *human ether-a-go-go-related gene*; ICH S7B, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Document S7B: The Non-Clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals; $I_{Kr}$, rapid component of the cardiac delayed rectifier current; KCNH2, alternative nomenclature for the gene encoding the hERG $K^+$ channel; LQTS, long QT syndrome; TdP, *torsades de pointes*; WT, wild type

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## Abstract

hERG (*human ether-à-go-go-related gene*) potassium ($K^+$) channels play a crucial role in electrophysiological activity in the heart, exerting a profound influence on ventricular action potential repolarization and on the duration of the QT interval of the electrocardiogram. hERG channels are strongly implicated in the acquired form of long QT syndrome (LQTS) as they exhibit a unique susceptibility to pharmacological inhibition by therapeutically and chemically diverse drugs. Investigations over a number of years provide compelling evidence that a comparatively large inner cavity and the presence of particular aromatic amino-acid residues (Y652 and F656) on the inner (S6) helices of the channel are important features that allow hERG to accommodate and bind disparate drugs. However, whereas functional hERG channels are comprised of four identical subunits, blocking molecules may not interact equally with aromatic residues from each of the four subunits. In this issue of *Molecular Pharmacology*, Myokai *et al* (pgs XXX-XXX) report for the first time the use of tandem dimers incorporating mutations to Y652 and F656 to elucidate asymmetric binding of the high affinity hERG inhibitor cisapride. Not only has this approach provided increased information on spatial arrangements involved in cisapride binding to the channel, but it offers a powerful means of refining the wider understanding of hERG channel structure-function in relation to drug binding.

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*Torsades de pointes* (TdP) is a polymorphic ventricular tachycardia that is strongly associated with delayed ventricular repolarization in the congenital and acquired (drug-related) forms of long QT syndrome (LQTS; (Modell and Lehmann, 2006; Sanguinetti and Tristani-Firouzi, 2006; Yap and Camm, 2003)). Although TdP can spontaneously resolve back into a normal cardiac rhythm it can also degenerate into potentially fatal ventricular fibrillation. It is the spectre of TdP that makes QT interval prolongation by either clinically used drugs or drugs in development a matter for some concern. Although the incidence of TdP in patients receiving a particular drug usually tends to be rather low (Yap and Camm, 2003), the attendant risk may nevertheless be unacceptable for many drugs, particularly in the case of medications used to treat non-life-threatening conditions. Two examples serve as useful illustrations here. In 1997 the United States' FDA announced withdrawal of approval for antihistamines incorporating terfenadine, due to cardiac safety issues (Cruzan, 1997; Josefson, 1997). In 2000, the FDA announced the voluntary withdrawal of US marketing of a treatment for severe heartburn called cisapride (Propulsid, Janssen Pharmaceutica) due to multiple reports of heart-rhythm abnormalities including fatalities, primarily among patients either taking other medications or with conditions predisposing towards cardiac arrhythmia (Henney, 2000).

In principle, delayed ventricular repolarization (and thereby QT interval prolongation) could result from drug-induced modulation of a number of different cardiac ion channels. Remarkably, however, virtually all drugs that delay ventricular repolarization share a common feature: an ability to inhibit the functional activity of ion channels responsible for the 'rapid' delayed rectifier potassium current ($I_{Kr}$), which plays a crucial role in ventricular action potential repolarization (Sanguinetti and Tristani-Firouzi, 2006; Witchel and Hancox, 2000). The association between $I_{Kr}$ channel inhibition and drug-induced QT interval prolongation is sufficiently strong that relevant non-clinical drug-safety testing guidelines (the International Conference on Harmonization document ICH S7B) recommend use of an *in vitro* $I_{Kr}$ assay as part of an integrated risk assessment of the propensity of pharmaceuticals to delay ventricular repolarization (ICH, 2005).

"hERG" came to prominence when the *hERG* (*human Ether-à-go-go-Related Gene*, alternative nomenclature *KCNH2*) product was shown to be a functional potassium

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channel that mediates ionic current with properties close to native $I_{Kr}$, and mutations to the gene were implicated in the familial long QT syndrome (LQTS; (Curran et al., 1995; Sanguinetti et al., 1995; Trudeau et al., 1995)). A link between hERG and acquired (drug-induced) LQTS was also suggested (Sanguinetti et al., 1995). Since that time numerous hERG mutations have been implicated in congenital LQTS (Modell and Lehmann, 2006) and a gain-of-function hERG mutation has been found to mediate one form of the recently identified short QT syndrome (Brugada et al., 2004). To return to our earlier drug examples of terfenadine and cisapride, despite differences in therapeutic class and chemical structure, both were found to be potent hERG $K^+$ channel inhibitors (Rampe et al., 1997; Roy et al., 1996; Suessbrich et al., 1996). Moving forwards to the current day, many structurally and therapeutically diverse drugs have now been identified as inhibitors of the hERG $K^+$ channel including both cardiac (Class I and III antiarrhythmic drugs) and numerous non-cardiac drugs (Sanguinetti and Mitcheson, 2005; Vandenberg et al., 2001). While many of these drugs have in common a central basic nitrogen group together with several hydrophobic/aromatic groups, it is the diversity of structures producing hERG $K^+$ channel blockade that is particularly striking. Given the strong association between $I_{Kr}$/hERG block, QT interval prolongation and risk of TdP, an obvious and important question arises: what makes $I_{Kr}$/hERG uniquely susceptible to pharmacological inhibition by chemically and therapeutically diverse drugs? Considerable insight into the nature of drug-hERG interactions has emerged since 2000 and in this issue of *Molecular Pharmacology*, Myokai and colleagues further this field (Myokai et al., 2008). The brief historical perspective below aims to place this important new study in context.

hERG is a six transmembrane domain voltage-gated $K^+$ channel that normally functions as a homo-tetramer (Fig. 1A) and the majority of $I_{Kr}$/hERG-blocking drugs appear to cross the cell membrane and enter the channel from the cytosolic side when it opens. Recovery from blockade by high affinity methanesulfonanilide drugs is slow and data from experiments using a hERG channel mutant (D540K) that opens on hyperpolarisation and a relatively bulky molecule, MK-499, provided compelling evidence that slow recovery results from drug retention (“trapping”) in the inner cavity once the channel has closed (Mitcheson et al., 2000b). The structural correlate here is a larger inner cavity of hERG than of other Kv channels due to the

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absence in hERG of a proline (P-x-P) motif that otherwise restricts inner cavity size likely by causing a kink in the inner (S6) helices (del Camino et al., 2000; Mitcheson, 2003). A second key structural determinant of hERG's drug-sensitivity is the presence of particular aromatic amino-acid residues in on S6 helices facing the inner cavity. In 2000, a phenylalanine residue at position 656 was found to be critically important for high affinity binding by the methanesulfonanilides, dofetilide and MK-499 (Lees-Miller et al., 2000; Mitcheson et al., 2000a). Alanine scanning mutagenesis, coupled with homology modeling based on the bacterial KcsA channel, mapped the methanesulfonanilide binding site (for MK-499) to the S6 and pore helices implicating F656, a nearby tyrosine (Y652) as well as several other residues in MK-499 binding (Fig. 1B; (Mitcheson et al., 2000a)). Importantly, Y652 and F656 were found also to be critical for binding of terfenadine and cisapride (Mitcheson et al., 2000a), suggesting that these residues may be consensus determinants of high affinity hERG channel blockade, via cation-π and π stacking interactions with the basic tertiary nitrogen and aromatic groups of the blocking drug.

Whilst F656 and Y652 are common to hERG and the related *EAG* channel, other voltage-dependent $K^+$ channels lack aromatic amino-acid residues at these positions (Sanguinetti and Mitcheson, 2005). hERG's uniquely fast 'C-type' inactivation (Smith et al., 1996) also likely participates in high affinity blockade, since introduction of C-type inactivation into the normally non-inactivating bovine EAG channel increased by 30-fold the channel's sensitivity to dofetilide (Ficker et al., 2001). For at least some compounds, including cisapride, it may be that conformational changes during hERG's unusually rapid 'C type' inactivation optimizes the positioning of these aromatic residues for drug binding, since repositioning of Y652 and F656 on the S6 helix of hERG has been shown to reduce cisapride sensitivity, whilst repositioning of the analogous residues in EAG induced cisapride sensitivity (Chen et al., 2002). Although there may be some exceptions (e.g. (Milnes et al., 2003; Ridley et al., 2004)), the majority of drugs that have been examined in detail effect hERG blockade via interactions with one or both of Y652 and F656 (Fig 1B; see (Sanguinetti and Mitcheson, 2005; Sanguinetti and Tristani-Firouzi, 2006) for reviews). Progressively more detailed knowledge of drug-binding mechanisms from structure-function analysis (Sanguinetti and Mitcheson, 2005; Sanguinetti and Tristani-Firouzi, 2006) and from *in silico* modeling of hERG-drug interactions

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(Recanatini et al., 2008) may ultimately facilitate removal of potential hERG blockade early during the drug design process.

Cisapride has figured repeatedly in structure-function analyses of hERG channel inhibition (Chen et al., 2002; Fernandez et al., 2004; Mitcheson et al., 2000a). Systematic mutation of Y652 and F656 to other residues has provided evidence that potency of blockade is well-correlated with hydrophobicity, particularly of the side chain of residue 656, whilst for residue-652; high affinity inhibition is contingent upon an aromatic side group, implicating cation-$\pi$ interactions with the drug's basic tertiary nitrogen (Fernandez et al., 2004). Functional hERG channels are tetrameric and to-date, structure function analyses have compared wild-type and mutant homotetrameric channels, which will presumably therefore have a rotational symmetry about an axis through the center of the pore. Drug structures may be asymmetric, however, and it follows that such molecules will not necessarily interact equally with particular residues on all four subunits. In this volume of *Molecular Pharmacology*, Myokai et al (2008) have investigated this issue for cisapride, using tandem dimers of hERG incorporating wild type (WT) and/or mutant subunits in which one or both of Y652 and F656 had been mutated to alanine. The use of tandem dimers is not without potential problems, as linking monomers could potentially influence conformational changes during channel gating and alter channel kinetics. However, even with a relatively short linking sequence, the voltage-dependent kinetics of WT hERG current ($I_{hERG}$) were little affected for WT tandem dimers, although the deactivation time-course was accelerated. Substitution of one tyrosine at position 652 on adjacent subunits markedly reduced the affinity of the channel for cisapride, suggesting that both Y652 residues on adjacent subunits were required for high affinity drug binding. In contrast, mutation of a single F656 on adjacent subunits did not necessarily result in a reduction in affinity. Most notably, the double mutants, in which, in addition to one of the Y652s, one F656 on each tandem subunit had also been mutated, has provided the first direct experimental evidence of the importance of the particular arrangement of the aromatic residues for high affinity binding: Tandem dimers in which F656 had been mutated on a subunit with an intact Y652, while the adjacent subunit contained Y652A and an intact F656, had a significantly lower binding affinity than tandem dimers consisting of a WT subunit in tandem with a subunit containing both Y652A and F656A. The

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authors employed a thermodynamic double mutant cycle analysis of changes in binding energy, in which each double mutant can be regarded as the consequence of two successive single mutations through different routes, to demonstrate that mutation of F656 on a subunit containing a mutated Y652 produced comparatively little further change in binding energy, while mutation of F656 on a subunit with intact Y652 but with Y652A on the adjacent subunit produced a marked reduction in binding affinity. The data are interpreted as suggestive of additive interactions between Y652 and F656 on the same subunit, but co-operative interactions between these residues on adjacent subunits. The findings of Myokai and colleagues are also broadly consistent with a recent simulation study involving simulation of cisapride docking to the hERG $K^+$ channel tetramer (using a template based on KvAP), which suggested T-shaped $\pi$–$\pi$ stacking interactions between cisapride and diagonally opposite Y652s and a parallel displaced interaction with a F656 (Farid et al., 2006). In summary, this important study not only provides unprecedented insight into the nature of cisapride blockade of the hERG channel but also establishes an exciting approach that can be expected to be invaluable in refining ideas as to how drugs may bind to functional tetrameric hERG channels.

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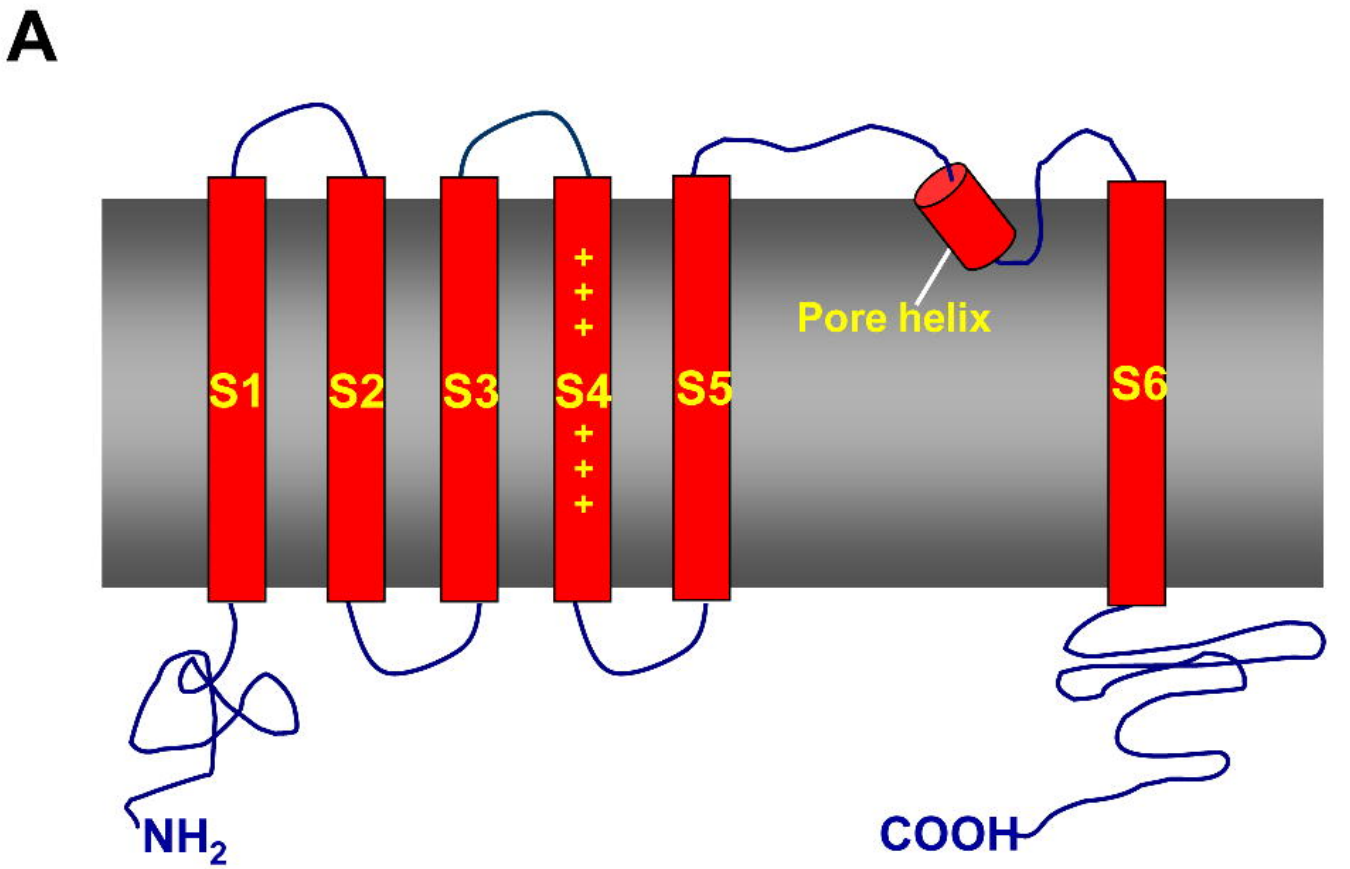
## Legends for Figures

**Figure 1. A** Schematic diagram of a single hERG channel subunit, showing transmembrane domains (red; labelled S1-S6 in yellow), lipid membrane (shaded grey) and intracellular 'N' ($NH_2$) and 'C' (COOH) termini. Four similar subunits combine to produce a functional hERG channel. **B** Schematic diagram illustrating selected amino acid residues (not to scale) found to be important for drug binding to the hERG channel. A vertical section shows the pore and inner (S6) helices from two of the four channel subunits; the position of the selectivity filter is also shown. The aromatic residues shown in blue (Y652 and F656) are key determinants of high affinity binding for a number of drugs including cisapride.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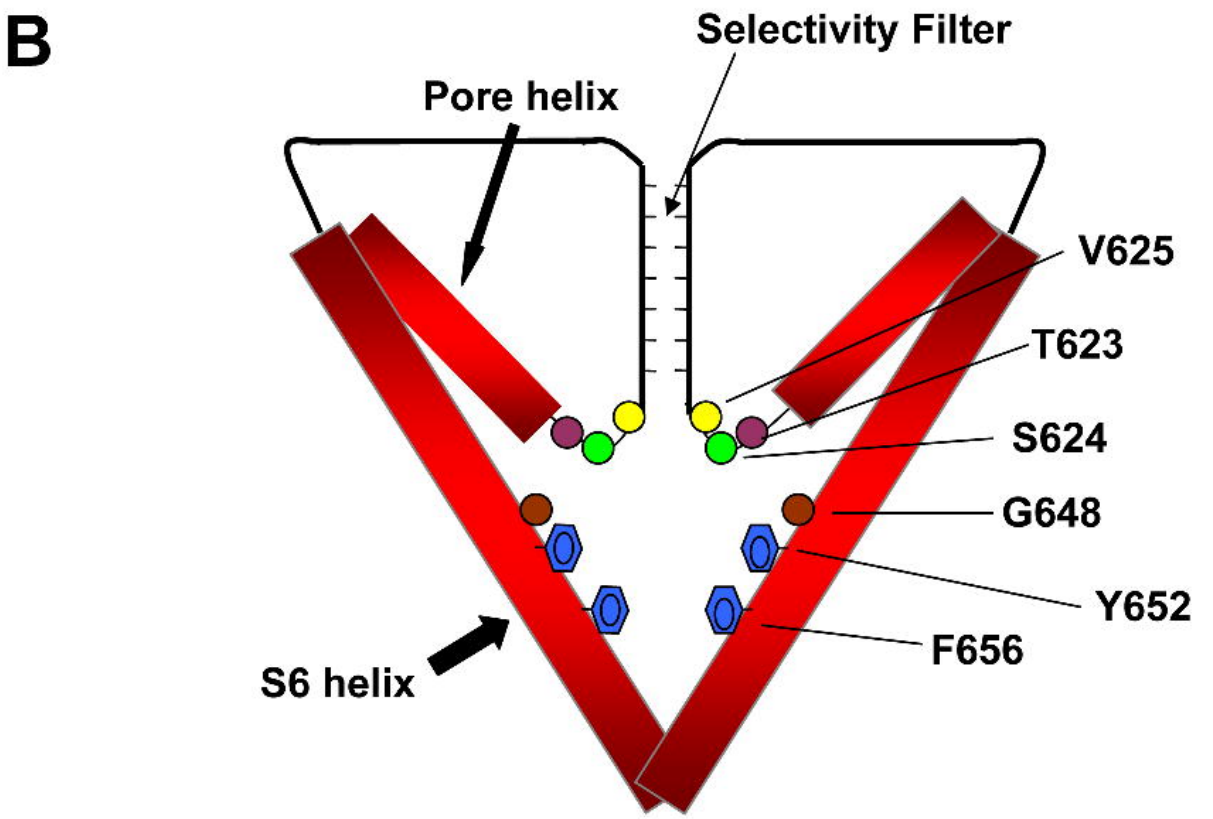
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Y652
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