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Review

Activation of human ether-a-go-go related gene (hERG) potassium channels by small molecules

Ping-zheng ZHOU[1, 2], Joseph BABCOCK[3], Lian-qing LIU[2], Min LI[1, 3], Zhao-bing GAO[1, *]

[1]*State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China;* [2]*State Key Laboratory of Robotics, Shenyang Institute of Automation, Chinese Academy of Sciences, Shenyang 110016, China;* [3]*Department of Neuroscience, High Throughput Biology Center and Johns Hopkins Ion Channel Center, School of Medicine, Johns Hopkins University, Baltimore, Maryland 21205, USA*

Human ether-a-go-go related gene (hERG) potassium (K^+) channels play a critical role in cardiac action potential repolarization. Mutations that reduce hERG conductance or surface expression may cause congenital long QT syndrome (LQTS). Moreover, the channels can be inhibited by structurally diverse small molecules, resulting in an acquired form of LQTS. Consequently, small molecules that increase the hERG current may be of value for treatment of LQTS. So far, nine hERG activators have been reported. The aim of this review is to discuss recent advances concerning the identification and action mechanism of hERG activators.

Keywords: Human ether-a-go-go related gene (hERG); ion channel; long QT syndrome (LQTS); activator

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Discovery and structure of hERG

In 1994, Warmke and Ganetzky first identified the human ether-a-go-go related gene (hERG) K^+ channel by screening a human hippocampal cDNA library with a mouse homologue of "ether-a-go-go" (EAG), a *Drosophila* K^+ channel gene[1]. Subsequently, Sanguinetti and colleagues reported similarities between the biophysical properties of the heterologously expressed hERG channel and the rapidly activating delayed rectifier K^+ current (I_{Kr}), a critical current in the phase 3 repolarization of the cardiac action potential, and confirmed that hERG encodes the α subunit of I_{Kr}[2, 3]. While hERG is predominately expressed in the heart, it is also found in diverse tissues including neurons, neuroendocrine glands and smooth muscle[3].

hERG (Kv11.1) was the 11th member of the voltage-gated K^+ channel family (Kv). Like other Kv channels, hERG is an obligate homotetramer, with each subunit containing six transmembrane domains (S1–S6): S1–S4 compose the voltage sensor domain, while the S5, P-loop, and S6 segments form the channel pore (Figure 1). However, hERG has several features that distinguish it from other Kv family members. First, a conserved tyrosine found in the GYG motif of other Kv channel pores is replaced by a phenylalanine[4]. This aromatic residue, along with others lining the hERG pore, has been proposed as a determinant of promiscuous small-molecule interaction. Second, hERG's protein sequence lacks the proline-X-proline (PXP) motif that often flanks the S6 helices of other Kv channels. It is generally believed that the PXP domain "kinks" this helix to reduce the volume of the pore cavity, and its absence in hERG has been suggested to play a role in promiscuous drug interactions as well. Finally, the hERG channel has a large "S5-P linker" domain between the S5 and P-loop segments that assumes an amphipathic helical arrangement in membrane mimetic sodium dodecyl sulfate (SDS) micelles and is believed to affect channel inactivation[5].

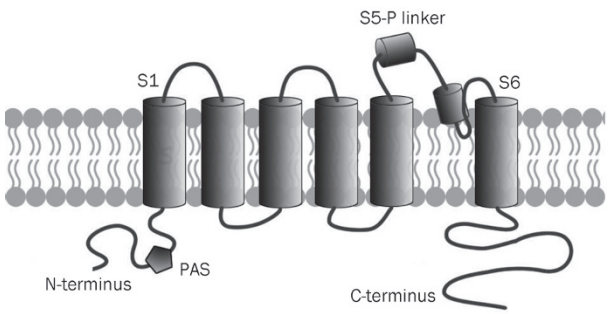

Figure 1. The basic structure of hERG channels. S1–S4 is the voltage sensor domain. S5, the P-loop, and S6 compose the pore of the channel; the termini of the protein are both intracellular.

* To whom correspondence should be addressed.
E-mail zb.gao@mail.shcnc.ac.cn
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The intracellular termini of the hERG channel are also important for function. The N-terminus contains a Per-Arnt-Sim (PAS) domain (about 135 amino acids), which, despite being a common motif in signaling proteins found in bacteria and plants, appears in no other mammalian ion channels. In hERG, the PAS domain modulates the deactivation of the channel following membrane depolarization. The C-terminus of the channel contains a cyclic nucleotide binding domain (CNBD), which has been linked to mutations affecting trafficking[5].

Gating of hERG K^+ channels

Although the overall structure of hERG is homologous to that of other potassium channels, its kinetic behavior is quite distinct and is characterized by comparatively slow activation and deactivation kinetics (on the order of hundreds of ms to s) but very rapid, voltage-dependent inactivation kinetics (on the order of ms to tens of ms)[5, 6]. The unusual kinetics of hERG are compatible with its function in cardiac repolarization. In the ascending phase of the action potential, as a result of slow activation and simultaneous fast inactivation, little outward current flows through hERG during depolarization. As the membrane repolarizes, hERG channels recover from inactivation much faster than they deactivate, thereby generating an outward current that peaks at about -40 mV. This outward current through hERG is the key determinant for termination of the plateau phase of the action potential[5] (Figure 2). Due to the importance and uniqueness of hERG gating kinetics, much of the recent work on hERG has focused on understanding the molecular rearrangements of the channel protein during the cardiac action potential.

Slow activation

As noted above, the voltage sensor of the hERG channel is the S1–S4 segment, in which the S4 helix is particularly important. Like other Kv channels, the hERG S4 has four periodically spaced arginine residues whose positive charges, which are repelled by changes in membrane potential, are thought to drive structural rearrangement of the protein during depolarization via the S4–S5 linker[7]. Sequence alignments and hydropathy plots suggest that the overall structure of this voltage sensor domain (VSD) is homologous to that of other K^+ channels. Why then are the kinetics of activation so different in hERG? To answer this question, Smith and Yellen (2002) attached a fluorescent molecule to the extracellular domain of S4 and examined the movement of this helix by fluorescence resonance energy transfer (FRET). They found that the fluorescence signal changed very slowly in response to membrane depolarization, suggesting that slow voltage sensor movement is responsible for the unusual activation gating kinetics of the channel[6, 8]. However, there still is no mechanistic explanation for the slow movement of the hERG S4 helix, though Subbiah and colleagues have identified residues K525, R528, and K538 as molecular determinants of this behavior using tryptophan scanning mutagenesis[9].

Fast voltage-dependent inactivation

Two major mechanisms have been proposed for the inactivation of voltage-gated potassium channels[10]. The first is N-type (also called "ball and chain" type), which involves rapid occlusion of the open channel by an intracellular segment of the protein. The second is C-type, which involves a slower change in channel conformation at the extracellular mouth. There is evidence that inactivation of hERG is similar to C-type and shows voltage-dependency, even though hERG inactivation is several orders of magnitude faster than C-type inactivation in Shaker K^+ channels[3, 5, 11]. Some studies have shown that this fast inactivation limits K^+ efflux during depolarization, allowing hERG to function as an inward rectifier of potassium concentration[11].

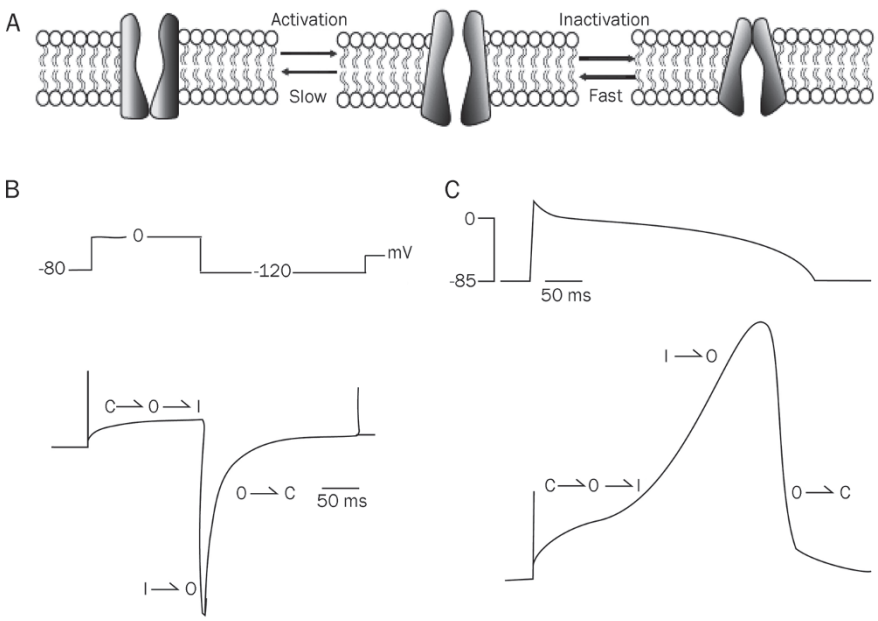


Figure 2. Gating of hERG channels. (A) The three main conformational states of hERG K^+ channels: closed (C), open (O), and inactive (I). Transitions between these states are voltage dependent. The transition from closed to open involves the opening of an intracellular gate, whereas transition from open to inactive involves the closing of a gate at the extracellular face of the permeation pathway. (B) Representative traces showing characteristic kinetics: slow activation and deactivation, coupled with rapid inactivation. (C) hERG currents recorded during cardiac ventricular action potential. (B&C adapted from Vandenberg, JI, *et al*. Eur Biophys J 2004; 32: 89-97).

The S5-P-loop region is critical for inactivation of hERG. Many mutations in this region, most critically at S620 and S621, will inhibit inactivation[5, 6]. S620T abolishes the inactivation of hERG, while S620A left-shifts the voltage-dependent inactivation approximately +100 mV. Besides S620 and S621, other residues such as W585, L586, H587, L589, G590, D591, I593, and G594 have also been reported as molecular determinants of hERG channel inactivation. Furthermore, Jiang *et al* reported that dynamic conformational changes in the S5-P linker occur during channel gating, suggesting that this domain's mobility is critically important for hERG kinetics[12].

It is currently still unclear why inactivation of hERG is much faster than C-type inactivation in other Kv channels. One explanation is that the previously mentioned tyrosine to phenylalanine substitution in the GYG pore motif may explain this isoform specific kinetics. This hypothesis comes from two experimental results: that mutation of the selectivity filter tyrosine to a phenylalanine (Y445F) in Shaker channels results in a channel with accelerated C-type inactivation and that members of the inward rectifier K^+ channel family, Kir6.x, contain a GFG selectivity filter and also undergo a rapid gating process that is analogous to C-type inactivation[6].

hERG and LQTS

hERG and congenital long QT syndrome

So far, at least 13 genes have been associated with congenital LQTS[13], including KCNQ1(LQT1), hERG(LQT2), SCN5A(LQT3), ANK2(LQT4), KCNE1(LQT5), KCNE2(LQT6), KCNJ2(LQT7), CACNA1C(LQT8), CAV3(LQT9), SCN4B(LQT10), AKAP9(LQT11), SNTA1(LQT12), and GIRK (LQT13)[13, 14]. Among these, hERG was the first reported and is the most prevalent. To date, nearly 300 different hERG mutations linked to LQT2 have been identified. Such mutations may cause loss of hERG function by one of four main effects: reduced or defective synthesis; defective trafficking from the endoplasmic reticulum (ER) to the plasma membrane (resulting in decreased surface expression); defective gating; or defective ion permeation[5].

hERG trafficking rapidly became a focus of interest for two reasons: (1) most hERG mutations cause trafficking defects, and (2) these trafficking defective mutants can be restored by high-affinity hERG channel-blocking drugs, which then give rise to a functional I_{Kr} current[15, 16]. Inhibitors of hERG act as molecular chaperones to rescue transport defective mutants[16]. However, the molecular mechanism by which these small molecules function as hERG chaperones is still largely unclear, with only a single report in 2005 linking E-4031 rescue of the N470D mutant to interactions with calnexin[17]. However, the mechanism of rescue by hERG blockers is likely heterogeneous, as evidenced by the fact that molecules such as E-4031, astemizole, and cisapride are able to restore trafficking of mutants N470D and G601S but not mutations in the C-terminal such as R752W, F805C, and R823W[17]. Based on this evidence, Elizabeth S Kaufman suggests that, although restoration of intracellular processing and transport is theoretically an attractive therapeutic strategy, most LQTS patients are heterozygous, and thus pharmacologic interventions that enhance the production and function of the healthy wild-type gene product may be more feasible[16].

hERG and acquired long QT syndrome

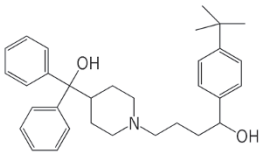
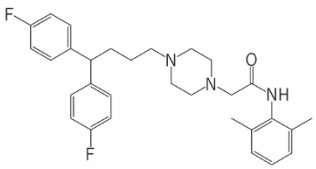
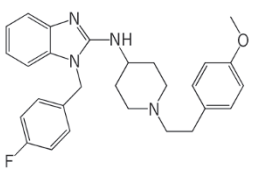
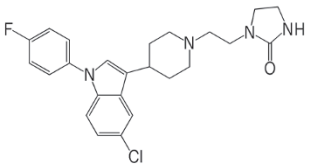
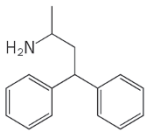
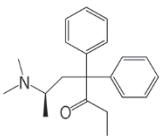
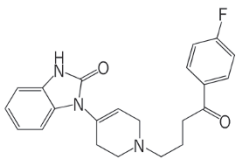
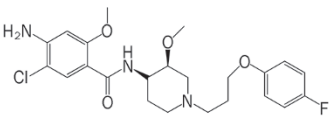
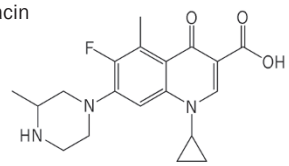
Unlike congenital LQTS, which may result from defects in numerous genes, almost all cases of pharmacologically induced LQTS (acquired long QT syndrome: aLQTS) have been linked to chemical blockade of the hERG channel. There is a recent paper reporting that single-nucleotide polymorphisms in other proteins that interact with hERG may be inhibited by small molecules, and thus also cause a LQTS[18]. Indeed, it is thought that hERG is a promiscuous target that binds structurally diverse small molecules.

Until now, aLQTS has resulted in many drugs being removed from the market or terminated during clinical development. Examples include several non-cardiac drugs that have been withdrawn or given strict limitation for use, including terfenadine, lidoflazine, astemizole, sertindole, levomethadyl, droperidol, cisapride and grepafloxacin (Table 1)[19]. Thus, potential blockade of cardiac I_{Kr} becomes a necessary pre-clinical assessment for candidate drugs. Structural determinants for this chemical blockade include a tyrosine at position 652 and a phenylalanine at 656[5]. Alanine scanning mutagenesis has demonstrated that these two residues dictate the high-affinity binding of many drugs that inhibit hERG. However, the docking mode of different drugs is not the same, and computational modeling suggests that many drugs may utilize diverse binding conformations, coordinating multiple residues both within and between the subunits of the channel. Thus, the complex interactions between drugs and hERG complicate *in silico* predictions of the hERG inhibition by novel therapeutic compounds.

hERG activators

As mentioned above, hERG mutations cause congenital LQTS and a wide variety of drugs of different classes and structures bind to hERG, leading to aLQTS. However, hERG defects have also been linked to other diseases such as stress-mediated arrhythmias, diabetes and myocardial ischemia induced arrhythmias[20]. Physiologically potentiating hERG would accelerate action potential repolarization and shorten the duration of the action potential. hERG channel activators can enhance channel function by accelerating myocardial repolarization, an effect that has been demonstrated by animal experiments, and they are considered potential therapeutics for LQTS. Indeed, hERG activators may become a novel class of antiarrhythmics, as reports have suggested that such compounds can reduce electrical heterogeneity in the myocardium and thereby the possibility of re-entry[20, 21]. However, due to the limitations of existing high throughput screening methods and difficulties in assaying the channel on a large scale, few activators have been reported. Most of the existing activators originated from combinatorial chemistry libraries, including RPR260243[22–24], PD-118057[25], PD-307243[26], NS1643[27], NS3623[28], A-935142[29], ICA-105574[30], and KB130015[31]. Addi-

Table 1. Summary of nine non-cardiac drugs withdrawn for hERG toxicity.

Terfenadine 	Lidoflazine 	Astemizole 
Sertindole 	Grepafloxicin 	Levomethadyl 
Droperidol 	Cisapride 	Grepafloxacin 

tionally, a natural product, mallotoxin, has also been shown to activate hERG[32]. Below, we review the literature on individual compounds.

RPR260243

RPR260243 [(3*R*,4*R*)-4-[3-(6-methoxy-quinolin-4-yl)-3-oxo-propyl]-1-[3-(2,3,5-trifluoro-phenyl)-prop-2-yn-1-yl]-piperidine-3-carboxylic acid] was the first reported hERG channel activator. RPR260243 dramatically slows current deactivation in patch-clamp experiments, and its effect is temperature and voltage dependent. Though it is a weak inhibitor of the L-type Ca^{2+} channel, RPR260243 has no significant effects on the human cardiac Na^+ channel or the KCNQ1/KCNE1 cardiac K^+ channel, which are also linked with LQTS, thus showing high selectivity for hERG[22]. Interestingly, RPR260243 inhibits the erg3 channel, which is in the same family as hERG, and a single S5 residue may account for this difference in pharmacology (Thr556 in hERG, Ile558 in rERG3). A Thr in this position favors agonist activity, whereas an Ile reveals a secondary blocking effect of RPR260243[23]. Additionally, RPR260243 enhances the delayed rectifier current in guinea pig myocytes and can, to some extent, reverse dofetilide-induced prolongation of action potential. Physiologically, it has been reported that RPR260243 can increase the T-wave amplitude, prolong the PR interval and shorten the QT interval in guinea pig hearts[22].

PD-118057

PD-118057 [2-(4-[2-(3,4-dichloro-phenyl)-ethyl]-phenylamino)-benzoic acid] was first reported by Zhou and colleagues[25]. It primarily enhances the peak amplitude of the hERG tail current in a dose-dependent manner. PD-118057 shows no major effect on I_{Na}, I_{Ca}, I_{K1}, or I_{Ks}, and it shortens the action potential duration and QT interval in arterially perfused rabbit ventricular wedge preparations and prevents QT prolongation by dofetilide. Mechanistically, Zhou *et al* reported that the compound did not affect the voltage dependence or kinetics of gating, nor did its activity require the open conformation of the channel. Later results by Sanguinetti and colleagues suggest that PD-118057 activates the hERG channel mainly by attenuating inactivation[33]. They also found that 10 μmol/L PD-118057 shifted the half-point of hERG channel inactivation by +19 mV, increased peak outward current amplitude by 136%, and enhanced K^+ conductance.

PD-307243

PD-307243 [2-[2-(3,4-dichloro-phenyl)-2,3-dihydro-1*H*-isoindolin-5-ylamino]-nicotinic acid] significantly enhances hERG currents by slowing channel deactivation and inactivation. At potentials from -120 to -40 mV, PD-307243 induces instantaneous hERG current with little decay. When the membrane potential is higher than -40 mV, PD-307243 induces an I_{to}-like upstroke of hERG current. This I_{to}-like current may result from slowed channel inactivation and deactivation, and this effect can be only observed once the channel is in the open state, which may also explain the compound's use dependence. Additionally, hERG reversal potential was not altered in the presence of 3 μmol/L PD-307243, suggesting that the compound does not affect the selectivity filter of the channel[26].

NS1643

Casis and colleagues (2006) reported that NS1643 [1,3-*bis*-(2-hydroxy-5-trifluoromethyl-phenyl)-urea] activates hERG channels expressed in *Xenopus* oocytes in a concentration- and voltage-dependent manner[27]. At a depolarization voltage of -10 mV, the compound's EC_{50} value is 10.4 μmol/L. While NS1643 strongly affects channel inactivation by right

shifting the voltage-inactivation curve by +21 mV at 10 μmol/L and +35 mV at 30 μmol/L, it has no effect on the activation of the channel. In the absence of inactivation, NS1643 does not enhance outward current magnitude[27]. However, Xu *et al* (2008) reported that NS1643 can also left-shift the voltage-dependent activation curve[34]. In guinea pig cardiac myocytes, 10 μmol/L NS1643 can activate I_{Kr} and significantly shorten the action potential duration[27].

NS3623

NS3623 [N-(4-bromo-2-(1*H*-tetrazol-5-yl)phenyl)-*N*′-(3-trifluoromethyl-phenyl)urea], originally identified as a chloride channel blocker[35], activates hERG channels expressed in *Xenopous* oocytes, with an EC_{50} value of 79.4 μmol/L[28]. NS3623 mainly affects the voltage dependence of channel inactivation by right-shifting the half point of inactivation by +17.7 mV. NS3623 also slows channel inactivation. Similar to results found using NS1643, inactivation defective mutants S620T and S631A are not sensitive to NS3623, supporting the conclusion that these two compounds have similar mechanisms of action on hERG[28].

A-935142

A-935142 [{4-[4-(5-trifluoromethyl-1*H*-pyrazol-3-yl)-phenyl]-cyclohexyl}-acetic acid)] was reported in 2009 as a hERG channel activator that enhances the amplitude of step and tail current in a concentration- and voltage-dependent manner. Current-voltage curves in the presence of 60 μmol/L A-935142 suggest that the compound enhances both the outward and the inward K^+ currents. Unlike previously reported activators, A-935142 simultaneously affects channel activation, deactivation and inactivation. Specifically, 60 μmol/L of A-935142 significantly accelerates the activation time constant of hERG channels from 164±24 ms to 100±17 ms and left-shifts the voltage-dependence of activation. A-935142 also reduces the rate of inactivation, right-shifts the voltage-dependence of inactivation, and slows hERG channel deactivation at voltage potentials from -120 to -70 mV[29].

ICA-105574

Gerlach and colleagues recently described a compound, ICA-105574 [3-nitro-N-(4-phenoxyphenyl) benzamide], that activates hERG channels, mainly by affecting channel inactivation, with a magnitude much larger than that of previously reported activators. Two micromolar ICA-105574 shifts the mid-point of the voltage-dependent inactivation by >180 mV from -86 mV to +96 mV. Consistent with this observation, 2 μmol/L ICA-105574 potentiates outward current amplitude ten fold, with an EC_{50} of 0.5±0.1 μmol/L. In addition to effects on channel inactivation, high concentrations of the compound (3 μmol/L) can also left-shift the voltage dependence of channel activation by -11 mV and slow channel deactivation 2-fold. Finally, ICA-105574 induces a concentration-dependent shortening of action potential duration (>70% at 3 μmol/L) in isolated guinea pig ventricular cardiac myocytes. This effect can be prevented by hERG inhibitor E-4031, supporting the conclusion that ICA-105574 increases hERG channel function through direct action on the protein[30].

KB130015

KB130015 [(2-methyl-3-(3,5-diiodo-4-carboxymethoxybenzyl) benzofuran)] is a derivative of amiodarone, a potent hERG blocker[36]. Because of the similarity, Gessner *et al* assumed KB130015 inhibited hERG[31]. Unexpectedly, they found that, while KB130015 does inhibit native and recombinant hERG at high voltages, it can activate both forms of the channel at low voltages. KB130015 accelerates activation by 4-fold and left -shifts the voltage-dependent activation curve by -16 mV, with an EC_{50} value of 12 μmol/L. Based on its similarity to amiodarone, KB130015 presumably binds to the hERG pore from the cytosolic side and functionally competes with blockade by amiodarone and other canonical inhibitors at this site[31].

Mallotoxin

Mallotoxin (MTX) [1-(6-(3-acetyl-2,4,6-trihydroxy-5-methylbenzyl)-7-hydroxy-2,2-dimethyl-2*H*-chromen-8-yl)-3-phenylprop-2-en-1-one], an extract of the tree Mallotus phillippinensis, is the only natural product reported to activate hERG. It was previously shown to inhibit protein kinase C (PKC), Ca^{2+}/calmodulin-dependent protein kinase II and III, and elongation factor-2 kinase. Zeng *et al* (2006) discovered that MTX can enhance both step and tail hERG current, with EC_{50} values of 0.34 and 0.52 μmol/L, respectively[32]. The potency of MTX is at least ten fold higher than previously reported hERG activators, including PD-118057, NS1643, and RPR260243. Furthermore, the mechanism by which MTX potentiates hERG is unique in comparison to these synthetic molecules. It mainly affects channel activation (left-shifting the activation curve by +24 mV at 2.5 μmol/L MTX) and deactivation without modulating inactivation. Using pre-recorded cardiac action potentials, 2.5 μmol/L MTX increases the total number of potassium ions passed through hERG channels by ~5-fold[32].

Binding site of small-molecule hERG activators

Understanding the binding site of agonists is helpful for investigating channel conformation and gating, particularly for rational drug design. However, the exact molecular determinants of hERG activator function remain unresolved, with most of the knowledge about possible binding sites derived from mutagenesis experiments.

Sangunetii *et al* found that two groups of residues have different effects on RPR260243[24, 37]. One group, including L553, F557 (S5), and N658, V659 (S6), affects the inactivation and deactivation effect of RPR260243 on the hERG channel, while mutations in the other group of residues, including V549, L550 (S4–S5 linker), and I662, L666, Y667 (intracellular S6 segment), only hinder the transition to the closed state of the channel. In another study by Sangunetii *et al* about possible binding sites of PD-118057[33], they focused on the S5-P-S6 domain. Through alanine scanning mutagenesis of this region, four mutants (F619A, L622C, I639A, and L646A) were identified that display the lowest agonist activity in all tested mutants. Molecular

simulations suggested that PD-118057 interacted with F619 in the pore domain of one subunit and L646 (S6) of an adjacent subunit, which together form a hydrophobic binding pocket, reducing channel inactivation and increasing channel open possibility.

Limited binding site information about PD-307243 and NS1643 was reported by Xu and Tseng in 2008[34]. Because both compounds act on the extracellular side of the channel and significantly slow channel inactivation, the authors speculated that these compounds may act on the pore domain. Perturbation of the conformation of the outer vestibule/external pore entrance (by cysteine substitution at high-impact positions or cysteine side chain modification at intermediate-impact positions) prevented the activation effect of NS1643 but not that of PD-307243, suggesting that NS1643 may bind to this domain. Further pharmacological experiments showed that the effects of PD-307243 could be abolished by $TPeA^+$ and dofetilide (both hERG inhibitors that block the pore), which supports the conclusion that PD-307243 may be a "pore-modifier" (Table 2)[34].

Outlook and challenges

LQTS is responsible for many sudden deaths before age 20. Current treatments for LQTS include beta-adrenergic receptor blockers, left cardiac sympathetic denervation or, in the worst cases, implantation of cardiac defibrillators[24, 38]. However, pharmacologic treatment is not always effective, and both surgery and implanted devices are expensive and require invasive procedures[24, 39]. Acute episodes of drug-induced LQTS are treated with magnesium sulfate administration and discontinued use of the suspect medication. Activation of hERG could provide an alternative and more specific treatment for acquired or congenital LQTS. In addition, hERG activators may become a novel class of antiarrhythmics because, as mentioned above, they can reduce electrical heterogeneity in the myocardium and, thereby, the possibility of re-entry[20, 21]. However, this idea has yet to be confirmed clinically. A recent report in PubChem described a co-drug screen aiming to protect against the development of aLQTS by a high–throughput method (AID: 1680). Compounds that exhibit such activity would be expected to include neutral antagonists that bind to the same site as hERG blockers but do not favor the "blocked conformation" of the pore. Alternatively, these compounds may be hERG agonists that competitively bind to the same site as hERG inhibitors or compounds that prevent the access of a hERG inhibitor to its cognate binding site at the intracellular aspect of the pore region. Although it remains to be verified whether these compounds would be effective, this strategy does provide a new idea for the treatment of aLQTS.

The structural and mechanistic heterogeneity of the currently reported hERG activators suggests that hERG channels may have different agonist binding sites, a phenomenon that has been observed in voltage-gated KCNQ channel activators. However, so far, studies on the binding site by hERG agonists are still descriptive, mostly relying on inference from mutagenesis data for RPR260243 and PD118057. However, activation of hERG by small molecules may be a double-edged sword from a clinical perspective because excessive potentiation may chemically induce short QT syndrome[40]. Moreover, there are no reports from animal models or human clinical data about drug-induced short QT syndrome, indicating that the safety of hERG agonists remains to be tested.

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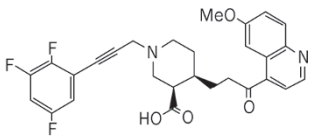
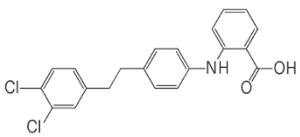
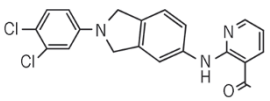
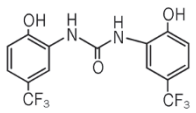
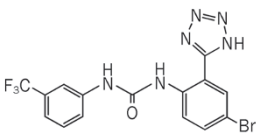
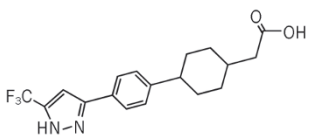
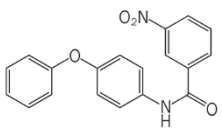
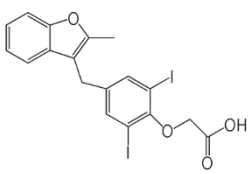
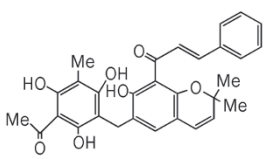
Table 2. Summary of 8 HERG K^+ channels activators.

Compound name	Structure	Mechanism of action	Binding site	Selectivity on channels	Refs
RPR260243		Slows deactivation	Interacts with residues in the S4–S5 linker or cytoplasmic ends of the S5 and S6 domains	No effect on human cardiac Na^+ channel, KCNQ1/KCNE1 channels, weakly inhibits L-type Ca^{2+} channels, inhibits erg3 channel	[22–24]
PD-118057		Attenuates inactivation	Contacts the pore helix of hERG channels to attenuates inactivation and enhances K^+ conductance	No effect on I_{Na}, $I_{Ca,L}$, I_{K1}, and I_{ks}	[25, 33]
PD-307243		Slows channel deactivation and inactivation	May works as a "pore-modifier" of the hERG channels	Activates I_{ks} and $I_{Ca,L}$; no effect on I_{to} and Nav1.5	[26, 34]
NS1643		Attenuates inactivation	Binds to the outer vestibule/pore entrance of hERG	No report	[27, 34]
NS3623		Attenuates inactivation	No report	No effect on I_{Ks}, I_{Kur}, Kv4.3, $I_{Ca,T}$, I_{Na}, activates $I_{Ca,L}$	[28]
A-935142		Accelerates activation Attenuates inactivation and slow deactivation	No report	No report	[29]
ICA-10557		Mainly attenuates or remove channel inactivation, besides at high concentration it also accelerates activation and slows deactivation	No report	No report	[30]
KB130015		Accelerates activation	Presumably binds to pore domain from the cytosolic-side	No report	[31]
Mallotoxin (MTX)		Left-shifts the activation curve, and slows deactivation process	No report	No report	[32]

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