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Perspective

Medicinal Chemistry of hERG Optimizations: Highlights and Hang-Ups

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1. Introduction

In recent years, withdrawal of marketed drugs including sertindole, grepafloxacin, and terfenadine owing to prolongation of the length of time between the start of the Q wave and end of the T wave on an electrocardiogram (QT interval) has prompted considerable effort in trying to establish the molecular basis of this potentially lethal phenomenon. In the majority of cases, those chemical entities that prolong the QT interval and lead to torsades de pointes (TdP[a]) preferentially interact with a product of the human ether-a-go-go related gene (hERG), the α-subunit of IKr channels responsible for the rapid component of the delayed rectifier potassium current in the heart.[1−3] Consequently, throughout the pharmaceutical industry efforts to predict QT prolongation risk have been focused on assays testing in vitro hERG channel activity in mammalian cell lines expressing the hERG channel. Medicinal chemistry groups engaged in both hit-to-lead and lead optimization activities have encountered blockade of the hERG channel as a significant hurdle along the drug discovery trajectory. Despite this, an increasing body of information has been accumulated on the strategy and tactics for overcoming inhibition of hERG. A brief summary of the in silico, in vitro, and in vivo approaches employed to measure blockade of hERG and QT prolongation is detailed in this report. The purpose of the following discussion is to summarize the approaches engaged to circumvent activity at hERG, identified through an extensive literature survey. The optimizations are recorded as pairs of compounds, which have been categorized in terms of the tactics employed to diminish hERG activity. Examination of the properties of the compounds within each category has enabled formulation of some empirical guidelines. Conclusions and recommendations for future activities are also presented.

1.1. In Vitro and In Vivo Approaches. The biophysics of cardiac action potential and relation to electrocardiogram (ECG) has been discussed extensively elsewhere.[4] It is well established that blockade of the hERG channel can lead to prolongation of the action potential duration and consequently an increase in the QT interval. It should be noted, however, that the cardiac proarrhythmic toxicity of drugs through interaction with other channels or subunits involved in repolarization of heart muscles (e.g., minK, Kir2 channels) to the best of our knowledge has not yet been reported but cannot be excluded. Nevertheless, the majority of drugs that cause TdP also substantially inhibit the hERG channel at therapeutic concentrations;[2] hence, significant effort has been invested in quantifying drug−hERG interaction. The 4-fold symmetry of the hERG subunit, together with the possibility of multiple binding sites, means the channel is particularly promiscuous with regard to drug interaction. This adds an extra layer of complexity in terms of the types of assay used in order to definitively assess the extent of block a drug candidate causes. Table 1 summarizes the widely applied techniques used to assess hERG blockade in both in vitro and in vivo settings.

1.2. In Silico Approaches. Several recent review articles have detailed the state of the art in terms of in silico hERG modeling.[4,12−14] Approaches can be broadly classified into

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[a] Abbreviations: hERG, human ether-a-go-go gene related product; TdP, torsades de pointes; minK, minimal potassium channel; K_vLQT1, potassium voltage-gated channel subfamily KQT member 1; Kir2, inward rectifier potassium channel 2; IKr, potassium voltage-gated channel ether-a-go-go protein; IKs, slow voltage producing potassium channel β subunit; KcsA, *Streptomyces lividans* potassium channel; MthK, *Methanobacterium thermoautotrophicum* potassium channel; K_vAP, *Aeropyrum pernix* voltage-dependent potassium channel; APD, action potential duration; MAP, monophasic action potential; GRIND, grid independent descriptors; MLR, multiple linear regression; DSM, discrete structural modifications; ZI, zwitterion.

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Table 1. Summary of in Vitro and in Vivo Techniques for Studying hERG Blockade

details	comments
In Vitro−Ligand Binding Assay	
Fast and sensitive assay that uses HEK293 cells stably transfected with the hERG channel and radiolabeled ligand, e.g., dofetilide[5] or MK-499.	Can correlate well with electrophysiology. No functional activity can be inferred.
High throughput of ≥100 compounds per day. Low-cost assay.	May not detect compounds that act at sites distinct to the ligand binding site, leading to lack of correlation with electrophysiology.
In Vitro−Electrophysiology hERG Assay	
Manual patch clamp electrophysiology used for more detailed work (e.g., temperature dependence of hERG block).	Can detect activity at any binding site and can offer information on state/use dependence of a channel.
Automated electrophysiology in the form of PatchXpress.[6]	Reported to correlate well with QT prolongation in vivo.
Manual patch clamp has low throughput of <50 data points per week. PatchXpress has high throughput of up to 1200 data points per week.	False negatives may arise as a result of hydrophobic interactions between compounds and the apparatus used in the experiment.
Ex Vivo−Langendorff-Perfused Isolated Heart Preparation[7]	
Records monophasic action potentials (MAPs) in isolated Langendorff-perfused rabbit heart and evaluates action potential duration (APD), conduction, instability, triangulation, and reverse-use dependency.	Offers information on several markers of proarrhythmia liability, which have been validated with drugs known to induce TdP and those not associated with proarrhythmia in a clinical setting.
High-cost, low-throughput assay.	
Ex Vivo−Purkinje Fibers[8]	
Measures transmembrane action potential. Prolongation of the QT interval and subsequent risk of arrhythmia is related to prolongation of the APD in Purkinje fibers.	The intensive use of animals (primarily dogs) is unattractive from an ethical perspective. Currents measured cannot be correlated to man, inhibiting a translational approach.
High cost, low throughput of around one compound per week.	
In Vivo−Animal Models	
The methoxamine-sensitized rabbit model[5] has been widely used to assess proarrhythmic liability.	Markers of repolarization liability other than APD or QT prolongation may have higher predictive value owing to better correlation with TdP.
The AV dog model[9,10] involves a structural, mechanical, and electrical remodeling process that increases the susceptibility to arrhythmia on exposure to drugs that delay ventricular repolarization.	The reported rabbit and dog models use anesthetized animals and have been mainly used for class III antiarrhythmics.
Other animal models include guinea pig (ECG of conscious restrained guinea pigs[11]), pig, and monkey.	Guidelines suggest that safety pharmacology should be studied in *conscious* animals, thereby excluding possible interference of anesthetics. Guidance is required on appropriate dose selection of nonantiarrhythmic drugs where clinical data on relevant exposures or efficacy are unavailable.
High-cost, low-throughput series of assays.	

structure-based approaches, which bring together homology modeling, mutagenesis data, and ligand-based approaches, which include both pharmacophore and QSAR methods.

Early mutation studies indicated that the aromatic residues F656 and Y652 of the hERG S6 domain are important for interactions with the hERG blocker MK-499, and this is further supported by a homology model derived from the KcsA potassium channel structure.[15] More detailed analyses of the roles of F656 and Y652 indicate that at position 652 an aromatic residue is required, leading to the hypothesis that Y652 participates in π−cation type interactions with basic amine containing hERG ligands and presumably π-stacking interactions for neutral hERG ligands (see Figure 1). In the case of position 656, the hydrophobic surface area was found to be the key determinant, suggesting that F656 participates in hydrophobic interactions.[16] Strategies that disrupt the ability of a ligand to interact with F656 and Y652 have been proposed as a possible means to remove hERG activity.[17] Further homology models of hERG based on the KcsA and MthK potassium channel structures have been described in support of mutation and mechanism of action studies.[18−21] Some of this work has been covered in recent reviews.[17,22] A homology model based on MthK has been used alongside a 3D QSAR alignment model to examine potential ligand−channel interactions and showed consistency with mutagenesis data.[23] A "drain−plug" analogy was proposed where ligands effectively occlude the pore from the intracellular face, interacting with F656 and Y652.

It is clear that hERG homology models have provided a qualitative insight into potential ligand−channel interactions. A consensus view of ligand binding has emerged with the ligand oriented along the axis of the channel pore making interactions with the aromatic residues of the S6 domain. Recent studies detail the quantitative use of models based on KcsA, MthK, and KvAP potassium channel structures in combination with linear interaction energy (LIE) approaches to predict ligand binding energies with some success.[24,25] Despite this initial success, the use of homology models in a quantitative manner to predict hERG affinities is speculative and is unlikely to provide easy to implement, quick predictions. Indeed, in a drug discovery optimization environment, there are very few disclosed examples where structure-based in silico approaches to minimizing hERG activity have been used. One recently reported example in the optimization of CCR5 receptor antagonists showed how ligand docking to a hERG homology model was used in guiding chemistry efforts to more polar substituents within the series.[26]

Many attempts to model hERG data in the form of a ligand-based pharmacophore or QSAR model have been described. Models tend to be global with a varying degree of structural diversity of ligands using the entire spectrum of QSAR methodologies. hERG data are either sourced from the literature or generated in-house and tend to be either patch-clamp electrophysiology hERG potency data or radioligand binding data.

3D QSAR models using CoMFA,[27] Catalyst,[28,29] and CoMSiA[23] methodologies have been described and suggest key pharmacophoric regions consisting of a number of hydrophobes and a positive ionizable center. These alignment-dependent models give key insight into the pharmacophoric requirements and reassuringly show consistencies with the homology models, but their use in a predictive sense is somewhat limited. Models

are based on small data sets and can be subject to unrealistic conformations and molecular alignment difficulties, rendering the prediction of novel structurally diverse ligands difficult. Experience suggests that alignment-dependent 3D QSAR models can be more useful in a predictive sense when a more congeneric series is used, for example, a local model around a lead series. Alignment independent methodologies can alleviate some of the difficulties associated with the alignment-dependent methods (e.g., use in a predictive setting), and such a model using alignment-independent GRIND descriptors has been developed for hERG.[30] Further ligand-based approaches using fragment-based QSAR methods,[31−33] and a model suggesting that the extent of charge shielding around the basic amine of hERG blockers correlates with hERG potency, have been described.[34]

An increasing number of publications detail numerical models based on larger data sets and a multitude of 1D, 2D, and 3D descriptors using an array of multivariate methodologies. These models are categorical and attempt to provide a classification output of whether a compound is a hERG blocker or otherwise. Although the qualitative predictions that the categorical models provide may not be sufficient to aid an optimization program, such models find utility in profiling and prioritizing compound arrays. Predictive categorical models using neural networks,[35] Bayesian,[36] support vector,[37] decision tree,[38] MLR,[39] and a combination of nearest-neighbor similarity and 3D pharmacophore fingerprint[40] methodologies have all been detailed. Numerous software vendors have also strived to make available predictive hERG QSAR models. However, results from vendor models must be compatible with in-house data for the models to be useful.[41] Therefore, it is essential to validate an externally developed model in the context of the internal data for which it is to be predictive prior to use.

The true test of any in silico model is the acceptance and application by medicinal chemists to guide decisions at the design stage in a drug discovery environment. Unfortunately, in the case of in silico hERG models the predictive value in chemical optimizations is uncertain because of the very few examples of use that have been published. This perhaps reflects the nature of the published models in that global models appear to be better suited as early warning systems to profile arrays of compounds flagging potential hERG blockers rather than for direct use in an optimization program. A local model providing quantitative predictions is likely to be better suited for the task. All in silico approaches to hERG have, however, contributed to a greater understanding of the structure of the channel, ligand−channel interactions, and SAR.

1.3. Classification of Literature Optimizations. An extensive survey of the medicinal chemistry literature up to December 2005 has identified a number of reports where the authors have successfully minimized hERG/IKr activity during a medicinal chemistry optimization program and reported significant SAR at hERG. In contrast to global hERG models where heterogeneous data sets are often used, the approach adopted here has the advantage of dealing with optimization pairs from the same chemical series and the data have been generated under identical assay conditions. Therefore, there exists a direct relationship between the start and end points of the optimization, which has enabled us to determine the most appropriate method to employ depending on the nature of the starting compound. The primary focus of the review is on efforts to remove hERG activity that may result in concomitant variation of the primary target potency, further emphasizing the multifactorial nature of hERG optimizations. The resulting analysis is aimed at producing a set of simple, easy to understand guidelines for attenuating hERG activity that will be of utility to the practicing medicinal chemist rather than developing statistically validated models or 3D pharmacophore information.

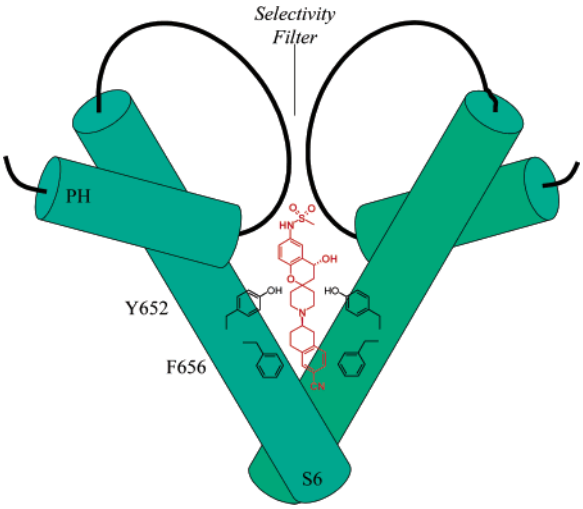


Figure 1. Schematic depiction of the putative interactions between a spiropiperidine-based antiarrhythmic agent and the hERG channel. The inner helices (S6) and loops extending from the pore helices (PH) to the selectivity filter form the inner cavity and the ligand-binding site. For clarity, S6 and PH domains of only two subunits of the tetrameric channel are shown (longitudinal view with respect to the cell membrane).

The reported optimizations were grouped into a series of categories based on common descriptors in order to formulate some simple, empirical guidelines on steering away from hERG activity. These categories are as follows: discrete structural modifications (DSM), formation of zwitterions (ZI), control of log *P* and attenuation of pK_a. Each of the categories is expanded upon in the following sections. Although we have attempted to focus on examples where there is only one clear site of modification, it should be noted that it is not possible to alter one parameter in isolation, and this may confound interpretation of the controlling factor mitigating hERG activity. Therefore, the categorization applied here is best regarded a mnemonic rather than a rigorous classification. What is apparent from our survey is that there is no such thing as a panacea in terms of detuning hERG activity. Indeed, many of the groups who encounter hERG issues employ several tactics to diminish this unwanted activity.

1.3.1. Discrete Structural Modifications. From consideration of the published SAR information against the hERG channel, discrete, often peripheral modifications to a drug molecule can have a dramatic effect on potency. A possible explanation of this phenomenon could be derived from the available mutagenesis data combined with homology modeling on the open and closed states of the channel.[15,23] These studies suggest that the major determinants of hERG−drug interaction are π-stacking and hydrophobic interactions between aromatic residues (F656 and Y652) lining the large cavity and aromatic moieties suitably positioned in a drug molecule. Figure 1 shows a schematic depiction of a spiropiperidine-based antiarrhythmic agent (MK-499) and its putative interactions with the hERG channel based on homology modeling.[15]

Modification, either subtly or in a wholesale manner, of such molecular features in the drug molecule could potentially disrupt these putative interactions and affect affinity for the hERG channel. Strategies adopted to mitigate hERG activity in this way not only are restricted to modifications to distal aryl rings but also can include introducing constraint, variation in stere-

ochemistry, and may even result in slight increases in lipophilicity. Several groups have employed such approaches with considerable success in removing hERG activity. For optimizations included in this classification we have ensured that there is no significant reduction (<1 log unit) in key physicochemical parameters such as clogP and pK_a.[42]

1.3.2. Formation of Zwitterions. Physically limiting the membrane permeability of a compound through formation of a zwitterionic species could prevent access of a drug molecule to the proposed transmembrane binding site, therefore minimizing any potential interaction with hERG. Some attention has been given to this approach, with the advantages and shortcomings of the zwitterion method discussed below.

1.3.3. Control of log *P*. SAR data can often show series-dependent correlation between potency of hERG block and measures of lipophilicity. This is supported by recent published hERG homology models and mutagenesis data[15,16] suggesting that a lipophilic ligand binding site exists that is accessed from the intracellular domain. When the polarity of a drug molecule is increased, interaction with the lipophilic cavity is destabilized. Such an approach has been employed in a number of optimization campaigns as a means of decreasing hERG activity, and several examples are presented herein. Optimizations included in this category have been restricted to those where a significant (>1 log unit) reduction in clogP has been observed.

1.3.4. Attenuation of pK_a. Many of the ligands that block hERG contain a basic nitrogen moiety that is likely to be protonated at physiological pH. It should be noted, however, that a basic amine is not a prerequisite to hERG blockade because a suitably placed aromatic or hydrophobic group in the ligand can interact with the channel equally well. Mutagenesis and homology modeling has led to the proposal that $\pi-$cation interactions between basic amine containing hERG blockers and aromatic residues within the cavity of the hERG channel contribute to ligand binding affinity.[15,23] Therefore, lowering the pK_a of a basic nitrogen would reduce the proportion of molecules in the protonated form at physiological pH and would be expected to disrupt any putative $\pi-$cation interactions with the channel. In a similar vein, shielding the protonatable center through incorporation of alkoxy/hydroxyl groups at the β-position with respect to the amine may also destabilize any $\pi-$cation interaction and thus lower affinity for hERG.[34] In general, we have observed that modification of pK_a can often have the effect of increasing the polarity of a substrate (e.g., piperidine to piperazine) and thus can be regarded as another manifestation of the effect of log *P*. Data presented in the optimization tables refers to the highest calculated basic pK_a.

2. Survey of Strategies Used to Detune hERG

2.1. Discrete Structural Modifications. Subtle structural modification was one of strategies employed in an effort to design out hERG affinity within a series of novel pyrrolidinone-based farnesyltransferase inhibitors (FTIs).[43] Several members of the series such as compound **1** (Table 2) were found to have significant hERG activity (FT IC_{50} = 1.9 nM and hERG IP = 0.44 μM), and selectivity was, therefore, a key goal in the medicinal chemistry strategy. Migration of the pyrrolidinone carbonyl group featured in **1** out of the ring resulted in the 1-acylpyrrolidine analogue **2** with more than 10-fold reduced hERG affinity (IP = 5.8 μM). However, this structural change had a similar effect on FT inhibition (IC_{50} = 27 nM), rendering analogue **2** with a similar selectivity window over hERG compared to the parent compound **1**. Similarly, deletion of a chlorine on the phenyl ring (**3**) had the effect of reducing hERG activity with concomitant reduction in potency at the primary target. In their further efforts to increase the therapeutic window, a range of constrained analogues of **1** were synthesized.[44] Compound **4** was one of the most potent FTIs in this series (IC_{50} = 0.15 nM); however, it was also one of the most potent hERG blockers with an IP of 0.08 μM. The compound was found to cause 10% prolongation of the QTc interval in the anesthetized dog at a plasma level of 2.4 μM, which, although not as severe as hERG binding data suggested, was deemed unacceptable. The hERG SAR within this macrocyclic series proved to be highly sensitive to minor structural and topological changes, as illustrated by diastereoisomer **5** and N-methylated analogue **6**, both showing significantly attenuated hERG binding (IP = 4.7 and 2.6 μM, respectively) in comparison to the parent compound **4** (IP = 0.08 μM). Expansion of the cyclopentyl to cyclohexyl ring was also successful, delivering tetrahydronaphthalene derivative **7**, one of the most potent and selective FTIs in the series (hERG IP = 7 μM; FT IC_{50} < 1 nM). Interestingly, insertion of oxygen into the cyclopentyl ring did not affect hERG binding, despite reduced lipophilicity of the resulting analogue **8** compared to **4**. However, (*S*)-analogue **7** showed a significant drop in hERG affinity where the oxygen is replaced by a methylene unit (cf. **8**), further illustrating subtle hERG SAR in this series. In the alkoxy analogues, reduction in hERG activity could be achieved by preparation of a diastereomerically related compound **9**, similar to the example discussed above.

A report from Blum et al. disclosed a series of 2,4-disubstituted imidazole neuropeptide Y5 receptor antagonists that exhibited significant hERG block. Notably, these compounds lack a basic nitrogen, which is often perceived to be a key determinant of hERG activity. A strategy of introducing polar functionality was adopted by modification of the imidazole C2 substituent.[45] However, several compounds with polar functionality still retained potent hERG activity. Indeed, hydroxyethylamide **10** shows 87% inhibition of hERG at 3 μM in an in vitro whole-cell electrophysiology assay. Introduction of a *gem*-dimethyl substitution to give the more conformationally constrained analogue **11** resulted in a reduced hERG potency of 6% inhibition at 3 μM, high NPY5 receptor affinity (K_i = 2.8 nM), and reasonable pharmacokinetic properties.

Researchers at Merck have reported the optimization of a benzimidazole series of NMDA NR2B antagonists.[46] Compound **12** derived from a screening hit has a high affinity for the NR2B channel (K_i = 180 nM) but also shows high hERG affinity in the MK-499 binding assay (IP = 0.12 μM). Similar to the example above,[45] compounds in this series lack a basic amine. Acetylation of the benzimidazole amine substituent to give acetamide **13** resulted in almost a 22-fold decrease in hERG affinity (IP = 2.6 μM) while slightly increasing NR2B affinity (K_i = 93 nM). Similarly, a simple change of the methylsulfonamide of **14** to the ethylsulfonamide in **15** resulted in a 13-fold decrease in hERG affinity. In a parallel series of benzylpiperidines the isopropylsulfonamide **17** showed a 10-fold reduced hERG affinity upon comparison with the methylsulfonamide **16**. In each of these examples, the reduction in hERG affinity may be attributed to specific structural changes in the periphery of the molecules, potentially disrupting the interaction of the adjacent aromatic ring with the hERG channel binding site.

In their CCR5 program, Kim and co-workers[47] have adopted a number of strategies in order to avoid hERG activity. It was found that modification of a distal aryl ring (**18** to **19**) by incorporation of a trifluoromethyl group significantly reduced activity at the hERG channel. In a related series of compounds,[48] peripheral structural effects were also important in negating hERG activity. Conversion of the *tert*-butyl group of **20** to the

Table 2. Application of Discrete Structural Modifications to Control hERG

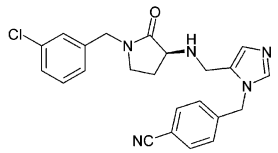
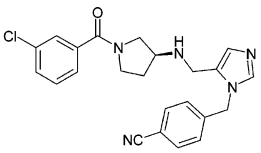
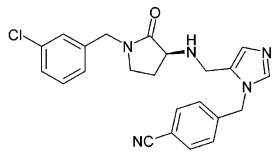
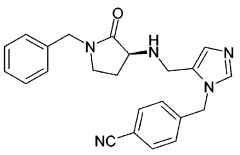
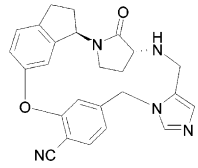
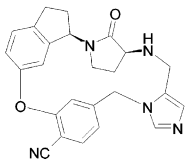
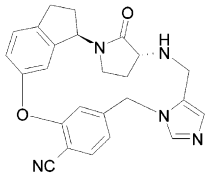
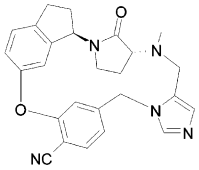
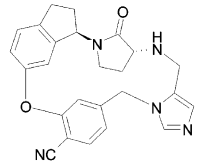
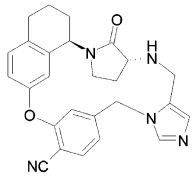
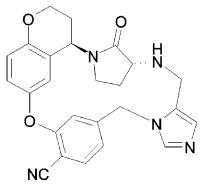
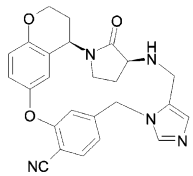
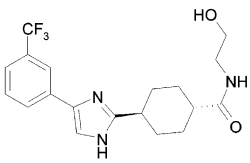
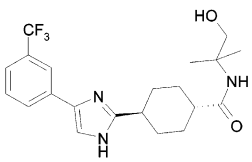
hERG active	hERG[a] (μM)	IC_{50}[a,b] (nM)	clogP[c]	pKa[c]	hERG optimised	hERG[a] (μM)	IC_{50}[a,b] (nM)	clogP[c]	pKa[c]	Ref
 **1**	**0.44**	**1.9**	2.49	6.46	 **2**	**5.8**	**27**	2.30	8.56	43
 **1**	**0.44**	**1.9**	2.49	6.46	 **3**	**7.2**	**29**	1.77	6.46	43
 **4**	**0.08**	**<1**	2.48	6.64	 **5**	**4.7**	**3.6**	2.48	6.64	44
 **4**	**0.08**	**0.15**	2.48	6.64	 **6**	**2.6**	**1.4**	3.06	6.38	44
 **4**	**0.08**	**<1**	2.48	6.64	 **7**	**7**	**<1**	3.04	6.65	44
 **8**	**0.15**	**<1**	1.93	6.61	 **9**	**9.1**	**<1**	1.93	6.61	44
 **10**	**87% @ 3 μM**	**11**	2.65	5.66	 **11**	**6% @ 3 μM**	**2.8**	3.36	5.67	45

Table 2 (Continued)

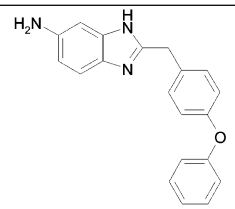
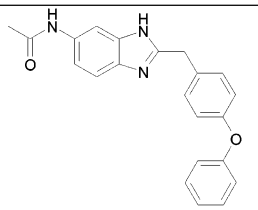
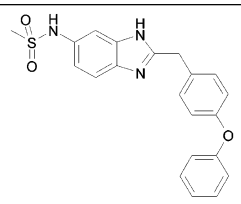
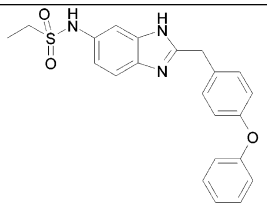
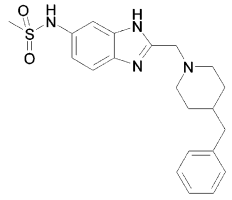
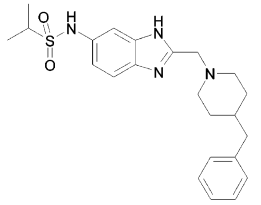
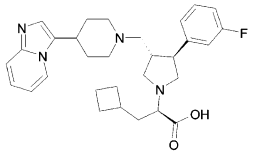
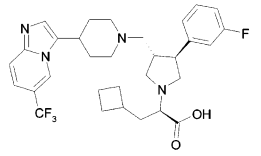
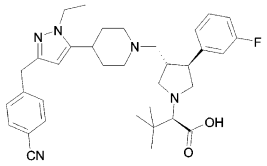
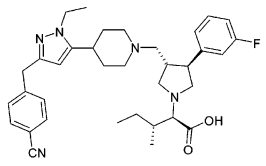
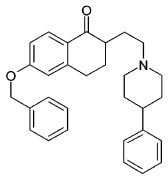
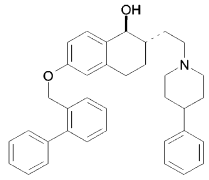
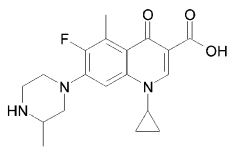
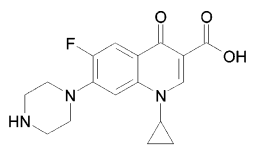
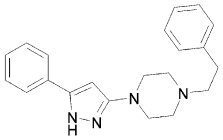
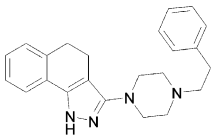
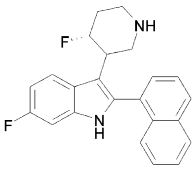
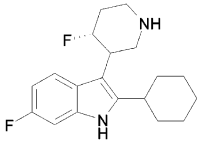
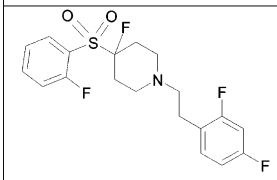
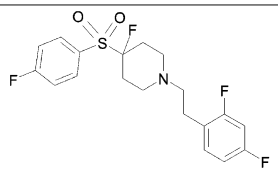
hERG active	hERG[a] (μM)	IC_{50}[a,b] (nM)	clogP[c]	pKa[c]	hERG optimised	hERG[a] (μM)	IC_{50}[a,b] (nM)	clogP[c]	pKa[c]	Ref
 **12**	**0.12**	**180**	4.95	6.79	 **13**	**2.6**	**93**	5.14	5.49	46
 **14**	**0.12**	**0.68**	4.78	5.18	 **15**	**1.6**	**3**	5.31	5.20	46
 **16**	**0.34**	**0.99**	3.81	10.98	 **17**	**3.3**	**240**	4.65	10.97	46
 **18**	**2.7**	**0.42**	3.79	10.07	 **19**	**>10**	**0.82**	4.73	10.07	47
 **20**	**0.99**	**1.3**	3.56	10.19	 **21**	**>10**	**1.3**	4.48	10.19	48
 **22**	**0.17**	**69**	7.06	9.14	 **23**	**1.5**	**37**	7.97	9.34	49
 **24**	**50**		-0.13	8.82	 **25**	**966**		-1.15	8.76	50

Table 2 (Continued)

hERG active	hERG[a] (μM)	IC_{50}[a,b] (nM)	clogP[c]	pKa[c]	hERG optimised	hERG[a] (μM)	IC_{50}[a,b] (nM)	clogP[c]	pKa[c]	Ref
 **26**	**EC25 5.8**	**23**	4.17	7.58	 **27**	**EC25 >30**	**5.3**	4.39	8.49	51
 **28**	**0.11**	**0.34**	5.46	9.00	 **29**	**5.4**	**0.25**	5.06	9.11	52
 **30**	**0.4**	**0.32**	3.50	5.51	 **31**	**5.6**	**3.9**	3.33	5.63	53

[a] Both hERG and primary target activity refer to K_i, IC_{50}, or IP unless otherwise stated. [b] IC_{50} refers to activity at the primary target. [c] clogP and pK_a are calculated according to ref 42. pK_a is reported as the highest basic pK_a. Where no value of pK_a is reported, calculation of this parameter was not possible.

sec-butyl system of **21** is accompanied by a greater than 10-fold reduction in the hERG activity with essentially conservation of potency at the primary target. Within this same series, the Merck group also employed control of log *P* to minimize hERG activity, and this is discussed below.

A particularly interesting example of how selectivity over IKr/hERG can be achieved against a related ion channel has been recently disclosed.[49] This report was centered around the development of selective inhibitors of the slow component of the delayed rectifier potassium current (IKs), which are of potential utility in prolonging action potential duration without the proarrhythmic side effects of compounds such as dofetilide. It should be noted that all compounds in this series were tested as racemates. Around 40-fold IKs/IKr selectivity could be achieved by modification of the terminal benzyl moiety of **22** to incorporate a biphenyl system (**23**). However, the improvement in selectivity cannot be ascribed solely to modification of the terminal aryl system, as incorporation of an additional H-bond donor may also have an effect on IKs/IKr activity.

Issues with QT prolongation in the fluoroquinolone class of antibiotics are well-known and have resulted in the removal of such drugs from the marketplace. Despite the apparently modest activity at hERG, compounds in this class can cause QT prolongation, and this has been attributed to the high concentrations of drug in plasma leading to significant block at hERG.[50] In an example of more subtle structural changes, the removal of two methyl substituents from compound **24** (grepafloxacin) resulted in an almost 20-fold reduction in hERG activity for compound **25**. Comparison of other fluoroquinolones in the series revealed that the most potent compounds against hERG were all substituted in the C5 position.

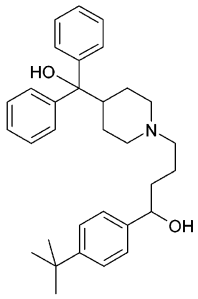
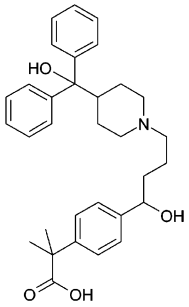
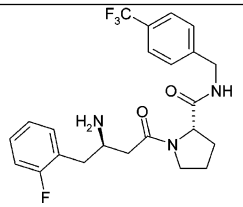
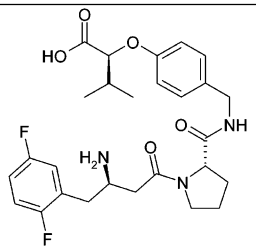
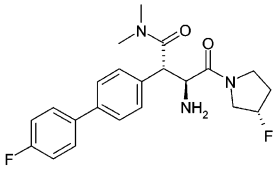
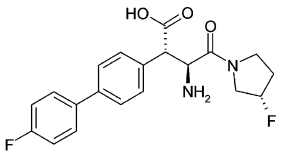
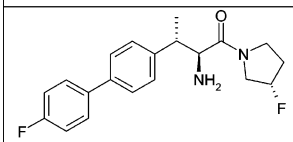
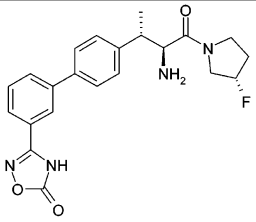
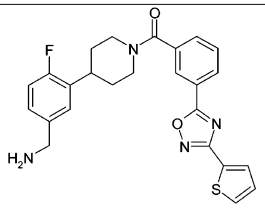
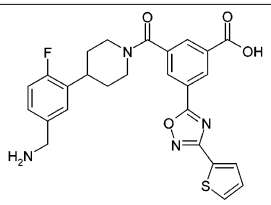
Workers at Merck, Sharpe & Dohme have been interested in the development of a pyrazolopiperazine family of dopamine D4 antagonists as potential antipsychotics. During their medicinal chemistry program, selectivity against numerous ion channels including hERG was encountered as an issue.[51] The first step in the optimization trajectory within the series was to control the pK_a of the basic nitrogen, and this is discussed below. The lowest reported hERG activity in the series was afforded by conformational constraint of the progenitor compound **26** by linking the pyrazole to the 5-phenyl substituent to form the 4,5-dihydro-1*H*-benzo[*g*]indazole **27**.

5-HT_{2A} antagonists also tainted by high hERG binding were recently reported by Rowley et al.[52] To improve selectivity over hERG, the authors adopted a lipophilicity-lowering strategy, which also proved to be successful (see Table 4), and made modifications to the distal aryl groups. In this case, a cyclohexyl replacement for a naphthyl system actually proved to be more effective than the tactic of controlling log *P* (**29**, hERG K_i = 5.4 μM; $5HT_{2A}$ K_i = 0.25 nM).

As well as using more wholesale structural changes and clogP to tune hERG activity, a recent report from Fish and co-workers describes the effect of subtle structural changes in enhancing the selectivity window in another series of 5-HT_{2A} antagonists.[53] Preparation of the isomeric fluorophenylsulfone **31** yielded more than a 10-fold reduction of hERG activity while retaining activity at the target of interest. Given the minimal impact of this modification on the relevant physicochemical parameters, this example serves to highlight the power that discrete structural modifications offer in terms of achieving selectivity over hERG.

2.2. Formation of Zwitterions. Terfenadine (**32**, Table 3), a second-generation antihistamine, was the first compound in the series to be launched in 1982. However, postmarketing analysis conducted some time after launch indicated its association with a number of TdP-related incidents. Terfenadine was shown to prolong the QT interval when used concomitantly with drugs or food that inhibit CYP3A4, resulting in elevated plasma levels of the drug.[54] Initially, in light of the cardiac side effects, the

Table 3. Application of the Zwitterion Approach To Minimize hERG Activity

hERG active	hERG[a] (μM)	IC_{50}[a,b] (nM)	clogP[c]	pKa[c]	hERG optimised	hERG[a] (μM)	IC_{50}[a,b] (nM)	clogP[c]	pKa[c]	Ref
 **32**	**0.056**	**1**	6.07		 **33**	**23**	**15**	1.96		55
 **34**	**0.87**	**721**	3.80	7.99	 **35**	**76**	**0. 48**	1.46	7.75	56
 **36**	**4.6**	**12**	2.48		 **37**	**76**	**6.6**	0.15		57
 **38**	**1.1**	**64**	3.33		 **39**	**28**	**3**	2.42		58
 **40**	**0.8**	**4.3**	3.82		 **41**	**76.8**	**88**	1.54		59

[a] Both hERG and primary target activity refer to K_i, IC_{50}, or IP unless otherwise stated. [b] IC_{50} refers to activity at the primary target. [c] clogP and pK_a are calculated according to ref 42. pK_a is reported as the highest basic pK_a. Where no value of pK_a is reported, calculation of this parameter was not possible.

FDA issued a black box warning. However, since instances of cardiac arrhythmia continued, terfenadine was withdrawn from the market in 1997. Interestingly, it transpired that the main metabolite of terfenadine, carboxylate **33** (later marketed as fexofenadine) is responsible for the therapeutic effect of its parent. In addition, the compound exhibits significantly reduced hERG affinity and no effect on QT interval.[55] The low hERG affinity of the zwitterionic fexofenadine could be attributed to the poor cell membrane permeability of the compound and consequently its limited access to the intracellular lumen of the channel, thought to be critical for hERG blockade. The zwitterion approach has since been widely employed as one of the strategies for designing out hERG activity. In addition, recent molecular modeling work comparing the two compounds has also suggested that differences in conformation could be invoked to explain this difference in hERG activity.[23]

In a number of reports on their series DPPIV inhibitors,[56−58] workers from Merck have shown that incorporation of an acid moiety (or related bioisostere) into an amine containing template to furnish a zwitterionic system has been of utility in minimizing hERG activity. Derivation of the zwitterionic system **35** from the original proline-derived homophenylalanine lead **34** gave a compound that was essentially inactive at hERG.[56] However, the improvement in the hERG profile was not without penalty, as the zwitterionic species has significantly reduced oral bioavailability (F = 1.2% for **35** vs 37% for **34**). Oral administration to portal vein cannulated rats indicated that the low bioavailability is due to poor absorption, which in turn can be ascribed to the presence of the acid group in the compound.

More recently, a related series of biaryl-β-methylphenylalanine DPPIV inhibitors have been disclosed.[57] Conversion of the carboxamide derivative **36** to the carboxylic acid **37** gave approximately a 16-fold reduction in hERG activity, with essentially retention of potency at DPPIV. As observed in the previous study, there exists a tradeoff between selectivity over hERG and oral bioavailability (cf. **37**, F = 16%; **36**, F = 67%). Incorporation of an acid bioisostere into the biaryl-β-methylphenylalanine template has also been shown to afford enhancements in selectivity over hERG (cf. compounds **38** and **39**).[58] Again, a concomitant reduction in oral bioavailability was observed between the two compounds, which emphasizes a possible limitation in this approach.

A communication related to a series of human β-tryptase inhibitors again shows how formation of a zwitterion can be used to attenuate hERG activity.[59] Introduction of a carboxylic acid moiety into **40** leading to compound **41** is successful as a means of minimizing hERG activity; however, this is accompanied by a reduction in activity at the primary target. No comment is made on any effect on absorption associated with this modification. The authors go on to demonstrate how hERG activity can be reduced while retaining potency at β-tryptase through control of log P.

2.3. Control of log *P*. Vaz and co-workers[59] have utilized a QSAR CoMSiA model of hERG activity in order to improve their selectivity profile in a series of inhibitors of human β-tryptase. From consideration of the model, incorporation of a carboxamide substituent was pursued as an approach to minimizing hERG activity. A comparison of **40** and **42**(Table 4) shows a significant reduction in clogP (**40**, clogP = 3.82; **42**, clogP = 2.73), with commensurate reduction in hERG activity. The Aventis group have also utilized the zwitterion approach as a means of enhancing selectivity over hERG, replacing the carboxamide moiety with a carboxylic acid (discussed above).

Fraley et al.[60] had identified hERG activity coupled with significant QT prolongation in dogs as an issue with their series of indolylquinolinone VEGFR-2 kinase inhibitors. Application of the zwitterion approach in modifying **43** to the isonipecotic acid derivative **61** (Figure 2) had only a limited impact on hERG activity. Introduction of groups capable of hydrogen bonding in the linker group connecting the basic amine with the indolyl core did offer enhanced selectivity over hERG (cf. compounds **43** and **44**). The log P was considered to be a controlling factor over potency at hERG because replacement of the amide with a sulfonamide moiety (compound **62**, Figure 2) resulted in greater activity at hERG. Inspection of the measured log P values (**44**, log P = 1.7; **62**, log P = 3.0) confirms that lipophilicity plays a role in this effect.

During optimization of a series of CCR5 antagonists, Shu and co-workers[48] adopted the approach of incorporating groups with hydrogen-bonding potential into their substrates as a means of controlling log P. Modifying the distal benzylpyrazole ring of compound **20** (clogP = 3.56) to include a sulfone derivative (compound **45**, clogP = 2.48) effectively ablated hERG activity. In this series, it is apparent that lipophilicity is a key determinant of hERG potency. Replacement of the methylsulfone of the benzylpyrazole **45** with the more lipophilic phenylsulfone (**63**, Figure 3, clogP = 4.41) maintained activity at CCR5 but resulted in an increase in hERG activity. It was found, however, that there was a disconnection between lipophilicity and acceptable DMPK properties. Although compounds such as **45** displayed suitable selectivity over hERG, this was generally accompanied by suboptimal DMPK parameters (e.g., compound **20** Clp (dog) = 22 mL min^{-1} kg^{-1}; compound **45** Clp = 108 mL min^{-1} kg^{-1}). Again, a compromise must be achieved between lipophilicity, selectivity, and DMPK parameters in order to identify a progressible compound.

As intimated in section 2.1, hERG activity has been an issue in Merck's series of 5-HT$_{2A}$ antagonists.[52,53] In this example, Fletcher and co-workers[61] have made significant efforts to minimize IKr activity in their series of compounds. A potent 5-HT$_{2A}$ hit **46** was obtained from an HTS campaign; however, the compound was found to be tainted with hERG activity. Control of clogP was exploited as an initial means of removing the undesired off-target activity. Replacement of the spirocyclic ether system of **46** (clogP = 4.93) with a sulfone moiety (**47**, clogP = 3.45) facilitated a greater than 25-fold reduction in hERG. From the same report, a related series of compounds was also optimized by controlling clogP. By use of compound **48** as a starting point, reduction in log P was facilitated through either modification of the two aryl rings to yield **49** or replacement with a primary carboxamide functional group to give **50**.

Workers at Bristol-Myers Squibb demonstrated previously how improved selectivity over IKr could be achieved through addition of a distal aryl ring system (see **22**−**23**, Table 2).[49] In the same report they also show how reduction of clogP through deletion of a phenyl ring system (**22**−**51**, Table 4) can be used to improve selectivity over IKr by a similar margin. Deletion of the aryl moiety not only has a beneficial effect on clogP but also removes a key pharmacophoric determinant of hERG activity.

In a 2002 report, Cooper et al.[62] made significant changes to the peripheral aryl rings in order to achieve separation between NK1 activity and IKr. Replacement of the terminal fluorophenyl ring of **52** with the spiroether system in **53** led to a reduction in IKr activity of around 50-fold, with conservation of activity at the primary target. From consideration of molecular overlays of **52** with dofetilide, it was postulated that changing the pK_a of the basic amine could also lower IKr activity. However, none of the modifications examined were as effective at reducing IKr activity compared to replacement of the distal aromatic system.

For the series of 5-HT$_{2A}$ antagonists reported by Rowley,[52] a general hERG pharmacophore model was considered as a means of improving selectivity. The authors hypothesized that the phenethyl group attached to the basic nitrogen of the lead compound **54** confers high activity at hERG (K_i = 0.08 μM). Indeed, deletion analogue **55** displayed significantly reduced hERG binding to K_i = 5.7 μM consistent with its lower clogP, thus providing an attractive point for further optimization of potency and bioavailability. The authors adopted a similar lipophilicity-lowering strategy by targeting other parts of the molecule, which also proved to be successful. For example, replacement of the naphthyl moiety in **28** with a pyridyl group led to analogue **56** (clogP = 2.84) with not only attenuated hERG (K_i = 2.5 μM) but also retained 5-HT$_{2A}$ affinity (0.35

Table 4. Control of log *P* To Effect Off-Target Activity at hERG

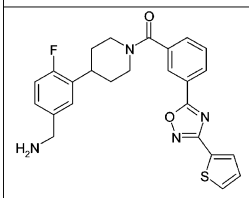
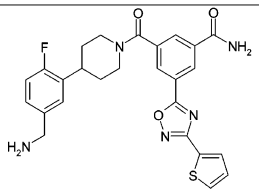
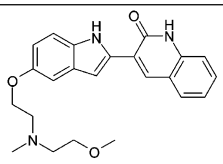
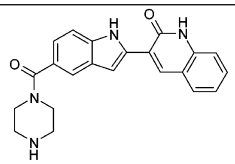
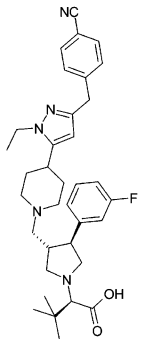
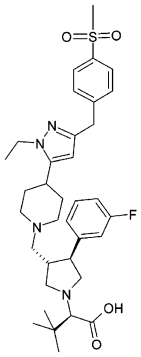
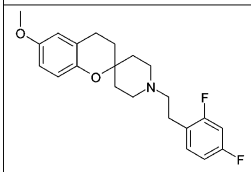
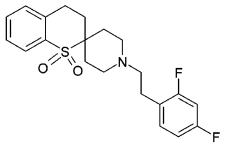
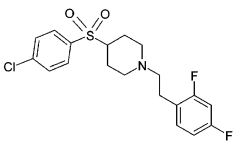
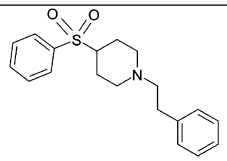
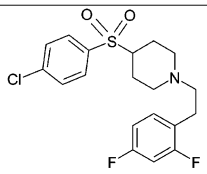
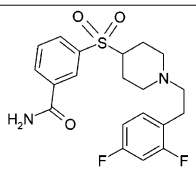
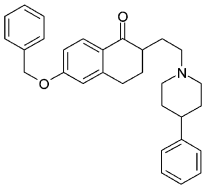
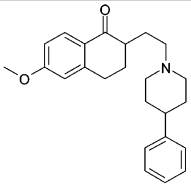
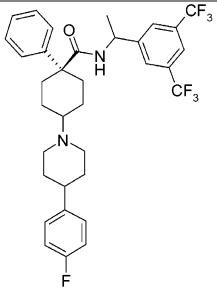
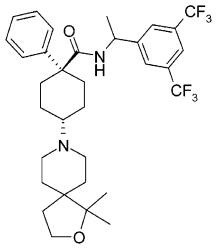
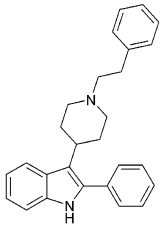
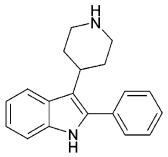
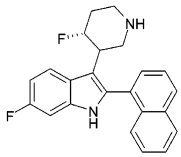
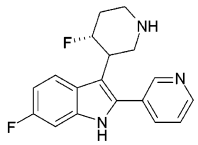
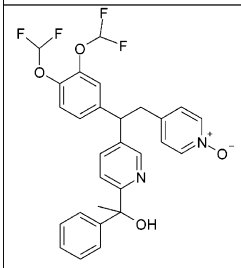
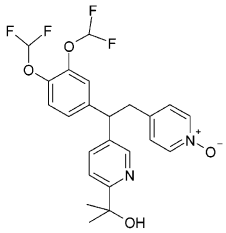
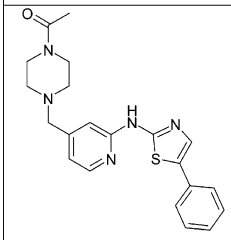
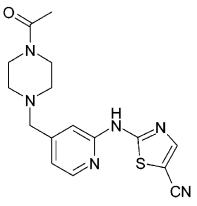
hERG active	hERG[a] (μM)	IC_{50}[a,b] (nM)	clogP[c]	pKa[c]	hERG optimised	hERG[a] (μM)	IC_{50}[a,b] (nM)	clogP[c]	pKa[c]	Ref
 **40**	**0.8**	**4.3**	3.82		 **42**	**17.1**	**1.3**	2.73		59
 **43**	**1.9**	**7**	3.47	7.86	 **44**	**>10**	**5**	2.06	7.71	60
 **20**	**1**	**1**	3.56		 **45**	**inactive**	**1.6**	2.48		48
 **46**	**0.02**	**1.8**	4.93	8.88	 **47**	**0.57**	**0.41**	3.45	8.57	61
 **48**	**0.15**	**1.4**	3.78	7.38	 **49**	**1.8**	**1.2**	2.49	8.13	61
 **48**	**0.15**	**1.4**	3.78	7.38	 **50**	**7.1**	**0.52**	1.85	7.33	61
 **22**	**0.16**	**69**	7.06	9.14	 **51**	**1.7**	**400**	5.29	9.15	49

Table 4 (Continued)

hERG active	hERG[a] (μM)	IC_{50}[a,b] (nM)	clogP[c]	pKa[c]	hERG optimised	hERG[a] (μM)	IC_{50}[a,b] (nM)	clogP[c]	pKa[c]	Ref
 **52**	**0.043**	**0.63**	8.33	9.19	 **53**	**2.2**	**0.26**	6.98	9.78	62
 **54**	**0.08**	**0.48**	6.50	9.60	 **55**	**5.7**	**12**	4.13	10.93	52
 **28**	**0.11**	**0.34**	5.46	9.00	 **56**	**2.5**	**0.35**	2.84	8.91	52
 **57**	**1.18**	**2.1**	3.69	4.63	 **58**	**22.7**	**35**	2.65	4.74	63
 **59**	**0.24**	**8**	3.23	4.80	 **60**	**10.6**	**13**	0.66	4.40	64

[a] Both hERG and primary target activity refer to K_i, IC_{50}, or IP unless otherwise stated. [b] IC_{50} refers to activity at the primary target. [c] clogP and pK_a are calculated according to ref 42. pK_a is reported as the highest basic pK_a. Where no value of pK_a is reported, calculation of this parameter was not possible.

nM). As described previously, modification of the naphthyl system to the cyclohexyl was also effective at attenuating hERG activity.

Compound **57**, a PDE4 inhibitor, was identified as a potential development candidate. However, cardiovascular safety studies in anesthetized dogs at a dose of 3 mg/kg showed a +8.9% prolongation of the QTc interval.[63] By use of an MK-499 binding assay to assess hERG affinity, **57** was optimized by modifying four key areas: tertiary alcohol substituents and 3-alkoxy substituents of the catechol, the 2,5-disubstituted pyridine, and the pyridine *N*-oxide. Initial SAR indicated that PDE4A inhibition but not hERG affinity was affected by stereochemistry. Replacement of the phenyl substituent of the tertiary alcohol by methyl resulting in the dimethyl alcohol **58** reduced hERG affinity by 19-fold (hERG K_i = 22.7 μM), but PDE4A inhibition decreased by 17-fold (PDE4A IC_{50} = 35 nM).

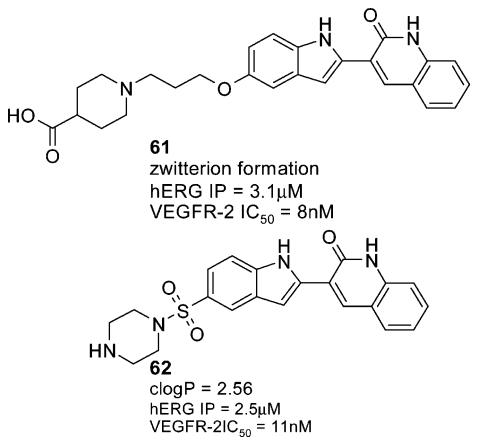


Figure 2. Attempted hERG optimizations on a series of VEGFR-2 inhibitors.

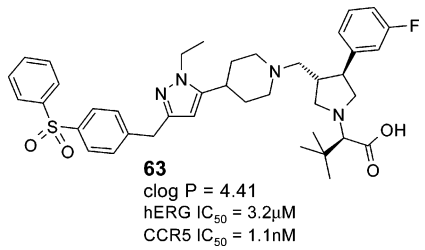


Figure 3. Effect of lipophilicity on hERG activity on a CCR5 antagonist.

The reduction of hERG affinity by replacement of the phenyl substituent could be attributed to the loss of a key aromatic pharmacophoric interaction with the hERG channel.

Another example from the kinase target class shows how hERG blockade and QT prolongation can be overcome through deletion of a phenyl group, thus removing an important pharmacophoric element at hERG.[64] Replacement of the phenyl of **59** with the nitrile in **60** resulted in a 44-fold reduction in activity at hERG. This modification was made in order to reduce the lipophilicity within the series, with the aim of improving the pharmacokinetics and selectivity over hERG. In this example a reduction in clogP from 3.23 in **59** to 0.66 in **60** was observed, although hERG SAR within the series indicated that piperazine with a polar N-substituent was also important in maintaining an optimal profile. Indeed, the existence of other examples with clogP similar to that of **59** but with lower hERG activity indicates that the structural change afforded by deletion of the phenyl ring was potentially more relevant to the hERG activity than the global lipophilicity of the compound.

2.4. Attenuation of pK_a. The group of Matasi et al.[65] identified a liability with hERG during optimization of their adenosine A_{2A} antagonists series. In this case, pK_a was considered to be of importance in governing hERG activity because compounds lacking a basic nitrogen were devoid of this unwanted side activity. This notion was strengthened further by the available homology model[15] suggesting a role for protonated amines interacting with aromatic residues lining the pocket of the channel. Efforts subsequently focused on modification of the benzylpiperazine linker in compound **64** to the phenylpiperazine moiety of compound **65** in order to reduce the pK_a of the side chain (Table 5). Although the reduction in hERG activity could be ascribed to control of pK_a, the authors could not discount the effect that structural modification of the linker could impart.

Elements of the multistep 5-HT_{2A}/hERG optimization from Fletcher et al.[61] have been discussed previously (vide supra). In a final example from this report, the authors demonstrate how control of pK_a can be used to gain additional selectivity over hERG. The initial stages of optimization relied on control of log *P* to furnish a greater than 25-fold window over hERG (see **46** to **47**, Table 4). Reducing the strain of the spirocyclic ring system (cf. **47** and **66**) furnished a further modest improvement in hERG activity. However, compound **66** was found to prolong the QT interval in the anesthetised ferret by more than 10% at doses of 3 and 10 mg kg^{-1} h^{-1}. A number of modifications were made to **66** in order to achieve selectivity over IKr. Notable success was achieved through introduction of a ketone at the position β to the amine (compound **67**). Such attenuation of the pK_a of the piperidine system yields a commensurate reduction in activity at IKr. The optimized compound did not exhibit QT prolongation in the anesthetized ferret model at doses up to 10 mg kg^{-1} h^{-1} and displayed acceptable pharmacokinetic properties. However, despite these improvements, compound **67** was found to degrade in polar solvents such as methanol because of the presence of the ketone moiety and therefore could not be progressed further.[53] Given that control of pK_a was found to be of utility in governing activity at hERG, the group sought alternative means of tuning pK_a other than using a ketone. Introduction of a 4-fluoro substituent in a piperidine ring system is known to reduce the pK_a; therefore, a series of 4-fluorosulfones were targeted. These were found to offer good levels of selectivity over hERG, with compound **74** (measured pK_a = 6.3, Figure 4) showing no increase in QT prolongation in anesthetized dogs at plasma concentrations up to 148 μM.

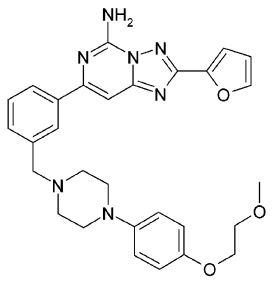
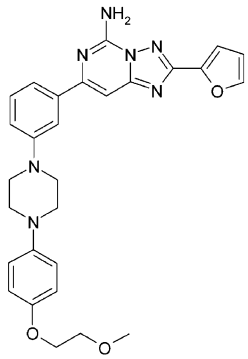
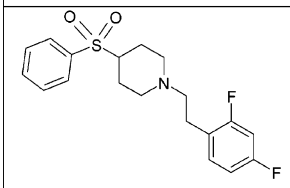
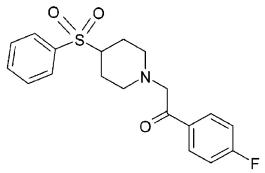
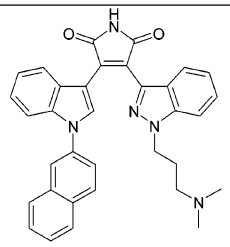
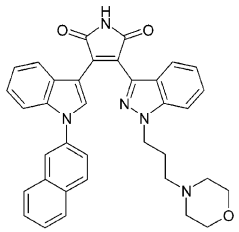
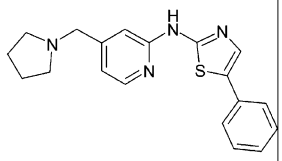
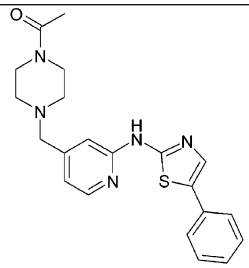
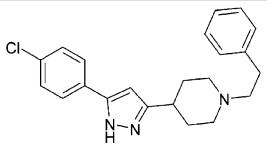
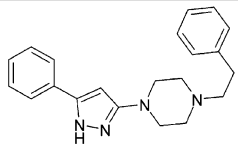
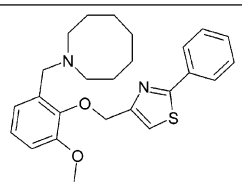
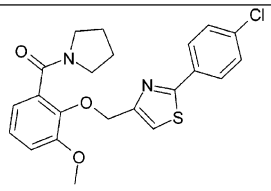
All compounds within an indolylindazoylmaleimide series of highly potent and selective protein kinase C-β (PKC-β) inhibitors recently reported by Johnson & Johnson were shown to have hERG activity.[66] Compound **68**, the most potent inhibitor in the series (IC_{50} = 5 nM) was found to have high activity at hERG (K_i = 25 nM). Intravenous infusion of this compound into anesthetized pigs caused a dose-dependent increase in the QTc interval by 7%, 11%, and 15% at 1, 3, and 10 mg/kg, respectively. According to the authors, the SAR in this series (not all reported) suggests that the basic amino group not only is critical for high PKC-β potency but also is important for hERG affinity, which raised a concern about tractability of this class of PKC inhibitors. Interestingly, the less basic morpholine analogue **69** showed significantly attenuated hERG activity (K_i = 850 nM) compared to the parent dimethylamine **68** with essentially conservation of activity at PKC.

For the series of VEGFR-2 kinase inhibitors discussed previously,[64] control of pK_a was of some use in achieving selectivity over hERG. Altering the pK_a of the pendent amine in compound **70**, by substituting an *N*-acetylpiperazine **59** for the pyrrolidine, resulted in a 10-fold decrease in hERG activity. However, a more substantial decrease in hERG was observed when deletion of a phenyl ring to control clogP was employed (vide supra).

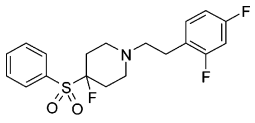
A further example on control of pK_a comes from a series of dopamine D4 antagonists discussed above.[67] The piperazine **26** (measured pK_a = 7.2), which is less basic than the piperidine **71** (pK_a > 7.5, exact measurement not possible because of low solubility), had improved selectivity over the hERG channel. In this case, the effect of clogP cannot be discounted because incorporation of the piperazine results in lowering of the lipophilicity of the compound. As detailed previously, further reductions in hERG activity within this series were realized by control of local conformation around the heteroaromatic ring.

Liang et al.[68] report another interesting example of how selectivity against hERG over other voltage gated ion channels

Table 5. Reduction in pK_a To Facilitate Selectivity over hERG

hERG active	hERG[a] (μM)	IC_{50}[a,b] (nM)	clogP[c]	pKa[c]	hERG optimised	hERG[a] (μM)	IC_{50}[a,b] (nM)	clogP[c]	pKa[c]	Ref
 **64**	**81% @ 5ug/mL**	**2.8**	4.34		 **65**	**(-4)% @ 5ug/mL**	**1**	4.78		65
 **66**	**1**	**0.33**	2.94	7.52	 **67**	**6.8**	**2.4**	2.19	5.25	61
 **68**	**0.025**	**5**	6.61	9.41	 **69**	**0.85**	**18**	6.54	7.45	66
 **70**	**0.022**	**3**	4.37	8.87	 **59**	**0.24**	**8**	3.23	4.80	64
 **71**	**EC25 0.4**	**2.3**	5.44	9.10	 **26**	**EC25 5.8**	**23**	4.17	7.58	67
 **72**	**0.18**	**4000**	6.16		 **73**	**7.7**	**1300**	4.07		68

[a] Both hERG and primary target activity refer to K_i, IC_{50}, or IP unless otherwise stated. [b] IC_{50} refers to activity at the primary target. [c] clogP and pK_a are calculated according to ref 42. pK_a is reported as the highest basic pK_a. Where no value of pK_a is reported, calculation of this parameter was not possible.



74
pKa = 6.3
hERG IP = 2.5μM
5-HT$_{2A}$ IC$_{50}$ = 0.68nM

Figure 4. hERG optimization through attenuation of pK_a.

can be achieved. An issue with hERG blockade at the outset of lead optimization on a series of Na_V 1.7 blockers was detected, with compound **72** having approximately 20-fold higher tonic potency at hERG than the ion channel of interest. The authors reasoned that the high degree of hERG activity could be ascribed to the presence of the basic amine in the progenitor compound. Preparation of a series of amide analogues (e.g., **73**) furnished compounds that reversed the selectivity profile with respect to hERG. It should be noted, however, that despite removal of the basic nitrogen, some members of the series did exhibit up to 90% inhibition of the hERG channel at 10 μM, suggesting that the basic amine is not the only structural determinant of hERG blockade in this series.

3. Summary and Analysis

3.1. Summary of Literature Optimizations. Our survey indicates that the most frequently encountered method of achieving selectivity over hERG is to make discrete structural modifications to the drug molecule. The effect of this change could result in disruption of any putative π-stacking interactions with the channel. This can be achieved either by subtle changes in the molecular architecture (e.g., incorporation of an electron-withdrawing moiety, removal of an electron-donating group to diminish π-stacking ability), which has minimal overall impact on molecular properties, or by making more significant alterations to the template (e.g., incorporation of a heterocyclic species, deletion of distal aromatic group).

Control of log *P* and pK_a do show some considerable promise as a means of controlling hERG activity and should form part of the first line of attack in seeking to optimize out this unwanted side effect. Indeed, these two approaches would appear to be intimately interlinked because reduction in pK_a is very often accompanied by a reduction of log *P*. Reduction in log *P* is undoubtedly a sensible strategy to adopt because it often confers beneficial effects on, for example, microsomal clearance and other crucial DMPK parameters. Of the four categories presented above, we suggest that the zwitterion approach is of lower utility than the other three owing to the attendant issues with lower permeability and hence reduced oral bioavailability that are often encountered.

3.2. SAR Analysis. Analysis of all hERG data did not identify consistent trends across publications with descriptors intuitive to the medicinal chemist. Only lipophilicity descriptors exemplified by clogP showed some consistency in their relationship with hERG activity.

For the presented optimizations, examination of the change in hERG activity per optimization category clearly indicates that the most frequent optimization types (DSM and log *P*) show similar properties (Figure 5). Only the ZI optimization category appears to be visually different from the other box plots. Pairwise comparisons of the mean hERG activity decrease using the *t*′ test indicate that at the 95% level no category is significantly more successful than the others. However, it should be noted that these observations are based on a small data set.

The relationship between starting point for optimization and optimization category can give guidance on the best strategy to

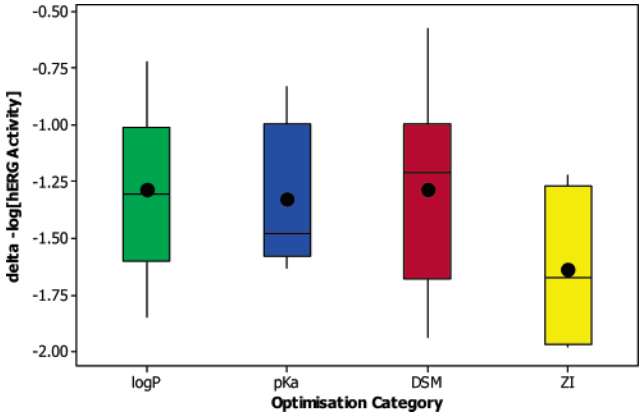


Figure 5. Box plot of Δ −log[hERG activity] versus optimization category. The mean Δ −log[hERG activity] for each category is denoted by closed circles.

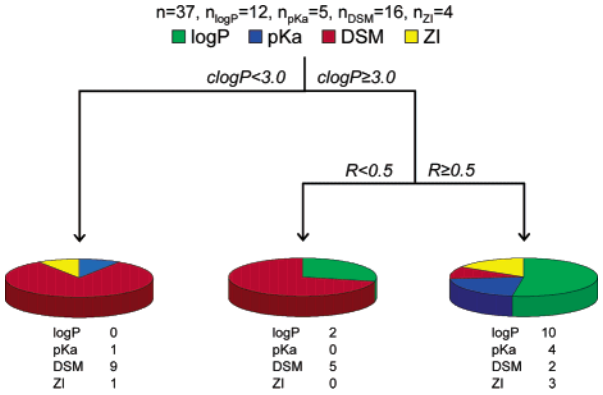


Figure 6. Optimization categories classified by clogP of the compound to be optimized and the correlation coefficient *R* between clogP and −log[hERG activity] for the series.

adopt. A decision tree based on the optimization data can be derived (Figure 6) as a mnemonic for selecting the most appropriate method to use. When clogP < 3.0 for the molecule to be optimized, the DSM category is most frequent. For molecules with clogP ≥ 3.0 then it should be established if a correlation between hERG activity and clogP exists within the series. For each of the optimizations belonging to a chemical series containing a minimum of four hERG data points, the correlation coefficient *R* was calculated between clogP and hERG activity for the series. In the case where $R \geq 0.5$, it is not surprising that the clogP category of optimization is most frequent, and for $R < 0.5$, the DSM category dominates. It is difficult to draw any conclusions related to ZI and pK_a optimizations because of the smaller number of examples, although pK_a optimizations tend to cluster with the log *P* optimizations. No relationship could be established between the calculated highest basic pK_a for the molecule to be optimized and the optimization category.

If it is assumed that a higher proportion of a particular optimization category within a defined starting point zone represents a greater likelihood of success of the optimization category, then some guidelines can be proposed for the optimization of unwanted hERG activity.

(i) For clogP < 3.0, make discrete changes to the structure (DSM).

(ii) For clogP ≥ 3.0, attempt to reduce log *P* and establish correlation *R* between log *P* and hERG activity within the series.

(ii.a) For clogP ≥ 3.0 and $R \geq 0.5$, pursue reduction of log *P* (lipophilicity). For log *P* optimizations the mean decrease in

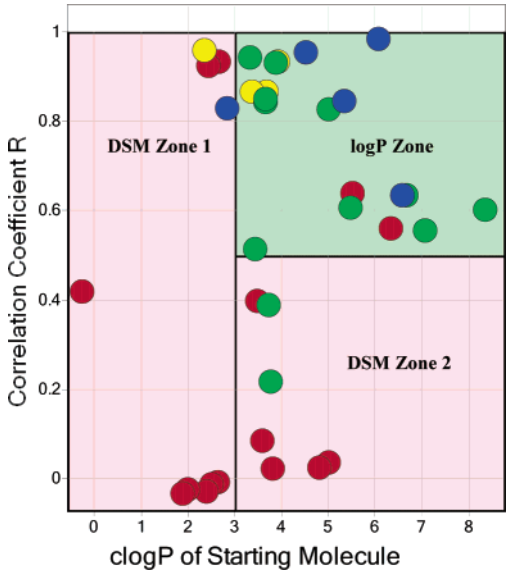


Figure 7. Optimization starting points on a plot of the correlation coefficient *R* between clogP and −log[hERG activity] versus clogP of starting molecule: (green) log *P*; (blue) pK_a; (red) DSM; (yellow) ZI. Optimization zones have been defined according to analysis of the literature data.

hERG activity per clogP unit is 0.8 log units. This should be used as a rough guide to assess success.

(ii.b) For clogP ≥ 3.0 and $R < 0.5$, pursue discrete changes to the structure (DSM).

These simple heuristics are highlighted graphically in Figure 7 and appear to be sensible from a medicinal chemistry perspective. If hERG activity is high and lipophilicity is low, then it is unlikely that further significant reductions in lipophilicity would be desirable because this may impair absorption and other important DMPK parameters.

3.3. Summary and Recommendations. From consideration of the collected literature in optimizing hERG, we can table the following recommendations.

(i) Four strategies for the removal of hERG have been identified: discrete structural modifications, control of log *P*, formation of zwitterions, and control of pK_a.

(ii) All four strategies were found to be equally effective at reducing hERG activity. Literature evidence suggests the zwitterions approach to be of lower utility owing to attendant issues with membrane permeability and oral bioavailability.

(iii) To identify the most appropriate approach with respect to starting compound, first consider clogP.

(iii.a) If clogP ≥ 3.0, seek to reduce this by incorporation of heteroatoms or polar groups or removal of lipophilic functions. *On average 1 log unit reduction in clogP leads to 0.8 log unit reduction in hERG activity*. However, if there is no correlation between hERG activity and log *P*, then pursue DSM strategy.

(iii.b) If clogP < 3.0, discrete structural modifications offer the highest probability of success.

(iv) A corollary to the above is to delete peripheral aryl moieties or include electron-withdrawing groups on aryl rings where possible. This may have the effect of reducing clogP and potentially disrupting π-stacking with the channel.

(v) Reducing pK_a of a basic nitrogen is often a successful strategy in attenuating hERG. This is often accompanied by a reduction in log *P*, which may be the more relevant parameter.

(vi) Some anecdotal evidence exists that constraint or generation of steric bulk or shielding around a basic nitrogen may benefit hERG selectivity.

(vii) Given that most hERG blockers contain a basic nitrogen, consider alternative solubilizing strategies.

(viii) Be aware of compounds with low aqueous solubility (<5 mg/L); the hERG binding and electrophysiology results for such compounds could be significantly underestimated.

(ix) Only test optically pure material in the hERG assay because the effects due to stereochemistry can be dramatic.

(x) Test a significant number of analogues within each chemical series against hERG to establish reliable SAR and to ensure that effects of discrete structural changes are picked up because the return can be significant.

(xi) When sufficient data are available, consider development of a local (series specific) in silico model to guide medicinal chemistry programs away from hERG activity. Application of global in silico models is best reserved for prioritization of compounds for synthesis/acquisition from larger arrays.

(xii) To ascertain effects of non-hERG-mediated QT prolongation, test key compounds in relevant ex vivo (e.g., Langendorff, dog Purkinje fibers) or in vivo models as early as possible.

3.4. Future Directions. Clearly there now exists a significant body of information on the tactics for optimizing against the hERG channel. Increasing amounts of data on the strategies for removal of this unwanted activity are being reported, and this could be used to further refine the classification scheme reported here. In particular, new SAR information could be used to create subzones within, for example, the DSM class of optimizations and will be followed up in due course. It is anticipated that the ever-burgeoning number of ion channel crystal structures, including mammalian channels,[69] will have considerable impact on our understanding of drug−hERG interactions. Other potential challenges in the area are likely to include the identification of additional ion channels implicated in drug-induced cardiac toxicity and subsequent optimization against these targets. Already there is evidence to suggest that drugs such as sevoflurane can exert cardiac toxicity not through hERG but via reduction in KvLQT1/minK potassium channel currents.[70] Similarly, increasing attention is being paid to other mechanisms that result in cardiotoxic end points such as inhibition of hERG channel trafficking, with compounds such as pentamidine producing QT prolongation via this mechanism rather than through inhibition of hERG.[71]

Biographies

Craig Jamieson completed his B.Sc. in chemistry at the University of Glasgow (1996) followed by doctoral studies at the University of Edinburgh (1999). After postdoctoral work at the University of Cambridge, he joined GlaxoSmithKline as a principal scientist before moving to Organon Laboratories in 2004, where he is currently a group leader in the medicinal chemistry department where he has continued his interest in the chemical biology of ion channels.

Elizabeth M. Moir studied chemistry at the University of Edinburgh where she completed both her B.Sc. (1998) and Ph.D. studies. She joined Organon Laboratories in 2001 and is currently a senior scientist in the medicinal chemistry group working on a variety of lead optimization and lead discovery projects in the CNS area.

Zoran Rankovic received his Ph.D. degree in organic chemistry from the University of Leeds (U.K.). In 1995 he joined Organon Laboratories in Scotland were he is currently a medicinal chemistry section head. His work on a number of CV and CNS-related projects has led to a keen interest in medicinal chemistry strategies and tactics for overcoming hERG inhibition.

Grant Wishart completed his B.Sc. (1993) and Ph.D. (1997) at the University of Abertay, Dundee, before joining the Wolfson Institute for Biomedical Research at University College London

as a medicinal/computational chemist. He joined Organon Laboratories in 2001 and is currently a senior research scientist within the computational medicinal chemistry section.

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