DRUG DISCOVERY TODAY BIOSILICO

Predictive *in silico* modeling for hERG channel blockers

Alex M. Aronov

hERG-mediated sudden death as a side effect of non-antiarrhythmic drugs has been receiving increased regulatory attention. Perhaps owing to the unique shape of the ligand-binding site and its hydrophobic character, the hERG channel has been shown to interact with pharmaceuticals of widely varying structure. Several *in silico* approaches have attempted to predict hERG channel blockade. Some of these approaches are aimed primarily at filtering out potential hERG blockers in the context of virtual libraries, others involve understanding structure–activity relationships governing hERG-drug interactions. This review summarizes the most recent efforts in this emerging field.

In recent years, there has been a revolution in philosophical approaches to drug discovery. Spiraling R&D costs along with higher failure rates of clinical candidates have contributed to the growing recognition by the drug discovery community that undesirable drug properties and toxicity are two of the major contributors to pipeline attrition. Many organizations have therefore moved to incorporate predictive absorption, distribution, metabolism, excretion, and toxicology (ADMET) into early discovery programs under the slogan of 'fail fast, fail cheap'. By identifying and removing undesirable leads early, more costly failures should be prevented downstream. Wider availability of commercial libraries, internal combinatorial chemistry capabilities, and improvements in high-throughput screening (HTS) campaigns have been providing medicinal chemists with multiple potential starting points, and application of predictive ADMET is seen as a way to narrow the scope of lead exploration.

Originally the domain of experimentalists, ADMET began to change in late 1990s, embracing the use of computational tools for predicting ADMET properties of compounds. As broader and higher quality proprietary datasets became available, computational approaches have advanced from 'first-generation' models, such as Lipinski's Rule of Five [1], and in a broader sense drug- [2,3] and lead-likeness [4,5], to 'second-generation' models that focus primarily on predicting pharmacokinetic parameters, such as solubility, intestinal permeability, oral bioavailability, and blood–brain barrier penetration [6,7]. The 'third-generation' models [6] have taken aim at major determinants of metabolic fate of xenobiotics (P450s) [8] and factors that induce them (pregnane X-receptor) [9], drug transport proteins affecting compound PK profile (P-glycoprotein) [10], and, more recently, ion channels implicated in QT interval prolongation (hERG) [11]. It is this predictive cardiotoxicity screening that is the focus of this review.

hERG-related cardiotoxicity

Sudden death as a side effect of the action of non-antiarrhythmic drugs is a major pharmacological safety concern facing the pharmaceutical industry and the health regulatory authorities [12]. In recent years, at least five blockbuster drugs (Figure 1) have been withdrawn from the market due to reports of sudden cardiac death, and several others were forced

Alex M. Aronov
Vertex Pharmaceuticals Inc.
130 Waverly St.,
Cambridge,
MA 02139-4242, USA
e-mail:
alex_aronov@vrtx.com

1359-6446/04/$ – see front matter PII: S1359-6446(04)03278-7

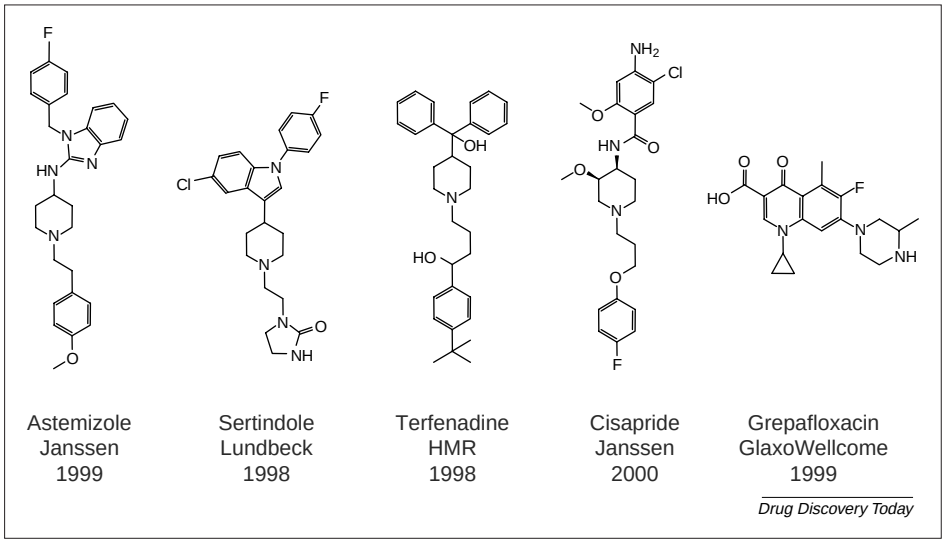

FIGURE 1

Drugs withdrawn due to QT prolongation concerns.

to carry strong 'black box' warning labels [11–13]. In all cases, long QT syndrome (LQTS), an abnormality of cardiac muscle repolarization that is characterized by the prolongation of the QT interval in the electrocardiogram, was implicated as a predisposing factor for torsades de pointes, a polymorphic ventricular tachycardia that can spontaneously degenerate to ventricular fibrillation and cause sudden death. Congenital LQTS can be traced back to several possible mutations resulting in defects in sodium channels, and two different potassium channels – the rapidly activating delayed rectifier I_{Kr} and the slowly activating delayed rectifier I_{Ks} [14,15].

Importantly, virtually every case of a prolonged duration of cardiac action potential related to drug exposure (acquired LQTS) can be traced to one specific mechanism – blockade of I_{Kr} current in the heart [13]. This current, a major contributor to phase 3 repolarization at the end of the QT interval, is conducted by tetrameric pores with the individual subunits encoded by Human ether-a-go-go related gene (hERG) [16]. With blockade of hERG K^+ channels widely regarded as the predominant cause of drug-induced QT prolongation, early detection of compounds with this undesirable side effect has become an important objective in the pharmaceutical industry.

Perhaps owing to the unique shape of the ligand-binding site and its hydrophobic character, the hERG channel has been shown to interact with pharmaceuticals of widely varying structure, often at concentrations similar to the levels of on-target activity of the respective compounds. While risk tolerance for QT prolongation may vary significantly depending on indication, development stage, etc., the documented hERG-blocking activity reduces the intrinsic value of the molecule, as it increases risk of clinical failure. Among the indications least tolerant of hERG blockade by the candidate compound are antivirals and antibacterials where high plasma concentrations of the drug are necessary to suppress resistance, and pain management drugs and antipsychotics where overdosing is likely. In addition, drug–drug interactions (e.g. via inhibition of P450 metabolism) may lead to unexpectedly high plasma levels of a drug, which are sufficient to prolong the QT interval. As a general rule, a safety window of greater than 30-fold has been recommended for the ratio of hERG IC_{50} to the expected compound C_{max} adjusted for its unbound fraction [17]. This ratio could be used as a 'go–no go' decision point in early discovery.

In recent years, several *in silico* approaches have attempted to mitigate the safety concern represented by hERG channel blockade. Some of these approaches have been aimed primarily at filtering out potential hERG blockers in the context of virtual libraries, others have involved understanding structure–activity relationships governing hERG–drug interactions. This review summarizes the most recent efforts in this emerging field.

Screening for hERG blockers

Any discussion of predictive modeling approaches would be incomplete without mention of the kind of data used to build predictive *in silico* models. Two excellent reviews in *Drug Discovery Today* have summarized the types of test systems and assays currently available for cardiotoxicity screening [18,19].

In vivo telemetry experiments in non-rodents (typically in conscious or anaesthetized dogs) are the ultimate preclinical test for cardiotoxicity, with data generated under physiological conditions and related to the pharmacokinetic profile of the drug. However, its high cost severely limits its use at the earlier discovery stage [18]. *In vitro* electrophysiology in primary cardiac tissue, such as Purkinje fibers, is sometimes used, but is relatively low throughput and variability precludes its widespread application.

Voltage clamp techniques represent the 'gold standard' in the field and provide real time mechanistic information on ion channels [19]. The experiments are performed in mammalian cells (e.g. chinese hamster ovary (CHO) cells or human HEK 293) transfected with the gene for hERG. The overwhelming majority of predictive hERG models have been built using mammalian patch clamp data. Electrophysiology data from experiments performed in *Xenopus* oocytes can be found in the literature. Unfortunately, the highly lipophilic environment in the oocytes appears to limit access of the drug to its site of action, leading to IC_{50} values being increased by as much as 100-fold [19,20]. While this could be tolerated in a classification model, QSAR approaches have tended to

avoid using data collected in the oocytes. Because of the moderate throughput of patch clamp experiments, many companies have been willing to compromise on data quality to increase assay throughput. Techniques such as fluorescence-based assays with cells stably transfected with hERG and radioligand (typically dofetilide or MK-499) displacement assays [18] have been successfully used to tune out hERG channel activity in lead series [21,22]. However, the variation in potency obtained in these experiments from patch clamp data and the frequent occurrence of false negatives makes these datasets less amenable to consistent model building.

A new and exciting development in ion channel screening has been the recent introduction of automated high-throughput patch clamp machines (e.g. planar patch technology) [19]. Several correlations to manual patch clamp data have been reported, but further validation is necessary. If successful, this approach could revolutionalize cardiac safety testing by increasing the rate of data acquisition by ~100-fold, ultimately providing larger and more diverse datasets for *in silico* modeling.

Predictive modeling of hERG blockers

Of the variety of hERG modeling approaches that have appeared in the literature, most can be broadly divided into three categories: homology modeling, QSAR models, and classification methods (Box 1).

Homology modeling of hERG

At least two groups [23,24] have reported hERG homology models using available atomic resolution structures of bacterial K^+ channels KcsA [25] (closed) and MthK [26] (open). These channels contain only two transmembrane domains (equivalent to helices S5/S6 in Figure 2a), and the models therefore only cover the predicted structure of the hERG pore. The basic architecture of hERG channel is expected to be similar to that of other voltage-gated K^+ channels, such as KvAP (Figure 2a) [27]. The channel pore domain is formed by tetramerization from helices S5 and S6, as well as the pore helix P and the selectivity filter loop. The selectivity filter lies on the extracellular side of the membrane. The movement of S6 helices with respect to each other in a crossover fashion renders the channel closed, with the water-filled cavity isolated from cytosol. The voltage-sensing paddles formed by helices S3b and S4 are responsible for the voltage dependence exhibited by KvAP. The debate about the exact position of the S4 segment and the details of voltage sensing at the molecular level is ongoing [28,29]. Although the structure and location of the paddles with respect to the membrane may be different in hERG, the basic structure of the pore is likely to be reasonably conserved.

Two bands of aromatic residues are predicted to line the cavity, with each monomer contributing Phe656 and Tyr652 (Figure 2b) [16,23,24]. These residues are both located on the S6 helix, with the tetrad of Phe656 situated closer to the mouth of the channel, and the four Tyr652 residues further toward the pore helix. The homology model is corroborated by earlier mutagenesis data. Sanguinetti and co-workers used alanine scanning to identify key residues responsible for hERG blockade by potent inhibitors terfenadine, cisapride and MK-499 [23]. Phe656 and Tyr652 appeared to be the primary interaction points. The current consensus implicates Phe656 in π-stacking interactions with the ligands, whereas Tyr652 is thought to participate in a cation–π interaction with the protonated basic nitrogen present in most of the reported hERG blockers. Recently, the potency for hERG blockade by these three drugs was shown by systematic mutagenesis to correlate well with measures of hydrophobicity of residue 656, such as its side-chain van der Waals hydrophobic surface area [30]. In the case of residue 652, the presence of an aromatic residue in this position is required for high affinity hERG blockade, consistent with the importance of the cation–π interaction predicted by ligand-based models.

Additionally, residues Thr623 and Val625, located near the pore helix, were implicated in hERG binding to MK-499, but the effect was moderate in the cases of terfenadine and cisapride [16]. Both Thr623 and neighboring Ser624 (Figure 2b) have been shown to have pronounced effects on hERG block by vesnarinone, clofilium and ibutilide. These polar residues may be able to interact with the polar tails present in many of the potent hERG blockers [13,16,24].

A combination of structural features is thought to be responsible for the 'binding promiscuity' of hERG relative to other K^+ channels. The aromatic residues critical for binding to structurally diverse drugs are missing in channels of the Kv1–4 families, replaced by Ile or Val in positions equivalent to Tyr652 and Phe656 [13,16,30]. The exact positioning of the aromatic residues is also of critical

BOX 1

In silico modeling methods

Homology modeling
a comparative modeling procedure whereby a three-dimensional model for a protein sequence is created based on the structures of homologous proteins.

QSAR (quantitative structure–activity relationship)
a computational approach that attempts to relate activity data for a set of molecules to a set of molecular descriptors. These descriptors are values associated with a two- or three-dimensional molecular structure and created through application of well-defined algorithms. Common types of descriptors include scalar values, structural fragment keys, connectivity and pharmacophore fingerprints.

Classification methods
approaches to distributing molecules into classes or categories of the same type on the basis of a functional observation. Typically, activity thresholds are employed in the classification.

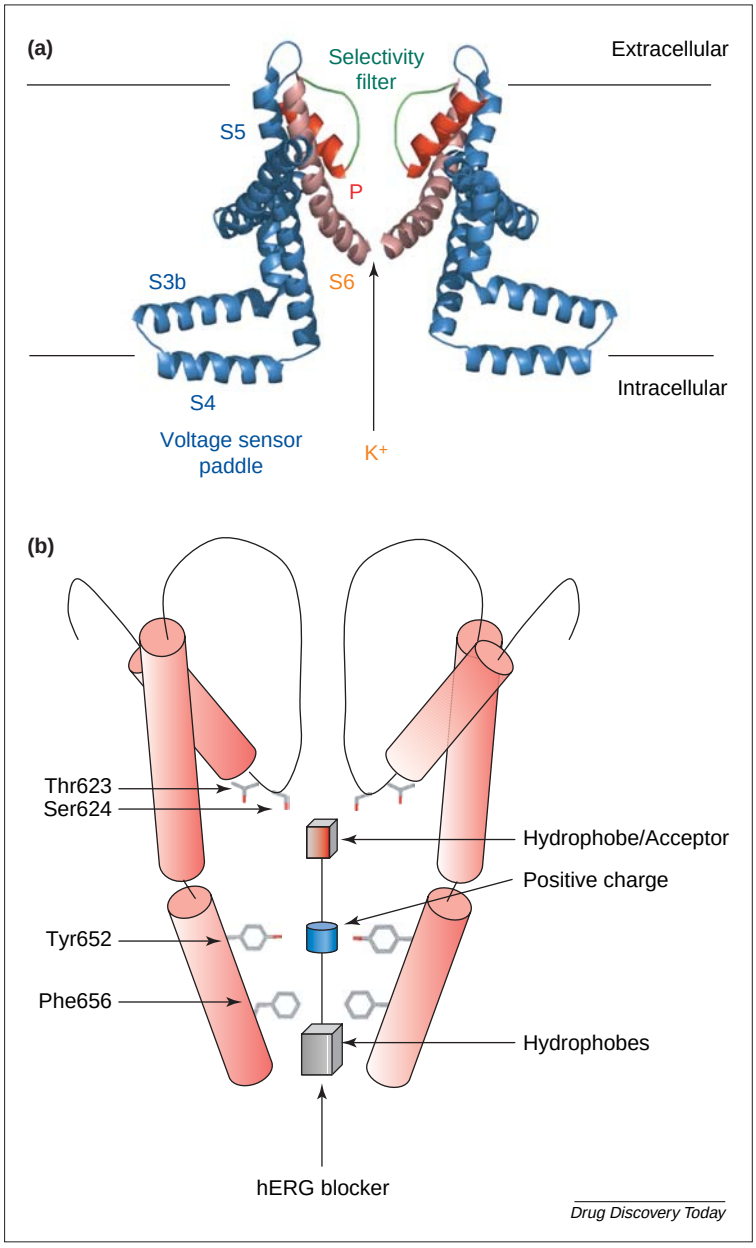

FIGURE 2

Structural model of hERG channels. (a) Key elements of hERG channel topology illustrated using the X-ray structure of KvAP [27]. Two of the four subunits comprising the tetrameric channel are shown. **(b)** Model of the pore portion of hERG channel. The P-S6 fragment is shown for a dimer. Aromatic residues Phe656 and Tyr652 are critical for hERG block by most known small molecule ligands. Polar residues Thr623 and Ser624 modulate the binding potency for a number of reported hERG blockers. A hERG blocker is represented schematically based on published evidence. One or two hydrophobes interact with Phe656, a positive charge is stabilized by cation–π interaction with Tyr652, and the generally hydrophobic tail contains an acceptor able to interact with the polar residues on the loop that connects the pore helix to the selectivity filter.

importance for binding. Structurally related EAG channels were made sensitive to the hERG blocker cisapride by moving the Tyr residue by one position along the S6 helix [31]. Finally, the hERG channel lacks the Pro–Val(Ile)–Pro motif on the S6 helix, which is present in Kv1–4 channels and is thought to decrease the size of the inner cavity by inserting a kink in the inner helices [16,30].

QSAR

Ligand-based approaches have been extensively applied to understanding SAR of hERG channel blockers. The first hERG pharmacophore was described by Ekins *et al.* using 15 molecules from the literature [32]. It contains four hydrophobes and one positive ionizable feature and produced an r^2 value of 0.90. Proposed distances between the positive center and the hydrophobes are 5.2, 6.2, 6.8 and 7.5 Å. The model was further applied to predict IC_{50} values for a test set of 22 mostly antipsychotic compounds known to inhibit hERG ($r^2 = 0.83$). More recently, the initial training set was expanded to include 66 molecules, resulting in an observed-versus-predicted correlation of $r^2 = 0.86$ [33]. This model produced a correlation of $r^2 = 0.67$ on addition of 25 molecules from the literature. Cavalli *et al.* [34] constructed a hERG pharmacophore based on a training set of 31 carefully selected QT-prolonging drugs from the literature. The proposed pharmacophore contains three aromatic moieties connected through a nitrogen function that is a tertiary amine throughout the whole set of molecules. The nitrogen and the aromatic moieties are separated by distances of 5.2–9.1, 5.7–7.3 and 4.6–7.6 Å. CoMFA analysis performed on the training set produced a correlation with $r^2 = 0.952$ ($q^2 = 0.767$), and its predictive ability was tested on a set of six additional compounds ($r^2_{pred} = 0.744$). Pearstein *et al.* [24] reported a CoMSiA model built using in-house patch clamp data for 28 compounds, 18 of them sertindole analogs ($q^2 = 0.571$). According to the model, decreasing the positive charge on the central nitrogen and increasing the steric bulk on the hydrophobic end of the molecule are two potential ways to reduce hERG blocking activity. The model was tested on four sertindole analogs with widely varying potency for hERG. Keseru [35] used literature data on 55 compounds to train a QSAR model based on calculated descriptors. Five descriptors were used: Clog*P*, calculated molar refractivity (CMR), partial negative surface area, and the Volsurf W2 (polarizability) and D3 (hydrophobicity) descriptors. A model of acceptable quality was obtained ($r^2 = 0.94$, SSE = 0.82) and tested on a 13 compound holdout set ($r^2 = 0.56$, SSE = 0.98). A hologram QSAR model was then created that made use of 2D fragment fingerprints ($r^2 = 0.81$, SSE = 0.67).

One of the larger hurdles for building QSAR models using literature data has been the large discrepancy observed for hERG IC_{50} values determined in different laboratories. Interlaboratory variability of greater than 10-fold is not uncommon, even in cases when inhibition was measured using the same cell line. Additional efforts in generating internally consistent hERG datasets that could be made available to the broad scientific community are sorely needed to propel the field forward.

Classification methods

Whereas QSAR methods aim to predict absolute compound activity, classification methods attempt to bin compounds by their potential hERG inhibition. The earliest example

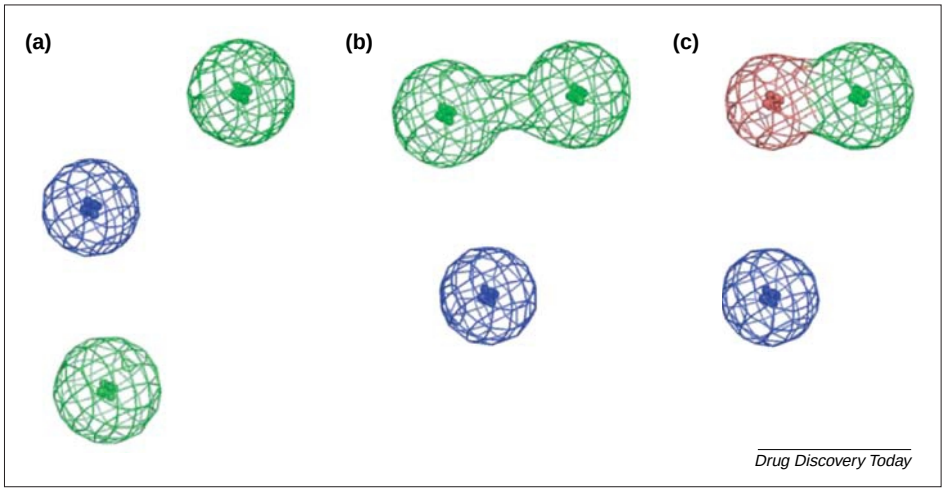

FIGURE 3

Three-point pharmacophores for *in silico* hERG block prediction (from [39]). Colored features correspond to positive charge (blue), hydrogen bond acceptor (red), and hydrophobic (green).

of a hERG-based classification was reported by Roche *et al.* [36]. A total of 244 compounds representing the extremes of the dataset (< 1 μM and > 10 μM for actives and inactives, respectively) were modeled with a variety of techniques such as substructure analysis, self-organizing maps, partial least squares, and supervised neural networks. The most accurate classification was based on an artificial neural network. In the validation set containing 95 compounds (57 in-house and 38 literature IC_{50} values), 93% of inactives and 71% of actives were predicted correctly. In a decision-tree-based approach to constructing a hERG model using calculated physicochemical descriptors, Buyck *et al.* [37] used three descriptors – Clog*P*, CMR and the pK_a of the most basic nitrogen – to identify hERG blockers within an in-house dataset. With IC_{50} = 130 nM as a cutoff, factors suggestive of hERG activity were determined to be Clog*P* ≥ 3.7, 110 ≤ CMR < 176, and pK_a max ≥ 7.3. A hologram QSAR [38] model utilizing 2D fingerprints was shown to be predictive for discriminating hERG actives from inactives (threshold hERG IC_{50} = 1μM) [35]. A combined 2D/3D procedure for identification of hERG blockers was proposed by Aronov and Goldman [39]. A 2D topological similarity screen utilizing atom pair [40] descriptors and an amalgamated similarity metric termed TOPO was combined with a 3D pharmacophore ensemble procedure in a 'veto' format to provide a single binary hERG classification model. A molecule flagged by either component of the method was considered a hERG active. In the course of 50-fold cross-validation of the model on a literature dataset containing 85 actives (threshold HERG IC_{50} = 40μM) and 329 inactives, 71% of hERG actives and 85% of hERG inactives were correctly identified. Additionally, five of eight (62.5%) hERG blockers were identified correctly in a 15 compound in-house validation set. Most of the statistically significant pharmacophores from the ensemble procedure were three-feature aromatic–positive charge–hydrophobe combinations (Figure 3a,b) similar to those reported by Cavalli *et al.* [34]; however, a novel three point pharmacophore containing a hydrogen bond acceptor was also proposed (Figure 3c).

Understanding SAR of hERG blockers

As various *in silico* methods are being brought to bear on the problem of hERG, some understanding of the structure–activity relationship relevant to hERG block is starting to emerge.

Ligand binding mode

The current consensus view of the proposed binding mode of hERG blockers within the channel pore is shown in Figure 2b. The inhibitor orients itself along the pore axis, with the lipophilic end facing the opening to the cytosol and the polar tail facing the selectivity filter. One or two hydrophobic moieties interact with Phe656 side chains, probably via π-stacking. The basic nitrogen is involved in cation–π interaction with Tyr652 residues. Although most known hERG blockers contain a basic nitrogen center that is expected to be protonated under biological conditions, some possess an aromatic ring in its place. The aromatic linker may be able to participate in favorable π-stacking with Tyr652 *in lieu* of a cation–π interaction. The tail end of the molecule appears in many of the potent hERG blockers. It is thought to extend deep into the pore and form a hydrogen bond between the acceptor feature [39] (Figure 3c) and the side-chain hydroxyls of hERG, which might explain ligand-dependent attenuation of hERG binding affinity by T623A and S624A mutations.

An important caveat to this discussion is a clear possibility that multiple ligand binding sites exist on hERG that are capable of modulating channel activity. The ability to locate and describe these alternative sites is likely to come from a concerted effort in radioligand competition assays, mutagenesis, homology modeling, and ultimately X-ray crystallography.

Structural lessons learned

Mapping the chemical space of known hERG blockers has shed some light on the structural aspects of ligands consistent with a hERG blocker profile.

(1) Physicochemical property profiling of hERG actives and inactives clearly points to the fact that the likelihood of hERG block decreases significantly for polar low molecular weight ligands. As seen in Figure 4a, few of the known hERG blockers have Clog*P* < 1 or MW < 250. This is in agreement with the Clog*P* ≥ 3.7 limit for potent hERG blockers from Buyck *et al.* [37], and may be due to the large pore size in hERG as well as the lipophilic character of the pore lining. Unfortunately, for a variety of reasons that include target binding affinity, absorption and clearance

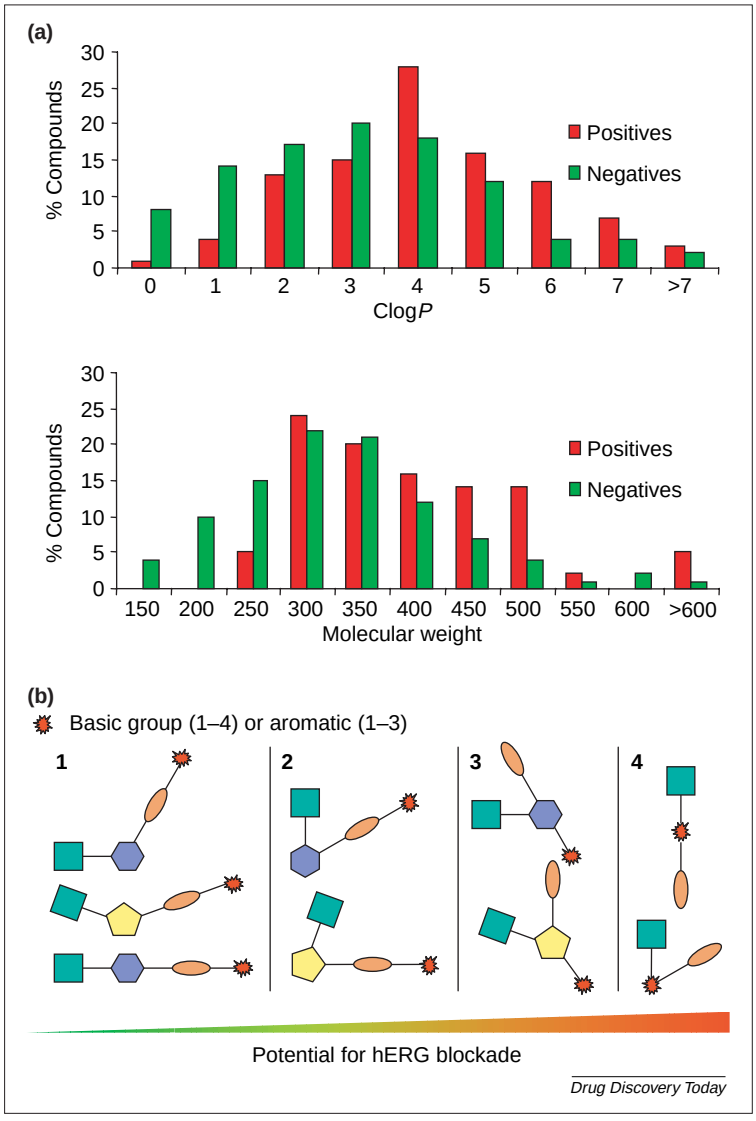

FIGURE 4

Reducing the risk of hERG interaction. (a) Clog*P* and molecular weight (MW) distributions for hERG actives and inactives (data set from [39] and references therein). Compounds with MW < 250 and Clog*P* < 1 are significantly less likely to block hERG. **(b)** Molecular shapes more likely to result in a potent hERG interaction. Linear topology consistent with *meta-/para-* attachments is seen less frequently in hERG active sets than V-shaped geometry stemming from *ortho*-substitution patterns. The role of the basic group in the ligand–hERG interaction can be played by an aromatic ring. The placement of the solubilizing group, if present, may have a strong impact on the hERG profile of the molecule.

considerations, few of the discovery compounds that move into the clinic fall in this category.

(2) The presence of a basic solubilizing group or a positively charged nitrogen in general increases the likelihood of hERG block. Conformational analysis of several hERG blockers from the literature led to the observation that shielding of the protonated form of the ligands decreases the amount of deprotonation, thus contributing to the increased potency for blocking hERG [41]. Tertiary amines, which form ammonium ions shielded by two structural fragments, block hERG more potently than compounds containing amines at the molecular periphery. Decreasing the pK_a of the basic amine may lower hERG activity by destabilizing the protonated species. A useful strategy for attaching solubilizing groups to a molecule of interest may involve identification of multiple suitable substitution sites, thus increasing the odds of finding ligands devoid of hERG activity.

(3) A large number of hERG blockers, both basic and neutral, contain flexible linkers that connect various molecular fragments. Flexibility appears to help the ligands find conformations compatible with the large binding site on hERG. Ligand rigidification may lead to removal of undesired hERG activity.

(4) Some general observations of the topology of ring-linker arrangements versus the potential for hERG blockade are shown in Figure 4b. Linear topology consistent with *meta-/para-* attachments is seen less frequently in hERG active sets than V-shaped geometry stemming from *ortho*-substitution patterns. The role of the basic group in the ligand–hERG interaction can be played by an aromatic ring. The placement of the solubilizing group, if present, may have a strong impact on the hERG profile of the molecule.

Conclusions

How useful have most of the *in silico* methods been so far? At this point it appears that they have contributed to a better understanding of the general SAR features implicated in hERG interactions. Some of the approaches may be able to link the prediction to interpretable features, such as presence or absence of certain molecular fragments. Many can be used as tools for the prioritization of compounds to be tested for hERG block due to their better performance in identifying hERG inactives. The main reason for that may lie in the datasets available for modeling. Naturally, they have tended to be global snapshots of structures associated with hERG activity, covering 'broad but shallow regions of chemical space' [13]. In other words, there is more known about non-blockers than blockers. This has rendered current *in silico* approaches less useful in making accurate predictions for each molecule, such as would be required in an ongoing medicinal chemistry optimization program. The drive toward better computational models may receive a boost in the near future from increasing availability of high-throughput hERG channel assays, such as planar patch. While more work is needed to validate the results from these assays versus the 'gold standard' of the patch clamp, these techniques are providing a window into local structure–activity datasets not available previously. Current computational methods do, however, identify trends, which may be sufficient to permit *in silico* identification of potential scaffold-based QT liabilities in the course of whole library screens, internal as well as commercial, and real as well as virtual.

Acknowledgements

The author would like to thank Esther Aronov for help in preparation of this manuscript.

References

1 Lipinski, C.A. *et al.* (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* 23, 3–25

2 Ajay *et al.* (1998) Can we learn to distinguish between "drug-like" and "nondrug-like" molecules? *J Med Chem* 41, 3314-3324

3 Egan, W.J. *et al.* (2002) Guiding molecules towards drug-likeness. *Curr. Opin. Drug Discov. Devel.* 5, 540–549

4 Teague, S.J. *et al.* (1999) The design of leadlike combinatorial libraries. *Angew. Chem. Int. Ed. Engl.* 38, 3743–3748

5 Oprea, T.I. *et al.* (2001) Is there a difference between leads and drugs? A historical perspective. *J. Chem. Inf. Comput. Sci.* 41, 1308–1315

6 Beresford, A.P. *et al.* (2004) In silico prediction of ADME properties: are we making progress? *Curr. Opin. Drug Discov. Devel.* 7, 36–42

7 Clark, D.E. (2003) In silico prediction of blood-brain barrier permeation. *Drug Discov. Today* 8, 927–933

8 Jalaie, M. *et al.* (2004) Prediction of drug-like molecular properties: modeling cytochrome p450 interactions. *Methods Mol. Biol.* 275, 449–520

9 Ekins, S. and Erickson, J.A. (2002) A pharmacophore for human pregnane X receptor ligands. *Drug Metab. Dispos.* 30, 96–99

10 Penzotti, J.E. *et al.* (2002) A computational ensemble pharmacophore model for identifying substrates of P-glycoprotein. *J. Med. Chem.* 45, 1737–1740

11 Ekins, S. (2004) Predicting undesirable drug interactions with promiscuous proteins *in silico*. *Drug Discov. Today* 9, 276–285

12 Brown, A.M. (2004) Drugs, hERG and sudden death. *Cell Calcium* 35, 543–547

13 Pearlstein, R. *et al.* (2003) Understanding the structure-activity relationship of the human ether-a-go-go-related gene cardiac K+ channel. A model for bad behavior. *J. Med. Chem.* 46, 2017–2022

14 Towbin, J.A. *et al.* (2001) Genotype and severity of long QT syndrome. *Drug Metab. Dispos.* 29, 574–579

15 Antzelevitch, C. and Shimizu, W. (2002) Cellular mechanisms underlying the long QT syndrome. *Curr. Opin. Cardiol.* 17, 43–51

16 Mitcheson, J.S. and Perry, M.D. (2003) Molecular determinants of high-affinity drug binding to HERG channels. *Curr. Opin. Drug Discov. Devel.* 6, 667–674

17 Redfern, W.S. *et al.* (2003) Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. *Cardiovasc. Res.* 58, 32–45

18 Netzer, R. *et al.* (2001) Screening lead compounds for QT interval prolongation. *Drug Discov. Today* 6, 78–84

19 Wood, C. *et al.* (2004) Patch clamping by numbers. *Drug Discov. Today* 9, 434–441

20 Cavero, I. *et al.* (2000) Drugs that prolong QT interval as an unwanted effect: assessing their likelihood of inducing hazardous cardiac dysrhythmias. *Expert Opin. Pharmacother.* 1, 947–973

21 Friesen, R.W. *et al.* (2003) Optimization of a tertiary alcohol series of phosphodiesterase-4 (PDE4) inhibitors: structure-activity relationship related to PDE4 inhibition and human ether-a-go-go related gene potassium channel binding affinity. *J. Med. Chem.* 46, 2413–2426

22 McCauley, J.A. *et al.* (2004) NR2B-selective N-methyl-D-aspartate antagonists: synthesis and evaluation of 5-substituted benzimidazoles. *J. Med. Chem.* 47, 2089–2096

23 Mitcheson, J.S. *et al.* (2000) A structural basis for drug-induced long QT syndrome. *Proc. Natl. Acad. Sci. U. S. A.* 97, 12329–12333

24 Pearlstein, R.A. *et al.* (2003) Characterization of HERG potassium channel inhibition using CoMSiA 3D QSAR and homology modeling approaches. *Bioorg. Med. Chem. Lett.* 13, 1829–1835

25 Doyle, D.A. *et al.* (1998) The structure of the potassium channel: molecular basis of K+ conduction and selectivity. *Science* 280, 69–77

26 Jiang, Y. *et al.* (2002) Crystal structure and mechanism of a calcium-gated potassium channel. *Nature* 417, 515–522

27 Jiang, Y. *et al.* (2003) X-ray structure of a voltage-dependent K+ channel. *Nature* 423, 33–41

28 Blaustein, R.O. and Miller, C. (2004) Ion channels: shake, rattle or roll? *Nature* 427, 499–500

29 Cohen, B.E. *et al.* (2003) Answers and questions from the KvAP structures. *Neuron* 39, 395–400

30 Fernandez, D. *et al.* (2004) Physicochemical features of the HERG channel drug binding site. *J. Biol. Chem.* 279, 10120-10127.

31 Chen, J. *et al.* (2002) Position of aromatic residues in the S6 domain, not inactivation, dictates cisapride sensitivity of HERG and eag potassium channels. *Proc. Natl. Acad. Sci. U. S. A.* 99, 12461–12466.

32 Ekins, S. *et al.* (2002) Three-dimensional quantitative structure-activity relationship for inhibition of human ether-a-go-go-related gene potassium channel. *J. Pharmacol. Exp. Ther.* 301, 427–434

33 Ekins, S. (2003) In silico approaches to predicting drug metabolism, toxicology and beyond. *Biochem. Soc. Trans.* 31, 611–614

34 Cavalli, A. *et al.* (2002) Toward a pharmacophore for drugs inducing the long QT syndrome: insights from a CoMFA study of HERG K(+) channel blockers. *J. Med. Chem.* 45, 3844–3853

35 Keseru, G.M. (2003) Prediction of hERG potassium channel affinity by traditional and hologram qSAR methods. *Bioorg. Med. Chem. Lett.* 13, 2773–2775

36 Roche, O. *et al.* (2002) A virtual screening method for prediction of the HERG potassium channel liability of compound libraries. *ChemBioChem* 3, 455–459

37 Buyck, C. (2003) An *in silico* model for detecting potential HERG blocking. In *EuroQSAR 2002. Designing Drugs and Crop Protectants: Processes, Problems, and Solutions* (Ford, M. *et al.*, eds.), pp. 86–89, Blackwell Publishing

38 Tong, W. *et al.* (1998) Evaluation of quantitative structure-activity relationship methods for large-scale prediction of chemicals binding to the estrogen receptor. *J. Chem. Inf. Comput. Sci.* 38, 669–677

39 Aronov, A.M. and Goldman, B.B. (2004) A model for identifying HERG K+ channel blockers. *Bioorg. Med. Chem.* 12, 2307–2315

40 Carhart, R.E. *et al.* (1985) Atom pairs as molecular features in structure-activity studies: definition and application. *J. Chem. Inf. Comput. Sci.* 25, 64–73

41 Zolotoy, A.B. *et al.* (2003) Physicochemical determinants for drug induced blockade of HERG potassium channels: effect of charge and charge shielding. *Curr. Med. Chem. – Cardiovasc. Hematol. Agents* 1, 225–241