Brief Articles

Common Pharmacophores for Uncharged Human Ether-a-go-go-Related Gene (hERG) Blockers

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In silico approaches are widely used to predict human ether-a-go-go-related gene (hERG) channel blockade. Published pharmacophore models of hERG blockers typically contain a basic nitrogen center flanked by aromatic or hydrophobic groups. However, hERG blockade has been observed in series lacking the basic nitrogen. By utilizing screening data for 194 potent uncharged hERG actives, we propose a pharmacophore for neutral hERG blockers, and provide guidance on eliminating hERG liability in an uncharged hERG active chemical series.

Introduction

Cardiac arrhythmia as a side effect of the action of nonantiarrhythmic drugs has become a major pharmacological safety concern for the pharmaceutical industry and the health regulatory authorities.[1] In recent years, a number of blockbuster drugs have been withdrawn from the market because of reports of sudden cardiac death and several others were forced to carry "black box" warning labels.[2] In all cases, long QT syndrome (LQTS[a]), 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.

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.[3] A major contributor to phase 3 repolarization at the end of the QT interval, this current is conducted by tetrameric pores with the individual subunits encoded by the human ether-a-go-go-related gene (hERG).[4] Blockade of hERG K^+ channels is widely regarded as the predominant cause of drug-induced QT prolongation, making early detection of compounds with this undesirable side effect 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 those required for efficacy. Indeed, the promiscuity of hERG appears astounding, with current estimates that between 30%[5] and 60%[6] of new molecular entities synthesized as potential therapeutic agents test positive for hERG blockade within the 30-fold window currently considered a safety concern.[7] Recently 10% of the molecules in development by a major pharmaceutical company representing a range of pharmacotherapeutic and chemical classes were found to cause QTc prolongation in vivo.[8] These hERG-related attrition rates in research are significantly higher than previously expected.

Unfortunately, the training sets used for pharmacophore modeling have tended to cover "broad but shallow regions of chemical space"[2] of mostly legacy compounds widely known to block hERG (e.g., antiarrhythmics, antihistamines, antiphychotics, antibacterials).[1] As a result, the published models typically contain variations on the now classic hERG motif of a basic nitrogen center flanked by aromatic or hydrophobic groups attached with flexible linkers.[9−15] In contrast, newly discovered hERG blockers have been sprouting up in many scaffold series not part of the initial training sets.[16] These scaffolds have all too often lacked the basic nitrogen feature, frequently taking medicinal chemists into previously uncharted waters in their efforts to design out the hERG activity.

hERG activity has been observed previously in ligands lacking the basic nitrogen center.[11,16−20] Well-documented cases involve widely available medications such as mizolastine and ketoconazole (Figure 1A). Here, we will refer to this type of compounds as the "neutral" or "uncharged" hERG blockers, i.e., ligands that lack a clearly recognizable highly basic amine motif. There have not been any published reports investigating the nature of neutral hERG blockade, a statement echoed by Delisle and co-workers in a recent review.[21] Hence, to our knowledge, this Brief Article constitutes the first study of the commonality of structure between various uncharged hERG blockers. By utilizing in-house data for a diverse set of 194 potent uncharged hERG actives, we propose a pharmacophore for neutral hERG blockers and demonstrate its ability to discriminate between known hERG actives and inactives from the literature. We also illustrate how mapping features of known hERG blockers onto the proposed pharmacophore can be used as guidance for chemists focused on eliminating hERG liability in an uncharged hERG active chemical series.

Data Set Description

We assembled the neutral hERG blocker data set from the hERG planar patch screening data compiled from our corporate database. A number of groups have demonstrated that planar patch technology is comparable to traditional whole-cell electrophysiology in reliability and data content and have applied this method to detailed studies of pharmacological profiles of

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[a] Abbreviations: hERG, human ether-a-go-go-related gene; LQTS, long QT syndrome; MMFF94, Merck molecular force field 94; QTc, corrected QT interval.

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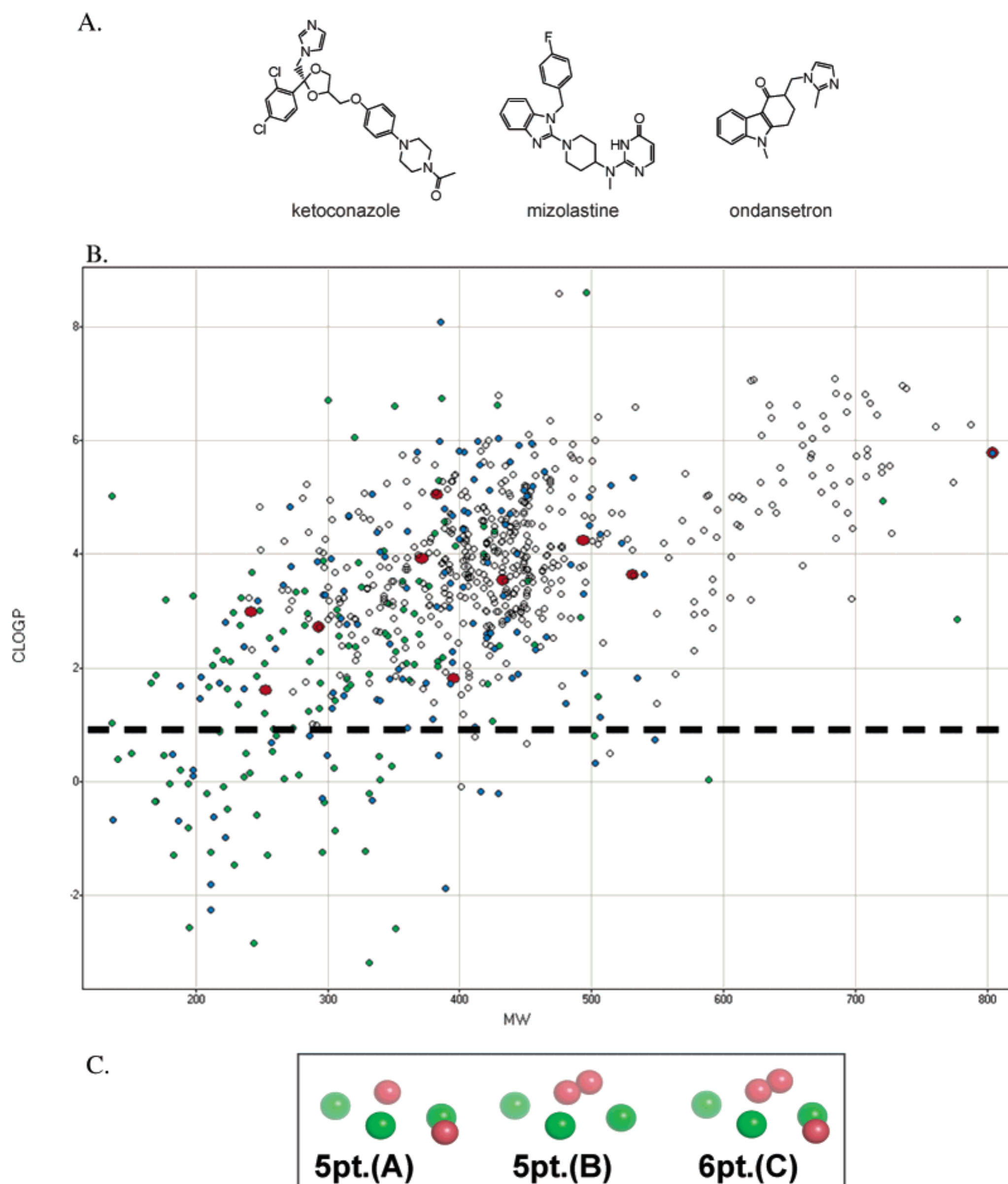

Figure 1. Uncharged hERG blockers. (A) Examples of marketed drugs belonging to the uncharged hERG blockers subclass that produced IC_{50} < 10 μM in the planar patch hERG assay. (B) ClogP and molecular weight (MW) properties of uncharged hERG blockers. The color-coding is as follows: literature uncharged hERG actives (red) and inactives (green) from Aronov and Goldman;[11] the complete internal set (519 compounds with hERG IC_{50} < 30 μM in the planar patch assay) of hERG actives (black); a random subset (123 neutral ligands that inhibit <25% of hERG activity at 10 μM in the planar patch assay) of internal hERG inactives (blue). Compounds with ClogP < 1 are unlikely to block hERG potassium current. (C) Common pharmacophores for uncharged hERG blockers. Pharmacophores were derived from the pharmacophore elucidation subset (194 compounds with hERG IC_{50} < 10 μM in the planar patch assay). The six-point pharmacophore is the union of two five-point pharmacophores. Color representation of pharmacophore features is green (hydrophobe/aromatic) and red (hydrogen bond acceptor). When used as a query for hERG blockers, default sphere radii from MOE were used: 1 Å for acceptors and 1.4 Å for hydrophobes. Intrafeature distances are available in Supporting Information.

QT prolonging drugs.[22-25] Internal data were collected using the planar patch instrument (IonWorks HT, Molecular Devices)[26] that allows medium-throughput electrophysiology measurements in a 384-well format. Compounds were added from a 3-fold aqueous addition buffer, and measurements were made at assay concentrations of 1.1, 3.3, 10, and 30 μM. The rank-ordering of compounds by potency of the hERG block observed in the gold standard patch clamp assay translates reasonably well into the planar patch experiments,[22] primarily because of the consistent shift of observed IC_{50} values to higher concentrations in IonWorks HT. The average 5-fold potency shift[27,28] serves to lower the number of false positives in the assay. Compounds were considered uncharged at biological pH when no protonation/deprotonation events were predicted with the MOE[29] wash routine. The complete internal set contained 519 neutral ligands with hERG IC_{50} < 30 μM. The subset representing the most active ligands (194 neutral ligands with hERG IC_{50} < 10 μM) was used for the purposes of pharmacophore elucidation, and the entire list was used for validation. An internal subset of hERG inactives (123 neutral ligands that inhibit <25% of hERG activity at 10 μM) was selected randomly from the corporate database.

Two additional validation sets were extracted from the literature. From the previously published lists of known hERG positives and presumed hERG negatives,[11] neutral ligands were extracted. The list of literature uncharged hERG blockers contained 10 compounds for which the neutral molecule is expected to be the dominant species at biological pH. Three of these

Table 1. Data Sets and Classification Results Using the Neutral hERG Blocker Pharmacophore(s)

data set	no. in set	no. matching[a]			% matching		
		5 pt (A)	5 pt (B)	6 pt (C)	5 pt (A)	5 pt (B)	6 pt (C)
potent hERG blockers[b] (IC_{50} < 10 μM)	194	152	134	40	78	69	21
all hERG blockers (IC_{50} < 30 μM)	519	427	413	227	82	80	44
potent literature hERG blockers[c] (IC_{50} < 10 μM)	3	2	2	1	67	67	33
hERG nonblockers (<25% inhibition at 30 μM),							
pharmacophore only	123	35	37	5	28	30	4
pharmacophore and ClogP > 1[d]		32	35	5	26	28	4
literature hERG nonblockers,							
pharmacophore only	140	30	40	9[e]	21	29	6
pharmacophore and ClogP > 1[d]		21	28	6[e]	15	20	4

[a] Pharmacophore match represents a full match to the pharmacophore queries in Figure 1C. [b] Potent hERG blocker subset (IC_{50} < 10 μM in the planar patch hERG assay) was used for pharmacophore elucidation. [c] Neutral hERG blockers from the literature[11] that produced IC_{50} < 10 μM in the planar patch hERG assay are ketoconazole, ondansetron, and mizolastine. [d] Pharmacophore match is combined with the observation that compounds with ClogP < 1 are unlikely to block hERG potassium current (Figure 1B). [e] One of the compounds, ritonavir, was recently implicated in blocking hERG (IC_{50} = 8.2 μM).[36]

compounds produced IC_{50} < 10 μM as measured in triplicate with the planar patch hERG assay: ketoconazole, 8.9 μM; mizolastine, 1 μM; ondansetron, 7.1 μM (Figure 1A). The list of presumed hERG inactives contained 140 neutral ligands for which no reports of QT prolongation existed as of 2003, based on warning labels and adverse effects cited in the literature.

A number of reports have linked hERG blocking ability of individual ligands to their physicochemical properties.[30,31] We have previously proposed ClogP = 1 as a cutoff for ligand lipophilicity below which binding to hERG is rarely observed.[3] The hydrophobic character of the hERG pore, lined with Phe656 and Tyr652 side chains, is likely to be responsible for this observation. As shown in Figure 1B, this notion holds true for neutral hERG blockers. All 10 of the uncharged hERG actives from the literature have ClogP > 1. The 140 literature hERG inactives span a wide range of lipophilicity values, with 42 ligands (30%) having ClogP < 1. Consistently, of the 519 in-house compounds with hERG IC_{50} < 30 μM in the planar patch assay, only four (<1%) fall below the cutoff. This is 10-fold lower than the 7.3% prevalence of compounds with ClogP < 1 among all hERG assay submissions (data not shown). The lipophilicity requirement appears to be general across various hERG-active chemotypes.

Three-dimensional coordinates for the molecules in the data sets were generated with CORINA.[32] Multiconformer databases were then prepared for pharmacophore query searching using OMEGA.[33] Up to 200 conformers per molecule were allowed. The energy window was set to 20 kcal/mol, and an rmsd cutoff of 1 Å was applied to ensure adequate and diverse conformational representation. Local strain energy was calculated using the MMFF94s[34] force field as implemented in MOE, and the highly strained conformations (>2 kcal/rotatable bond) were removed. This threshold is 3-fold higher than the 0.7 kcal/bond cutoff shown by Perola and Charifson[35] to retain 90% of bioactive conformations and was used to ensure adequate conformer coverage for the ligands, resulting on average in 58 conformers per ligand across the multiconformer databases.

Elucidation of the Uncharged hERG Pharmacophore

We chose to utilize the information on 194 diverse uncharged hERG blockers from our corporate collection to facilitate the search for pharmacophores consistent with observed blockade of hERG K+ channel by uncharged small-molecule ligands. Pharmacophore elucidation was performed using the new elucidation module within MOE. Each molecule was subjected to full conformational search using the Conformation Import option. Feature annotation was performed using the CHD scheme comprising five feature types: hydrophobe/aromatic, anion, cation, and putative points projected from hydrogen bond acceptors and donors. The putative points from donors or acceptors are projected in the approximate direction of a hydrogen bond based on the geometry of the lone pairs and occupy the space of the respective hydrogen bond partners. They are thus expected to better capture the physical reality of donor/acceptor identity and directionality. The constraint on the frequency of occurrence of the hydrophobe feature in generated queries was removed, and the upper limit on the number of features in a query was adjusted to 8.

Pharmacophore elucidation is performed in MOE using a "build-up" method, whereby two-point queries are built first and used to construct more complex three-point queries. Then four-point queries are generated from three-point queries, etc. In other words, larger queries are built from popular small queries. Query cluster parameter was set to 1 Å, and the active coverage required at each stage of pharmacophore assembly was lowered to 0.5 to ensure that the search was exhaustive.

The procedure resulted in 62 pharmacophore queries: 33 four-point queries and 29 five-point queries. All 33 four-point queries and 10 five-point queries matched over two-thirds of the 194 ligand pharmacophore elucidation set, and they were further analyzed for their coverage of hERG actives and the ability to discriminate between actives and inactives. The pharmacophore query searches were performed using the pharmacophore matching routine in MOE. Eventually, the two best five-point pharmacophore queries were chosen (Figure 1C, structures **A** and **B**). Both pharmacophores contain three hydrophobe/aromatic features and two hydrogen bond acceptors. Interestingly, they share four of the five features and differ only in the location of the second acceptor functionality.

Pharmacophore Validation

Search results for the data sets described above using the two pharmacophores (**A** and **B**) are shown in Table 1. The coverage of the complete 519-ligand in-house hERG data set was consistent with the results for the 194-ligand subset for both queries. Percent of compounds matching the pharmacophore query stayed approximately the same for the query using **A** (78% for the compounds with hERG IC_{50} < 10 μM versus 82% for the complete 519-ligand data set) and increased somewhat for the query using **B** (69% for the 194 compound hERG IC_{50} < 10 μM subset versus 80% for the complete in-house set). Both queries were able to recover two of three uncharged literature hERG blockers with IC_{50} < 10 μM (ketoconazole and mizolastine). In the internal set of 123 neutral hERG inactives that

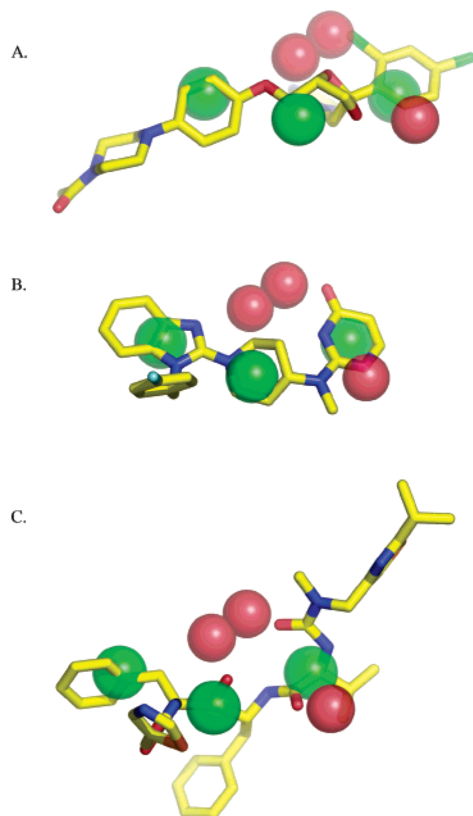

Figure 2. Six-point pharmacophore for hERG blockers mapped onto conformations of ketoconazole (A), mizolastine (B), and ritonavir (C). Color scheme for pharmacophore features is from Figure 1C: green (hydrophobe/aromatic) and red (hydrogen bond acceptor).

served as the primary control, 28% and 30% of compounds matched the two pharmacophore queries. When the requirement for pharmacophore match is combined with the observation from Figure 1B that compounds with ClogP < 1 are unlikely to block hERG K^+ current, the number of hERG inactives incorrectly identified as potential hERG blockers due to a pharmacophore match is reduced slightly to 26% and 28%, respectively. When challenged with the literature hERG inactives, only 21% and 29% of the inactives matched the two queries. When combined with the ClogP < 1 cutoff, the number of hERG inactives incorrectly identified as potential hERG blockers is reduced further to 15% and 20%, respectively.

Since the two five-point queries share most of their features, they were then combined to form a union six-point pharmacophore (Figure 1C, structure **C**). A more specific query, it produces matches between 21% and 44% cases of uncharged hERG actives while flagging 6% of literature hERG inactives and 4% of internal inactives and is thus able to provide the highest enrichment of hERG blockers of all queries considered in this study. Ketoconazole is the only potent literature active that matches this pharmacophore. As shown in Figure 2A, the central ketal oxygens of ketoconazole match the acceptor features, with the phenyl, the dichlorophenyl, and the ketal ring providing the three hydrophobes. In the case of mizolastine (Figure 2B), benzimidazole, piperidine, and pyrimidone contribute the hydrophobic components, while two of the three acceptor features are fulfilled with benzimidazole nitrogen and pyrimidone oxygen. When the six-point pharmacophore match is combined with the ClogP > 1 cutoff, the number of hERG inactives incorrectly identified as potential hERG blockers is reduced further to 4% in the case of the six-point query for both sets of hERG inactives (Table 1). Three of the nine initial matches (busulfan, dyphylline, and pentoxiphylline) have ClogP < 1, which apparently is incompatible with hERG blockade even in the presence of the aforementioned pharmacophore. Interestingly, one of the remaining six compounds, ritonavir, was recently implicated in blocking hERG. The reported patch clamp $IC_{50} = 8.2\ \mu M$[36] for ritonavir translates closely into planar patch $IC_{50} = 22.9\ \mu M$.[27] The mapping of ritonavir onto the six-point pharmacophore is shown in Figure 2C. The urea carbonyl, the amide carbonyl of P2 valine, and the hydroxyl group provide the necessary acceptor functionality, with the hydrophobes being distributed along the backbone.

Conclusion

We have identified a six-point pharmacophore as being effective for identifying those hERG K^+ channel blockers that are uncharged at biological pH. This pharmacophore and the smaller five-point pharmacophores contained within it appear with higher frequency in hERG blockers relative to nonblockers and can be used for rapid scaffold prioritization in the context of QT prolongation risk. Our results emphasize the importance of hydrophobic and hydrogen bond acceptor molecular features in binding to hERG. The acceptors are thought to be making interactions with the polar side chains, such as those of Thr623 and Ser624, to stabilize the hERG-ligand complex.[3,4] The role of acceptors in hERG binding was noted previously. We reported[11] on the presence of an acceptor in the most information-rich three-point pharmacophore obtained in the course of a binary classification procedure. Testai et al.[12] also observed that an acceptor atom, typically carbonyl oxygen or heteroaryl nitrogen, is usually present within the 4−6 Å range of the basic center. However, it is in the case of uncharged ligands that their role becomes more prominent. It appears that partial occupancy of the pharmacophore space may be sufficient for hERG blockade, as evidenced by the two five-point subqueries, especially in cases when physicochemical properties favor hERG binding. We expect that the ability to map the ligands onto the proposed pharmacophore in the course of ligand optimization will provide chemists with guidance for designing out the hERG liability.

On the basis of our findings, we suggest the following three approaches to mitigate hERG binding in an uncharged small-molecule series. The first is to make the ligand sufficiently polar (ClogP < 1) so that binding to hERG becomes unlikely (Figure 1B). Often there are limitations on the ability to lower ClogP in a project, as this may tend to limit ligand affinity for the primary target and the extent of gastrointestinal absorption. In this case, a mere decrease in lipophilicity often results in weaker hERG binding. If decreasing hydrophobicity is not feasible, excision of a moiety responsible for one of the interactions described by the hERG pharmacophore(s) (Figure 1C) may weaken hERG binding. In cases where neither of the previous approaches is applicable (e.g., all of the matching features are necessary for on-target activity of the lead molecule), it may be possible to introduce sufficient additional components into a molecule that could render it hERG inactive despite the presence of the pharmacophore matching features. In our experience, this three-pronged strategy has proven to be successful in steering lead structures clear of hERG binding.

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Supporting Information Available: The complete list of intrafeature distances for the neutral hERG pharmacophore. This material is available free of charge via the Internet at http://pubs.acs.org.

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