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Symmetric Kv1.5 Blockers Discovered by Focused Screening

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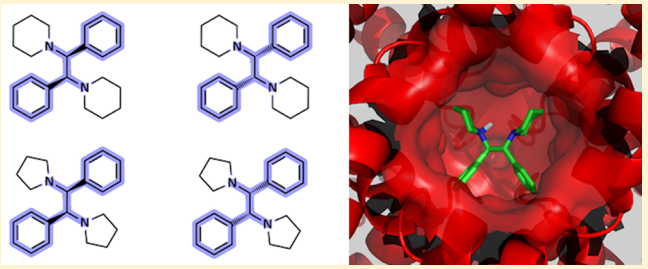
Supporting Information

ABSTRACT: Guided by computational methods, a set of 1920 compounds were selected from the AstraZeneca corporate collection and screened for Kv1.5 activity. To facilitate rapid generation of structure−activity relationships, special attention was given to selecting subsets of structurally similar molecules by using a maximum common substructure similarity-based procedure. The focused screen hit rate was relatively high (12%). More importantly, a structural series featured by the symmetric 1,2-diphenylethane-1,2-diamine substructure was identified as potent Kv.1.5 blockers. The property profile for the series is shown to meet stringent lead-optimization criteria, providing a springboard for the development of a new and safe treatment for atrial fibrillation.



KEYWORDS: *atrial fibrillation, Kv1.5 blockers, focused screen, maximum common substructure, structural series, symmetric ligands*

Atrial fibrillation is the most commonly sustained cardiac arrhythmia in clinical practice and is implicated in approximately 15% of all strokes in the United States.[1] A promising therapeutic approach is to selectively block the ultrarapid delayed rectifier potassium ion current (IKur) and thereby delay atrial repolarization and convert atrial fibrillation to normal sinus rhythm.[2] The voltage-gated potassium Kv1.5 ion channel underlies IKur, and its functional activity is predominantly found in the human atrium.[3] By blocking IKur, an atrial-selective increase in action potential duration and effective refractory period (ERP) is understood to be achieved, which in turn can decrease atrial fibrillation inducibility.[4] The discovery of structurally diverse Kv1.5 blockers was recently reviewed by Bilodeau and Trotter.[5] Some representative compounds that inhibit the Kv1.5 channel are shown in Figure 1.

To provide an existing Kv1.5 lead optimization (LO) program with series of new, potent structures having favorable physicochemical properties, a lead generation (LG) back-up program was initiated. Many different approaches can be used to find attractive leads to start a drug discovery project.[10] High-throughput screening (HTS) of large corporate compound collections is one approach typically used, but it is arguably not always necessary.[11] When extensive knowledge of the target and active ligands is available, approaches such as focused screening are often superior alternatives.[11] We therefore decided to opt for the more cost- and time-efficient virtual screens (VS), allowing for testing in the existing high-quality low-throughput LO screening assays. In short, the chosen approach was a limited focused screen guided by computational methods.

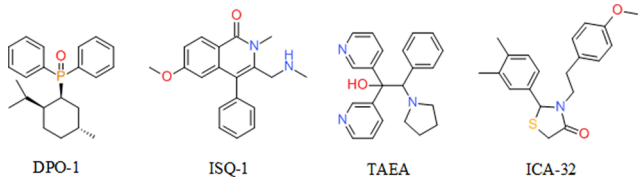


Figure 1. Molecular structures of potent Kv1.5 blockers: DPO-1,[6] ISQ-1,[7] TAEA,[8] and ICA-32.[9]

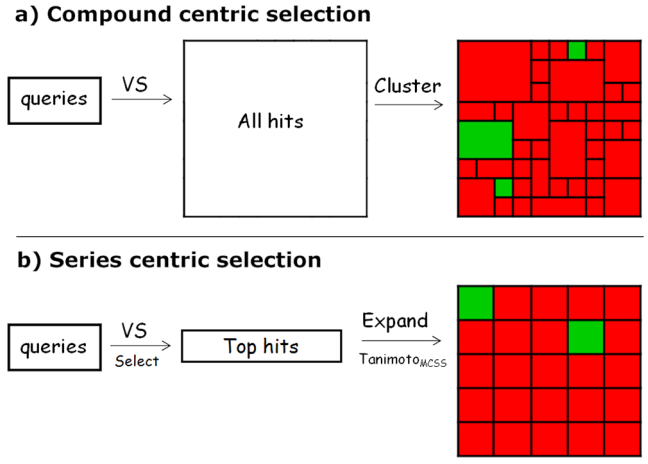


Figure 2. (a) Commonly used compound-centric virtual screening strategy may result in the selection of many singletons and big clusters, as visualized schematically by the different box sizes. The series-centric strategy (b), on the other hand, focuses on expanding the top VS hits into a more even distribution of compounds per series to allow for a speedier LG phase.

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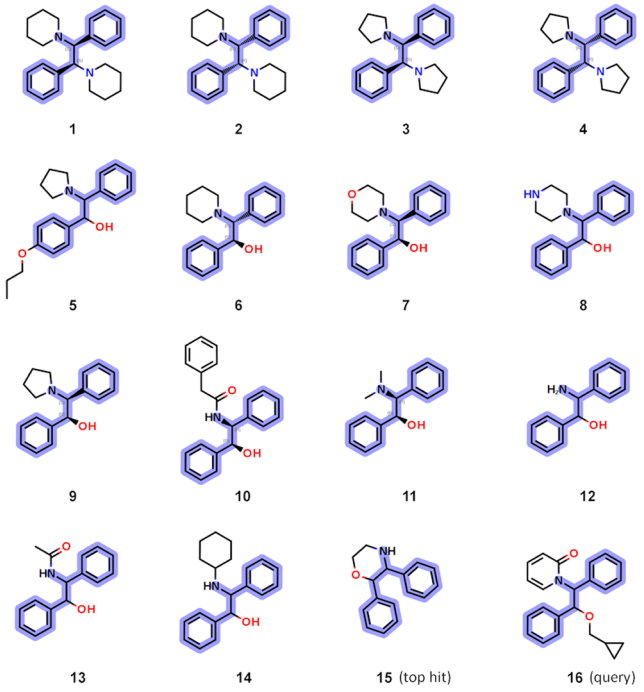

Figure 3. VS using **16** as the query resulted in the top hit compound **15**, which in turn was expanded into the structural series shown. The MCS for the series is the 1,2-diphenylethanamine as highlighted in blue. Compounds **1**−**4** were found to be potent Kv1.5 blockers.

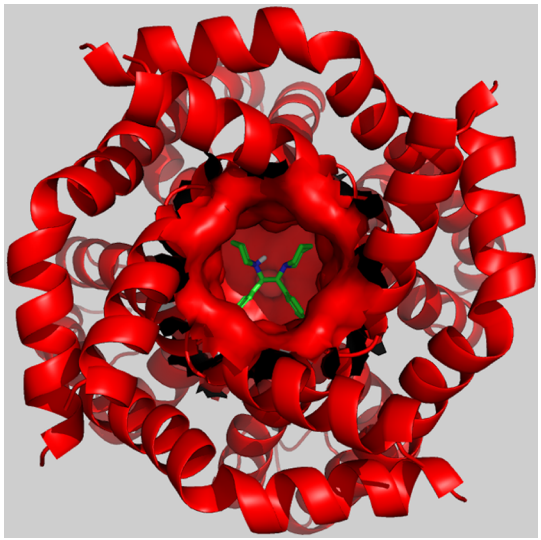

Figure 4. Symmetric compound **1** docked in a homology model of the Kv1.5 ion channel illustrating the high shape complementarity between protein and ligand.

Peukert et al. have described use of various virtual screening methods as successful approaches to identify Kv1.5 blockers.[12,13] Their approaches may be viewed as "compound-centric", whereas our strategy may be described as "structural series-centric". During virtual screening, little attention is typically made to arrive at series of compounds, and the number of near-neighbors in a cluster is seldom monitored. As a result, such focused screens may contain unnecessarily large clusters and an unreasonable large fraction of singletons (Figure 2a).

In an attempt to alleviate the uncertainty associated with obtaining singletons as hits and to ensure the economical use of limited screening resources (e.g., avoid screening big clusters), special attention was given to selecting series of similar compounds (Figure 2b). Twenty compounds were arbitrarily chosen as the "optimal" cluster size for a structural series, anticipating grounds for informed decision making. A potential advantage of this strategy is that the sets of near-neighbors are especially suitable for deriving structure−activity relationships. Another benefit is that that the number of screen iterations can be kept to a minimum, leading to a shorter LG phase. On the other hand, active compounds with very few near-neighbors will by definition not be discovered using this strategy, and the approach may not be optimal for difficult targets where low hit rates are expected.

Rather than depending on one particular computational method, a wide variety of methods were used in the initial VS: fingerprints,[14] shape- and electrostatic complementarities,[15,16] lingo similarities,[17] and maximum common substructure (MCS)-based techniques.[18] The results from each method will depend on the query structures, and the results are typically complementary.[19] The query structures were carefully selected from relevant sources (e.g., our internal LO program, literature and competitor patents). The VSs were performed on the AstraZeneca corporate collection, filtered according to typical drug-likeness criteria.[20] In addition, compounds registered with low purity (<85%) and compounds containing known toxophores or reactive groups were removed to provide high-quality hit structures to allow for an efficient LG phase.

The 500 highest ranked compounds from each VS search method were visually inspected and assessed in relation to the parent query. General physicochemical descriptors, with an extra emphasis on selecting compounds with low lipophilicity, and medicinal chemistry knowledge were used to select the most attractive starting points—top hits. The top hits thus selected were subjected to MCS-based similarity searches to expand the hits into structural series of near-neighbors. The method used was a MCS-based similarity measure (Tanimoto$_{MCSS}$) calculated using the OpenEye OEChem-toolkit,[15] according to eq 1.[18]

$$\text{Tanimoto}_{MCSS} = \frac{N_{AB}}{(N_A + N_B) - N_{AB}} \quad (1)$$

where N_A and N_B are the number of atoms in molecules A and B, respectively, and N_{AB} is the number of atoms in the MCSS of A and B. A Tanimoto$_{MCSS}$ value of 1 is obtained if two molecules are identical. The reason for using a substructure-based similarity measure was to ensure that core features of the hit molecules were preserved and that only molecules with small modifications were retrieved. Such small modifications have a strong correspondence with the type of functional group replacement common in medicinal chemistry projects. This is in contrast to fingerprint-based similarity comparisons where the structural similarity features are not always straightforward to rationalize and thus produce clusters that look unnatural to a medicinal chemist. As a consequence, key determinants of biological activity may be difficult to pinpoint and even more difficult to exploit in the design of improved analogues.

A final number of 82 structural series were selected, with an average cluster size of 23 compounds, leading to 1920 compounds in total. These compounds were tested for Kv1.5 activity in an Rb+ efflux assay. As many as 230 compounds

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Kv1.5 - "1,2-diphenylethane-1,2-diamine" Series

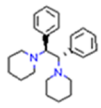
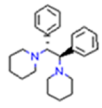
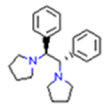
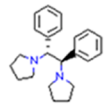
				Representative Compounds			
							
Properties	**L**	**M**	**H**	**1**	**2**	**3**	**4**
Potency							
Kv1.5 IC50 (μM)	≤10.0	≤50.0	>50.0	0.2	0.2	0.3	0.6
Ligand Efficiency	>0.30	>0.25	≤0.25	0.35	0.35	0.37	0.36
Ligand Lipophilicity Efficiency	≥3.0	≥2.0	<2.0	2.6	2.7	3.2	3.0
Selectivity							
hERG pIC50	<5.0		≥5.0	4.5	4.6	4.5	4.5
Physical Chemistry							
MW	≤450	≤500	>500	349	349	320	320
logD	≤4.0	≤5.0	>5.0	4.1	4.0	3.3	3.2
pKa	>8.4	>6.4	≥6.4	11.4	11.0	10.5	10.4
Solubility pH7.4 (μM)	>10.0	>1.0	≤1.0	81	95	100	92
Purity - LCMS (%)	≥85.0		<85.0	NV	96.0	97.0	91.0
In vitro DMPK							
CYP3A4 pIC50	<5.0	<6.0	≥6.0	4.8	4.7	4.9	4.9
CYP2C9 pIC50	<5.0	<6.0	≥6.0	4.7	4.7	4.7	4.7
CYP1A2 pIC50	<5.0	<6.0	≥6.0	4.7	4.7	4.7	4.7
CYP2C19 pIC50	<5.0	<6.0	≥6.0	4.7	4.7	4.7	4.7
CYP2D6 pIC50	<5.0	<6.0	≥6.0	6.5	6.9	6.5	6.7
HLM - CLint (μl/min/mg)	<13.0	≤70.0	>70.0	17.6	14.1	21.6	12.0
Hu Hep - CLint (μl/min/1E6)	<4.0	<22.0	≥22.0	<4.0	<4.0	<4.0	<4.0
React. Met. (CYP)	No		Yes	No	No	No	No
Caco-2 - PappAB (10-6cm/s)	>1.20	≥0.15	<0.15	10.2	10.3	13.3	14.0
PPB human (%free)	>10.0	≥1.0	<1.0	9.9	6.5	17.0	17.0
In vivo DMPK							
Bioav. rat PO (F) (%)	≥10.0		<10.0	26.6	103.3	48.9	15.6
Clearance iv rat (F) (ml/min/kg)	≤35.0		>35.0	NV	13.1	10.8	91.8
Half-life iv rat (F) (h)	>0.5		≤0.5	NV	15.1	7.1	1.0
Vss iv rat (F) (l/kg)	≤10.0		>10.0	NV	16.4	6.4	7.9

Figure 5. Summary of the properties of the novel series of 1,2-diphenylethane-1,2-diamine Kv1.5 blockers. The boxes are color-coded to monitor the distance from the target profile, where the green color denotes that the particular compound fulfills that particular project criterion for the next milestone transition.

were defined as actives (>40% inhibition at 10 μM), leading to a hit rate of 12%. The actives obtained from the primary screen were retested for purity (LC/MS) to confirm their molecular identity, and IC_{50} values were then determined. The great majority (90%) of the actives showed IC_{50} values less than 30 μM, and 17% showed IC_{50} values less than 10 μM. Roughly 20 series contained four or more active compounds.

Figure 3 depicts the structures of one of the most interesting hit series. It contained 15 compounds, and the MCS is the 1,2-diphenylethanamine fragment highlighted in blue. This particular structural series was identified using the MCS similarity-based search and the competitor compound **16** (1-[2-(cyclopropylmethoxy)-1,2-diphenylethyl]pyridin-2-one)[21] as a query, to provide the top hit (**15**). Compound **15** was subsequently expanded into the structural series shown in Figure 3. The calculated $Tanimoto_{MCSS}$ between the top VS hit **15** and the query **16** is relatively high (0.63). There were only a handful of compounds in the AstraZeneca corporate collection with higher values at the time. The $Tanimoto_{MCSS}$ for the remaining compounds in the 1,2-diphenylethanamine series as calculated from the query **16** end up in the mean distribution, meaning that they were not "top VS hits". On the other hand, compounds **1**−**14** are all structural near-neighbors of the top hit **15**, with $Tanimoto_{MCSS} \geq 0.63$.

The four 1,2-diphenylethane-1,2-diamine stereoisomers (**1**−**2** and **3**−**4**)[22,23] showed high affinity (0.2−0.6 μM) for the Kv1.5 channel and are equipotent with the more lipophilic reference compound DPO-1 (Kv1.5 IC_{50} = 0.5 μM, and log *D* = 6.0). The remaining compounds **5**−**15** were found to be inactive. The Kv1.5 IC_{50} values were determined by electrophysiological studies using the high-throughput planar patch clamp assay and IonWorks described by Schroeder et al.[24]

From a structural perspective, compounds **1**−**4** are atypical since they are symmetric. Some medicinal chemists have prejudices against symmetric compounds.[25] One common argument is that high symmetry will lead to stable crystals and thus low solubility. Another argument is that binding sites are rarely symmetrical. Nevertheless, there are many symmetric drugs in clinical use,[26] and in this case, symmetric ligands seem to be in harmony with the 3D structure of the target protein. There is currently no experimentally determined crystal structure of the Kv1.5 ion channel, but the complete Kv1.2 channel−β2 subunit complex structure has been determined, revealing a highly symmetric tetramer (PDB entry: 2A79).[27] The sequence identity between Kv1.2 and Kv1.5 is very high, and importantly, the amino acids in the pore domains are highly conserved (90%), allowing for the construction of a Kv1.5 homology model.[28] Figure 4 displays a Kv1.5 homology model based on the Kv1.2 crystal structure, proposing a conceivable ligand binding mode for compound **4**. The high shape complementarity between the protein and the docked ligand is notable.

Unlike previously reported potent Kv1.5 blockers, compounds **1**−**4** include an ionizable functional group. The presence of a basic nitrogen flanked by aromatic moieties prompted us to test the compounds for hERG inhibition, as

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many ligands that block the hERG channel are known to contain this motif.[29] From a regulatory perspective, there is a general requirement for selectivity against binding to the hERG channel, and this is particularly true for new and safe antiarrythmics. Figure 5 shows the measured hERG inhibition data, and compounds **1**−**4** were fortunately found to be inactive, exhibiting IC_{50} values above the highest assay concentration (>33 μM) and thus reducing our concern for cardiac arrhythmic side effects.

Furthermore, the above-mentioned pharmacophore of the 1,2-diphenylethane-1,2-diamine compounds **1**−**4** is sometimes associated with inhibition of one common cytochrome P450 enzyme: the 2D6 subtype.[30] Consequently, the compounds were tested for CYP2D6 inhibition, and submicromolar IC_{50} values were discovered (Figure 5). This information was included in the subsequent LO strategy with the goal of eliminating any such effect to increase metabolic stability and to minimize the possible effect of drug−drug interactions.

In addition to the already mentioned experiments, the diamine structural series, together with a small number of other interesting series, were exposed to extensive profiling. That is, several parameters frequently used for decision-making in drug discovery were determined; see Figure 5. For example, the four representative compounds (**1**−**4**) were found to be potent in the secondary Kv1.5 voltage clamp assay. Most other parameters, such as aqueous solubility, reactive metabolite formation, and CaCO-2 permeability, were found to be well within our defined thresholds. Moreover, in vivo pharmacokinetic studies were conducted in rat, providing promising results. Figure 5 summarizes the experimentally determined properties of the novel diamine series and shows the corresponding criteria for LO transfer. Such tables may be used to aid decision making at milestone transitions, as they simplify risk identification, as well as allow easy comparison between lead series.

In summary, a limited focused screening effort guided by computational methods, using a number of known Kv1.5 blockers as starting points, resulted in a novel series of symmetric Kv1.5 blockers with favorable properties in a timely fashion. The time between LG start and having the necessary data to demonstrate that the stringent LO criteria were met was a relatively short 9 months. This provides support for the use of focused screening as the method of choice when the aim is to identify new series for existing LO projects. The strategy of selecting series instead of compounds was successful in this case. In addition, feedback that the structural series were considered to be well-defined clusters of near-neighbors was received. On a more general note, selecting series based on substructures is very much aligned to the way medicinal chemists perceive SAR. Other benefits of selecting series based on substructures and using defined quality criteria are that it enables an appreciation of the series feasibility and its optimization potential and assesses any risks associated with it. The results of further medicinal chemistry work on the 1,2-diphenylethane-1,2-diamine structural series will be reported in the near future.

■ ASSOCIATED CONTENT

Supporting Information

Coordinates for the homology model and descriptions of the experimental methods used to measure Kv1.5 IC_{50}, log *D*, solubility, hERG inhibition, pK_a values, CYP inhibition, and HLM Clint. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

HTS, high-throughput screen; VS, virtual screen; MCS, maximum common substructure; LG, lead generation; LO, lead optimization; ERP, effective refractory period; IKur, ultrarapid delayed rectifier potassium ion current; Kv1.5, voltage-gated potassium (Kv) channel subunit 5

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