Journal of Medicinal Chemistry

Brief Article

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Computational Design and Discovery of "Minimally Structured" hERG Blockers

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Supporting Information

ABSTRACT: Molecular knowledge of hERG blocking liability can offer the possibility of optimizing lead compounds in a way that eliminates potentially lethal side effects. In this study, we computationally designed, synthesized, and tested a small series of "minimally structured" molecules. Some of these compounds were remarkably potent against hERG (**6**, IC_{50} = 2.4 nM), allowing us to identify the minimal structural requirements for hERG blocking liability.

INTRODUCTION

Binding of different drugs into the pore domain of human ether-a-go-go-related gene (hERG) channels (Kv11.1) can be the concurrent cause of a severe tachyarrhythmia (torsades de pointes), which is recognized as a very dangerous side effect of drugs, eventually leading to sudden cardiac death.[1] Several approved drugs that induce this effect, referred to as drug-induced long-QT syndrome, LQTS (e.g., astemizole, cisapride, terfenadine, haloperidol, sertindole, etc.), have been withdrawn from the market or restricted in their use because of their effects on QT interval prolongation.[2] In retrospect, it has been shown that all these drugs are able to interact with and block hERG channels and that hERG binding is probably one of the key molecular events for drug-induced LQTS.[3−7] Therefore, the hERG binding liability of new chemical entities has greatly challenged several drug discovery programs.

hERG blocking liability is usually assessed quite early in a drug discovery program, and several relatively fast experimental approaches are now available, spanning from planar electrode patch clamp techniques and radiolabeled drug binding assays to high-throughput fluorescent dye assays using Chinese hamster ovary (CHO) cells stably transfected with hERG ion channels.[8] In this scenario, in silico predictions of hERG binding have also played an important role in drug discovery. Over the years, a plethora of computational studies have appeared in the literature.[9,10] The first pharmacophore models developed about 10 years ago by Ekins et al.,[11] Cavalli et al.,[12] Aronov et al.,[13] and Pearlstein et al.[14] were characterized by a central positive ionizable feature linked to aromatic or hydrophobic groups. Conversely, other models have been based on uncharged ligands,[15] accounting for additional features responsible for hERG block. In addition, more recent in silico studies took advantage of remarkably large data sets, defining the pool of molecules and the associated physicochemical features responsible for binding to hERG.[16−18] All these investigations, carried out using ligand- or structure-based approaches, have greatly contributed to achieving a superior understanding of hERG−drug binding and a potential identification of pharmacophoric functions responsible for the interactions between small organic molecules and hERG. All this information has then been applied to the rational design of new chemical entities in several drug discovery programs.[19,20]

One of the accepted pharmacophoric models for hERG binders, mentioned above, shows (i) two aromatic rings (C0 and C1) almost coplanar and at a distance of ∼4.5−6.5 Å, (ii) one basic nitrogen atom (N) ∼5−9 and ∼5.5−7 Å far away from the rings C0 and C1, and (iii) a further aromatic moiety (C2), which is required for increasing potency against hERG, ∼4.5−7.5 Å from the nitrogen atom (see Figure 1). This model

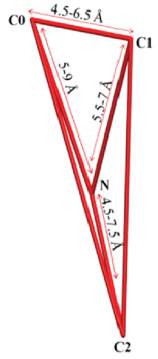


Figure 1. Pharmacophore model of hERG K^+ channel blockers. C0, C1, and C2 are the centers of mass of aromatic moieties, while N is a protonable nitrogen atom. Potent hERG blockers fit with this pharmacophore model if they adopt an extended conformation.

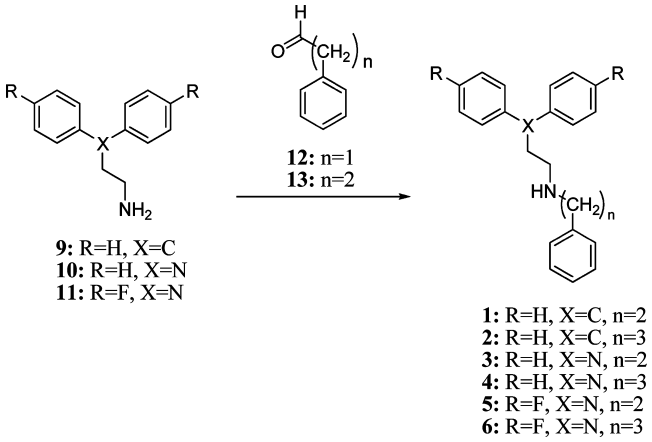
can account for the hERG blocking activity of several quite flexible drugs (e.g., astemizole, terfenadine, cisapride, etc.), which should adopt a kind of "extended" conformation to properly fit with the pharmacophore.[12] Such an extended

Received: September 9, 2011
Published: March 28, 2012

conformation was subsequently confirmed by structure-based studies, where docking simulations have shown that flexible molecules binding into the pore domain of hERG can adopt a conformation very similar to that reported in the model of Figure 1.[21−23] Furthermore, this model was also used to align a series of 31 hERG blockers to carry out a comparative molecular field analysis (CoMFA)[24] and to develop a 3D QSAR model for predicting hERG binding potencies of novel drug candidates.[12]

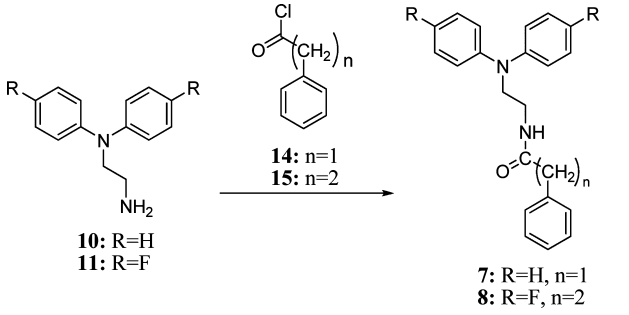
In the present study, we designed a small set of "minimally structured" molecules to fulfill the pharmacophoric hypothesis of Figure 1. Eight new derivatives were synthesized as reported in Schemes 1 and 2. Six of these molecules (**1**−**6**, Table 1)

Scheme 1[a]



[a]Reagents and conditions: $NaBH_3CN$, MeOH dry, room temperature for 7 days.

Scheme 2[a]



[a]Reagents and conditions: diisopropylethylamine, MeOH dry, 0° C for 4 h.

displayed the three aromatic moieties (C0, C1, C2) and the basic nitrogen atom (N), spatially arranged to fit the pharmacophoric scheme, whereas **7** and **8** carried a methanamide instead of the methylenamine group to evaluate the role of a positive charge on hERG block potency. The activity of the new compounds was predicted using the 3D QSAR equation derived from a CoMFA model, which represented an extension of that reported in 2002.[12] Finally, all molecules were tested to assess the hERG block activity using patch clamp experiments carried out in human embryonic kidney cells. The new compounds were active against hERG, and some of them turned out to be nanomolar inhibitors of this channel. This study confirms the pharmacophoric hypothesis of Figure 1 and further highlights the key role of the positively charged nitrogen for high hERG blocking potency. On this basis, we propose that three phenyl rings and one basic nitrogen, suitably spaced, represent the stereotype of a minimally structured potent hERG blocker.

RESULTS AND DISCUSSION

hERG Inhibitor Design. A small set of "minimally structured" hERG blockers was designed on the basis of the pharmacophoric model of Figure 1. Compounds **1** and **2** are diphenylpropanamines substituted by an ethyl- or a propyl-phenyl chain, respectively. In **3** and **4**, the methine carbon atom of the benzydryl moieties was replaced by a tertiary nitrogen atom. In addition, in **5** and **6**, a 4-4′di-fluoro substitution was introduced in the diarylamino moieties to modify the electronic features of the C0 and C1 aromatic rings, without significantly altering volume and lipophilicity of the molecules. Compounds **7** and **8** were the amide derivatives of **3** and **6** and were designed and synthesized to investigate the role of the positive charge on hERG block.

The rational design of **1**−**6** had also been strongly supported by known mutagenesis[25,26] and docking[23] studies showing that Tyr652 and Phe656 play a pivotal role in the hERG drug binding by promoting cation−π, π−π, and hydrophobic interactions with the basic nitrogen and aromatic rings of drugs.

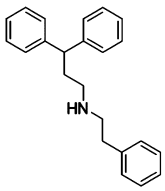
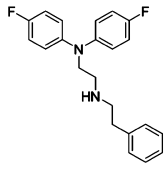
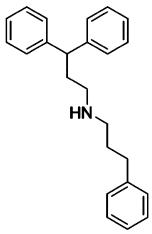
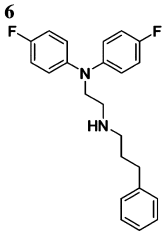
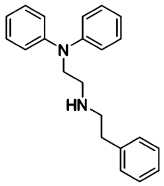
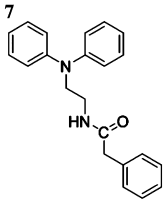
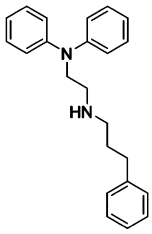
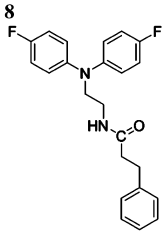
CoMFA Model. An extended CoMFA model for hERG K^+ channel block based on that previously developed in 2002[12] was constructed (see Supporting Information for computational details). The training set was composed of 75 (vs 31 of the 2002 model) hERG blockers selected from the literature as representative compounds of different therapeutic classes (antipsychotics, antihistamines, antibacterials, etc.) able to block hERG. These molecules showed a homogeneous distribution of drug activities within the pIC_{50} range 3.8−9.0. The CoMFA model was then used to support the above-reported design strategy and to help the definition of the proper length of the chain connecting N to C2. Finally, the model was utilized to predict the activity of **1**−**8** (Table 1). All the statistical parameters and the CoMFA contour maps are reported in Supporting Information.

Chemistry. **1**−**6** were synthesized in a parallel fashion, taking advantage of a reductive amination between the appropriate amines **9**−**11** and the commercial aldehydes **12**, **13** with $NaBH_3CN$ as reducing agent (Scheme 1; see Supporting Information for experimental details). **7** and **8** were obtained by a nucleophilic substitution between the amines **10**, **11** and the corresponding commercially available acyl chlorides **14**, **15** (Scheme 2; see Supporting Information for experimental details). The 3,3-diphenylpropan-1-amine **9** was commercially available. The N^1,N^1-diphenylethane-1,2-diamine **10** was prepared as described in the literature.[27] The N^1,N^1-bis(4-fluorophenyl)ethane-1,2-diamine **11** was obtained from hydrazine cleavage of phthalimidic derivative **17**, which was synthesized from *N*-(2-chloroethyl)-4-fluoro-*N*-(4-fluorophenyl)aniline **16**[28] through a nucleophilic substitution reaction with potassium phthalimide (Scheme S1 and Supporting Information for experimental details).

Biology. hERG K^+ current inhibition by **1**−**8** was determined from whole-cell voltage clamp recordings made in HEK cells stably expressing hERG channels (see Supporting Information for experimental details). The molecules all inhibited hERG currents in a concentration dependent manner. Inhibition was open state dependent, and repetitive pulsing to 0

dx.doi.org/10.1021/jm201194q | J. Med. Chem. 2012, 55, 4010−4014

Table 1. Experimental and Predicted hERG K^+ Channel Blocking Activity of 1−8

Compound	IC_{50} (nM)	pIC_{50exp}	pIC_{50pred}	Δ	Compound	IC_{50} (nM)	pIC_{50exp}	pIC_{50pred}	Δ
1 	2790	5.5 ± 0.06	6.3	-0.8	**5** 	44	7.4 ± 0.04	6.2	1.2
2 	17	7.8 ± 0.06	7.0	0.8	**6** 	2.4	8.6 ± 0.08	7.0	1.6
3 	62	7.2 ± 0.07	6.1	1.1	**7** 	7860	5.1 ± 0.07	5.1	0.0
4 	580	6.2 ± 0.15	6.9	-0.7	**8** 	192	6.7 ± 0.07	5.6	1.1

mV was required until steady-state inhibition was achieved (see Figure 2A for representative current traces). The mean concentration−response relationships for **1**−**6** and **7**, **8** are shown in parts B and C of Figure 2, respectively, and the IC_{50} values are reported in Table 1. In addition, Figure 2C shows the mean concentration−response relationships of **3** and **6** (in dashed lines) to compare hERG blocking activity with corresponding amide derivatives **7** and **8**, bearing methanamide instead of methylenamine. As predicted by the CoMFA model, the biological activities of the molecules were in the nanomolar to micromolar range.

Structure−Activity Relationships. Comparison of the biological activity of **1**, **2** and **5**, **6** showed the importance of the length of the N−C2 linker for potency, which is in agreement with in silico predictions. However, the same correlation could not be observed for **3** and **4**. Experimental data for **1**, **3**, and **5** also revealed that a positive contribution to the activity could be achieved by substituting the carbon between C0 and C1 with a tertiary nitrogen. Moreover, adding fluorine atoms at para positions of the C0 and C1 rings had a positive effect on the activity, probably as a consequence of their electronic effect on aromatic rings. In particular, the electron-withdrawal behavior of fluorines decreased the electron density of the phenyl groups, thus likely strengthening possible $\pi-\pi$ interactions with Tyr652 and Phe656. All these features (i.e., three methylenes between N and C2, and fluorine atoms on both C0 and C1) were present on **6**, which turned out to be the most potent hERG blocker of this series (IC_{50} = 2.4 nM) and one of the most potent hERG blockers ever reported in the literature.

To investigate the role of the positive charge on the hERG blocking activity, we designed and synthesized **7** and **8**, which represented the amide derivatives of **3** and **6**. While both compounds preserved a certain activity against hERG, the drop in potency of 2 orders of magnitude (7860 nM vs 62 nM for **7** vs **3**; 192 nM vs 2.4 nM for **8** vs **6**) clearly showed that, although not strictly necessary, the positive charge plays a major role in determining the overall potency of a compound toward hERG. In parallel, these data show that a possible way to lower the hERG blocking liability of a newly designed compound could be "switching off" a possible positive charge, located on the new molecule in the way reported in Figure 1. Notably, our CoMFA model nicely predicted the quite low pIC_{50} of **7**, while it underestimated the potency of **8** (see Table 1). This could be due to the underestimation of the role of the fluorine atoms and the linker (as occurred for **6**) and to the overestimation of the role of the positive charge, which was present on the most of the most potent derivatives of the training set. In addition, we observed that the activities of **1** and **4** were overestimated whereas **2**, **3**, **5**, **6**, and **8** were underestimated by the 3D QSAR, probably as a consequence of their "minimal" chemical structures. In fact, these new molecules lacked additional functional groups protruding into the CoMFA steric and electrostatic regions (see Supporting Information), despite a perfect fit with the pharmacophore of Figure 1.

dx.doi.org/10.1021/jm201194q | *J. Med. Chem.* 2012, 55, 4010−4014

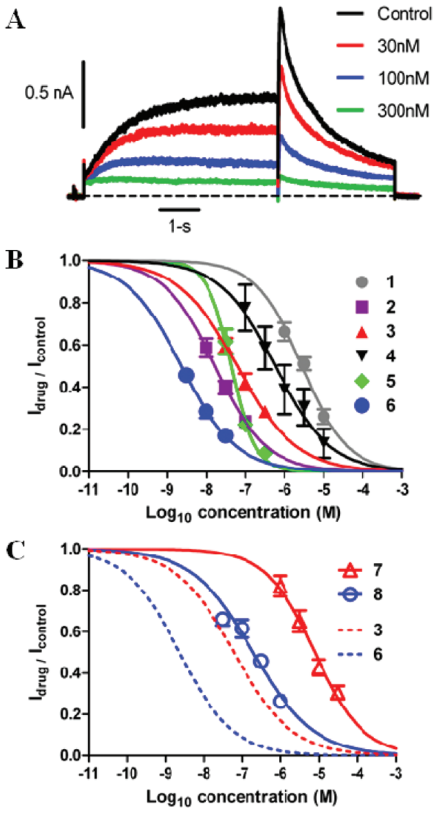


Figure 2. (A) Representative traces of hERG currents before (control) and after steady state inhibition by indicated concentrations of **5**. (B) Mean (±SEM.) concentration response relationships for inhibition of hERG currents by compounds **1**-**6** (n = 5 - 9 cells). Hill function fits of the data are shown by solid lines. (C) Mean ± SEM concentration–response relationships for inhibition of hERG currents by **7** and **8** with **3** and **6** (charged analogues) for comparison.

In Figure S1, the 3D structure of a low energy conformation of **2** was superimposed onto the pharmacophore model (Figure S1A) and the CoMFA contour maps (Figure S1B) are shown. It is readily apparent that **2** shows the minimal structural requirements for hERG block, as this molecule carries three phenyl rings and one basic nitrogen atom suitably spaced to fit C0, C1, C2, and N. Despite the CoMFA-based prediction pointing to this compound as a low micromolar blocker of hERG, **2** actually turned out to be a nanomolar inhibitor of this channel. Therefore, we can speculate that the pharmacophoric functions C0, C1, C2, and N are required for favorable interactions with hERG, while accessory groups, such as the fluorine atoms of **6**, can be important for increasing the hERG blocking potency. We also comment that the present CoMFA model was greatly influenced by the chemical nature of the training set, and therefore, it was only partially successful in predicting the hERG inhibition potencies of structurally different compounds, such as those here synthesized and tested. In addition, other physicochemical features, such as pK_a, membrane permeability, etc., which were not taken into account by the CoMFA model, can play a role in drugs applied to the extracellular side of the membrane binding to hERG. In fact, binding sites for hERG blockers have been mapped within the inner cavity of the channel, which drugs can only access from the intracellular side of the membrane.[29] Therefore, lipophilicity and the presence of formal charges are relevant features that can positively or negatively affect drug penetration into the cell and interaction with hERG.

In conclusion, by synthesizing and testing a small set of hERG blockers, designed on the basis of the pharmacophoric hypothesis of Figure 1, we demonstrate that three phenyl rings, suitably spaced, and one protonable nitrogen atom can represent the minimal structural requirements for high potency for hERG block.

■ ASSOCIATED CONTENT

Supporting Information

Computational details of the CoMFA model generation; experimental chemical procedures and compound characterization; details of patch clamp experiments. 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.

■ ACKNOWLEDGMENTS

The work has received funding from the European Community's Seventh Framework Programme (FP7/2007–2013) under Grant No. 241679 (ARITMO Project; L.C., M.Re.), Istituto Italiano di Tecnologia, Genova, Italy (Seed Project HERGSYM; R.B., C.I., M.M., M.Ro., M.Re.), and Alma Mater Studiorum, University of Bologna, Bologna, Italy.

We greatly thank Prof. Craig January (University of Wisconsin, U.S.) for the kind gift of HEK cells stably transfected with hERG.

■ ABBREVIATIONS USED

CHO, Chinese hamster ovary; LQTS, long-QT syndrome; pIC_{50}, negative logarithm of IC_{50}; pK_a, negative logarithm of K_a

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