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Pharmacophore-based search, synthesis, and biological evaluation of anthranilic amides as novel blockers of the Kv1.5 channel

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Abstract—The search for novel, potent Kv1.5 blockers based on an anthranilic amide scaffold employing a pharmacophore-based virtual screening approach is described. The synthesis and structure–activity relationships (SAR) with respect to inhibition of the Kv1.5 channel are discussed. The most potent compounds display sub-micromolar inhibition of Kv1.5 and no significant effect on the HERG channel. In addition, good oral bioavailability is demonstrated for compound **3i** in rats.

1. Introduction

Currently available drug treatment for atrial fibrillation (AF) is less than satisfactory and fraught with severe difficulties. The presently used antiarrhythmics of classes I (sodium channel blockers) and III (potassium channel blockers) can terminate AF and reduce its recurrence, but may increase mortality due to a variety of adverse effects, including the risk of potentially lethal ventricular proarrhythmia (CAST-trial,[1] SWORD-trial[2]). The gold standard of anti-arrhythmic therapy, amiodarone, a very unselective drug, is hampered by many noncardiac side effects.[3] Therefore, there is an unmet medical need for the development of safer and more efficient drugs for the treatment of atrial arrhythmias.[4]

Most of the available class III antiarrhythmics are blockers of IKr (e.g., dofetilide, ibutilide). The new antiarrhythmic agent azimilide is a combined blocker of IKr and IKs.[5] However, since IKr and IKs are both present in the atrium and ventricle, undesired ventricular effects may be observed for these drugs, which limit their use for the treatment of atrial arrhythmias. In contrast to IKr and IKs, the ultra-rapid delayed rectifier IKur, which is based on the Kv1.5 channel, has been found only in human atrial cells and not in the ventricles.[6] Therefore it is regarded as a promising target for the development of new atrial selective antiarrhythmics. Since then, many companies have filed patents claiming compounds as blockers of the Kv1.5 channel.[7] In addition, Aventis, Merck, and Nissan have published animal trial data supporting the use of this new approach but no clinical trials have yet been carried out to our knowledge.[7]

In the present study, we report the discovery and subsequent structure–activity investigation of novel anthranilic amides with favorable pharmacokinetic properties as blockers of the Kv1.5 channel.

2. Pharmacophore-based lead identification

So far, our efforts to identify Kv1.5 blockers have focused on two chemical series, namely the bisaryls and the *meta*-substituted benzene sulfonamides (Fig. 1).[8] Seven Kv1.5 blockers from the two series were selected

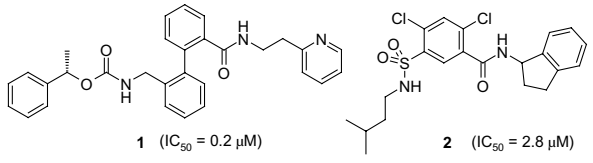

Figure 1. Representatives of the two compound series used for Kv1.5 pharmacophore modeling.

Keywords: Kv1.5 blockers; Anthranilic amides; Atrial fibrillation.
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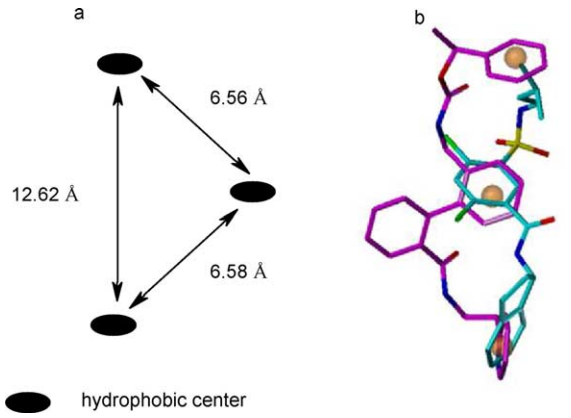


Figure 2. (a) Pharmacophore model, (b) matching of compound **1** and **2** with pharmacophore model.

to identify a pharmacophore model.[9] The derived pharmacophore consists of three hydrophobic centers in a triangular arrangement as depicted in Figure 2a. As illustrated in Figure 2b, one phenyl of the bisaryl core of compound **1** and the central phenyl moiety of compound **2** matches the middle hydrophobic center, while within each structure class, both ends of the side chains correspond to the remaining hydrophobic centers of the model. The model is consistent with the SAR previously observed for bisaryl Kv1.5 blockers.[8a] Furthermore, the pharmacophore model served as a query to screen an in-house data bank of 423 Kv1.5 blockers, which resulted in the retrieval of 58% of the known Kv1.5 blockers. This performance was deemed satisfactory for lead identification. Therefore, we ran a 3D search within the Aventis compound collection using this model as a query. This strategy resulted in 4234 virtual hits, 1975 compounds of them passed the sequence of filters described in Ref. 10. These 1975 compounds were submitted to a hierarchical cluster analysis resulting in 27 clusters. Representatives from 18 clusters were actually available for in vitro screening in *Xenopus* oocytes, which yielded one active compound with an IC_{50} of 5.6 μM[‡] (Fig. 3), belonging to a new class of Kv1.5 blockers. Although compound **3a** is less potent than the best representatives of the previously investigated chemical classes of Kv1.5 blockers, it exhibits a favorable pharmacokinetic profile. Therefore, a main goal was to improve in vitro potency of the anthranilic amides while keeping the pharmacokinetic profile of **3a**.

3. Chemistry

To systematically evaluate the structure–activity relationships in the boxed regions shown in Figure 3, we employed two different synthetic routes (Scheme 1). Compounds **3** were prepared from anthranilic acids **4** by conversion with sulfonyl chlorides, followed by activation of the acid functionality of **5** to the acid chloride

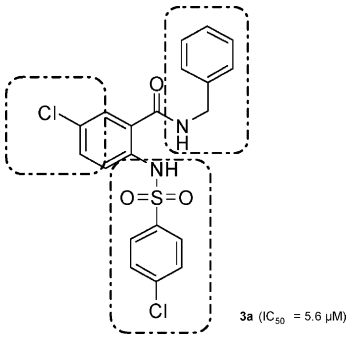
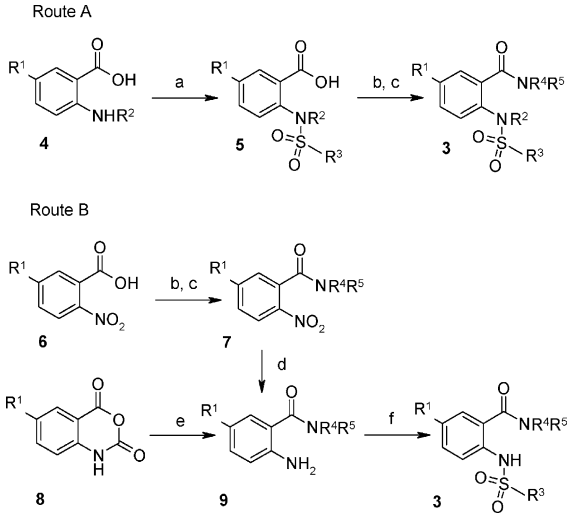


Figure 3. Chemical structure of the anthranilic amide lead **3a**.



Scheme 1. Reagents and conditions: (a) R^3SO_2Cl, Na_2CO_3, water, 60 °C; (b) $SOCl_2$, 60 °C; (c) HNR^4R^5, Et_3N, DCM, 20 °C; (d) H_2, Pd/C, THF/MeOH, 1 bar, 20 °C; (e) HNR^4R^5, DMF, 60 °C; (f) R^3SO_2Cl, pyridine, DCM, 20 °C.

and subsequent reaction with amines (route A). Requisite secondary amines, which were not commercially available, were obtained by reductive amination with $NaCNBH_4$ in methanol.[11] Whereas the route A described above, is ideal to probe the influence of the amine substituents R4, R5 as it is introduced in the last step, this is not true for R3. In order to rapidly evaluate substituent R3 we relied on route B where we used nitro benzoic acids **6** or anhydrides **8** and permutated the sulfonyl group R3 in the last step. Anhydrides **8** were opened in DMF with amines to yield anilines **9**. These intermediates could be also prepared by palladium-catalyzed hydrogenation of the nitro compounds **7**, which for their part were formed by coupling amines to the nitro benzoic acids **6** after activation with thionyl chloride. Finally, conversion of the anilines **9** with sulfonyl chlorides in the presence of pyridine afforded **3** (route B).

4. Biological results and discussion

As a first step in our investigation of the structural requirements for Kv1.5 inhibition we examined the role

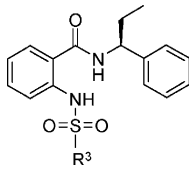
[‡] All IC_{50} values in this manuscript are determined in *Xenopus* oocytes expressing human Kv1.5 channels. Electrophysiology is described in detail in Ref. 8a.

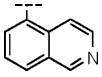
of the amide substituents (Table 1). In accordance with the pharmacophore model at least one hydro-phobic contact is needed. The distance of this hydrophobic moiety does matter for potency (**3b** vs **3c**) with an optimum for the phenyl group at the benzylic position. Further increase of activity is obtained by small α-substituents X (**3d**–**g**) with a preference for the (*S*)-stereochemistry at this position. Next to these secondary amides, tertiary amides were potent, which carried heterocyclic substituents (**3i**–**j**) or a cycloalkyl substituent (e.g., **3k**) in addition to the benzyl group. Smaller substituents such as the methyl in compounds **3l** or an end group with an additional linker as in **3m** result in a decrease of potency. The benzyl group can be readily replaced by substituents containing heterocycles (**3n**,**o**) without significant loss of activity, which can be used for fine tuning physicochemical and ADME properties.

We next turned our attention to the influence of the sulfonyl substituent R3 on the activity (Table 2). In addition to our *p*-toluyl probe from Table 1, the *p*-methoxyphenyl (**3p**) and 8-quinolinyl (**3q**) substituents turned out to be excellent moieties. Thereby, the nitrogen and its position proved critical as all other related substituents R3 (**3r**–**t**) gave rise to compounds with diminished activity. However, the aryl group can be replaced successfully by an aliphatic group, isopentyl (**3u**) being the among the best. Reduced steric bulk at this position resulted in lower potency as seen by comparison of compounds **3u**–**w**.

Finally, the findings, which were obtained with various R1 substituents indicate that this residue influences the inhibitory activity by steric and electronic factors: Increasing its size from methoxy (**3x**) to acetyl (**3ac**) and larger ether residues (**3ad**,**ae**) has a clearly detrimental effect on the potency (Table 3). If a basic nitrogen is incorporated into this site a further loss in activity is observed (**3af**). On the other hand, R1 plays together with substituent Z an electronic role by determining the acidity of the sulfonamide functionality. Remarkably, the inhibitory activity showed a correlation with the pKa values of the compounds. A pKa difference of 1.3 (**3x** vs **3ab**) results in an eightfold drop in potency. Replacement of the acidic hydrogen by a methyl group (**3ag**) resulted in further reduction of activity. These results suggest that the hydrogen might be involved in

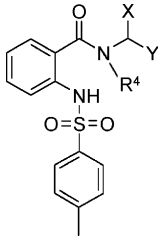
Table 2. Inhibitory activity of anthranilic amides against the Kv1.5 channel



Compound	R3	IC_{50} [μM]
3p	*p*-Methoxyphenyl	0.6
3q		0.6
3r	1-Naphtyl	3.5
3s		9.0
3t	3-Pyridyl	36%@10μM[a]
3u		1.7
3v	Propyl	3.1
3w	Ethyl	10

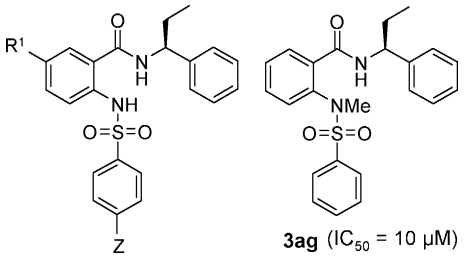
[a] Percent inhibition at 10 μM concentration.

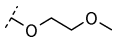
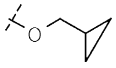
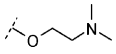
Table 1. Inhibitory activity of anthranilic amides against the Kv1.5 channel



Compound	R4	X	Y	IC_{50} [μM]
3b	H	H	CH_2Ph	6.7
3c	H	H	Ph	4.1
3d	H	*rac*-Me	Ph	3.2
3e	H	*S*-Et	Ph	0.7
3f	H	*R*-Et	Ph	2.7
3g	H	*rac*-Cyclopropyl	Ph	1.6
3h	CH_2Ph	H	3-Pyrazolyl	0.5
3i	CH_2Ph	H	3-Pyridyl	0.7
3j	CH_2Ph	H	2-(N-Me)-imidazolyl	0.7
3k	CH_2Ph	H	Cyclopropyl	1.9
3l	CH_2Ph	H	Methyl	5.0
3m	CH_2Ph	H	CH_2CH_2(1-imidazolyl)	8.5
3n	CH_2(3-Me-pyridyl)	H	3-Pyridyl	2.2
3o	CH_2(2-furanyl)	H	2-(N-Me)-imidazolyl	1.2

Table 3. Inhibitory activity of anthranilic amides against the Kv1.5 channel



Compound	R1	Z	pKa	IC_{50} [μM]
3x	OMe	Me	7.5	0.5
3y	H	Me	7.2	0.7
3z	H	H	6.9	1.3
3aa	F	Cl	6.5	3.8
3ab	H	CF_3	6.2	4.0
3ac	Acetyl	Me	—	3.8
3ad		Me	—	46%@10μM[a]
3ae		Me	—	41%@10μM[a]
3af		Me	—	15%@10μM[a]

[a] Percent inhibition at 10 μM concentration.

an intramolecular hydrogen bond with the carbonyl oxygen, which stabilizes a favorable active conformation.

To be considered as safe antiarrhythmics selectivity toward the HERG channel is mandatory. As could be shown with lead **3a** and compounds **3i**, **3n**, **3q**, and **3v**, there was no significant effect on the IKr current: 6%, 11%, 14%, 2%, and 1% inhibition at 10 μM concentration, respectively. Finally, for compound **3i** oral bioavailability in rats of 43% was found.

In conclusion, we have discovered and optimized a new class of potent Kv1.5 blockers based on a simple anthranilic acid scaffold. The compounds show no significant effect on the IKr current and are orally bioavailable, and are therefore promising drug substances for a new and safe treatment of atrial fibrillation.

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9. The conformational space of the seven Kv1.5 blockers selected as training set for pharmacophore elucidation was explored, using a Monte Carlo Multiple Minimum (MCMM) search protocol. This search protocol involved the generation of maximum 5000 conformers by random modifications of minimum 2 and maximum 7 torsion angles at a time. Each generated conformer was submitted to 250 steps of conjugate gradient minimization. Then, we discarded strained (more than 50 kJ mol^{-1} above the minimum) as well as duplicate conformations (distance cut off of 0.25 Å between equivalent heavy atoms). Conformers passing these filters were minimized until convergence (convergence criterion 0.05 kJ $Å^{-1}$ mol^{-1}). The energy was evaluated using the MacroModel 6.5 (www.schroedinger.com) implementation of the OPLS All–Atom force field. All the calculations were carried out in water, as modeled by the generalized Born/solvent accessible surface solvation model. The next step was to select representative sets of conformers, using hierarchical clustering on distances between all the potential pharmacophoric centers. Within each cluster, we chose the minimum energy conformation as input for the automated

pharmacophore elucidation tool, DISCO (www.tripos.com). After visual inspection of the DISCO solutions, one of them was selected as query for 3D database searches with UNITY (www.tripos.com).
10. Compounds with the following features were removed from the 3D search hit list: (a) compounds with reactive moieties or groups known to be detrimental for Kv1.5 activity; (b) known Kv1.5 blockers; (c) molecular weight outside the 200–500 range, hydrophilic solvent-accessible surface area greater than 120 $Å^2$, calculated logPoctanol/water outside the −2.0–5.0 range. QikProp 1.5 (www.schroedinger.com) was used to compute the physical properties.
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