Design and Synthesis of Novel Isoquinoline-3-nitriles as Orally Bioavailable Kv1.5 Antagonists for the Treatment of Atrial Fibrillation

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Abstract: Novel 3-cyanoisoquinoline Kv1.5 antagonists have been prepared and evaluated in in vitro and in vivo assays for inhibition of the Kv1.5 potassium channel and its associated cardiac potassium current, I_{Kur}. Structural modifications of isoquinolinone lead **1** afforded compounds with excellent potency, selectivity, and oral bioavailability.

Atrial fibrillation (AF) is the most common sustained arrhythmia observed in clinical practice. The prevalence of AF is rising as the population ages, and the disease has been identified as a major contributor to stroke and resultant morbidities and mortalities.[1] Pharmacological approaches to AF treatment have centered on ion channel blockers, as reentrant electrical excitation appears to play a major role in disease physiology.[2] Currently available antiarrhythmic drugs inhibit multiple ion channels and therefore exhibit frequent adverse affects, including potentially lethal ventricular proarrhythmia.[3] This limitation has led to a recent focus on "atrial selective" therapy as a potentially safer treatment for AF.[4]

The ultrarapid delayed rectifier potassium current I_{Kur}, which is observed in the human atrium but not in ventricle,[5] has emerged as a target for atrial selective therapy. The voltage gated Kv1.5 channel is responsible for I_{Kur} and is also more prevalent in human atrium than ventricle.[6] I_{Kur} is a component of the repolarization phase of the atrial action potential, and its inhibition is known to effect prolongation of the atrial refractory period and consequent restoration of normal sinus rhythm to arrhythmic tissue. Thus, selective blockade of Kv1.5 may result in termination and/or prevention of AF without exerting undesired ventricular effects. Recent publications, most notably from researchers at Sanofi-Aventis, have detailed the effects of selective Kv1.5 antagonists in animal models of arrhythmia.[7,8] Our efforts in this area have focused on the development of compounds suitable for chronic oral dosing that would represent a viable approach to prevention of AF recurrence in patients. Here, we detail the discovery of a novel series of orally bioavailable isoquinoline Kv1.5 blockers that have evolved via optimization of isoquinolinone **1** (ISQ-1, Figure 1).[7,9]

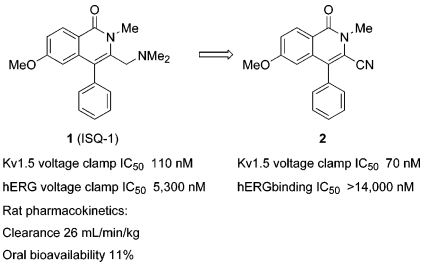

Figure 1. Profiles of isoquinolinones **1** and **2**.

Screening efforts identified compound **1** as a potent Kv1.5 antagonist[10] with moderate plasma clearance and low bioavailability in rat pharmacokinetic experiments. Of primary concern was the moderate selectivity observed with respect to the human ether-a-go-go-related gene (hERG) channel as measured by voltage clamp experiments (Figure 1). hERG and the I_{Kr} current that it mediates are present in human atrium and ventricle. Because the proarrhythmic potential of existing antiarrhythmic drugs is chiefly due to their high potency against I_{Kr},[11] high selectivity for Kv1.5 over hERG was considered a key feature for atrial selective therapy. Structure−activity relationships (SAR) in this series suggested that the dimethylaminomethyl moiety was associated with the undesired hERG blockade by **1**, and synthetic modifications identified replacement of this functional group with a cyano substituent as a viable strategy for increasing selectivity over hERG.

Accordingly, cyanoisoquinolinone **2** (Figure 1) exhibited Kv1.5 potency similar to that of **1** and no measurable activity in a hERG binding assay.[12] An effort to expand the SAR around this lead found that substitutions and replacements of the phenyl substituent did not improve potency or physical properties nor did alterations of the substitution pattern of the fused aryl region. The best opportunity for alterations of potency and physical properties was found in the modification of the *N*-methyl group of **2**. Because of the empirically observed low solubility of **2**, which limited its use in animal experiments, polar groups were incorporated with the aim of decreasing overall compound lipophilicity. Synthesis of these compounds proceeded as outlined in Scheme 1.

Ortho-lithiation of 4-methoxybenzoic acid (**3**) and treatment with ethyl benzoate or methyl 3-fluorobenzoate provided addition products **4a**,**b** without recourse to protection of the carboxylic acid moiety.[13] Thionyl chloride treatment gave **5a**,**b**, which were treated with allylaminoacetonitrile[14] to provide the corresponding amide derivatives.[15] Treatment with sodium methoxide resulted in cyclization to provide key isoquinolinone intermediates **6a**,**b**. Osmium-catalyzed dihydroxylation and periodate cleavage of **6b** provided aldehyde **7**, which underwent reductive transformations under standard conditions to give adducts **8c** and **8d**. Alternatively, dihydroxylation of **6a**,**b** gave diols **8a**,**b**, which could be transformed via standard protecting group manipulations and displacement chemistry to amines such as **8e**. **8a** was also readily resolved by chiral HPLC to provide enantiomerically pure diols **8f** and **8g**. Use of alternatively substituted benzoate electrophiles in the first step of this sequence provided analogues such as **8h** (Table 1).

Evaluation of compound potency was aided by the use of high-throughput patch clamp (HT-clamp) measurements. This technology provided determinations of potency versus the Kv1.5 channel with throughput many times that possible using

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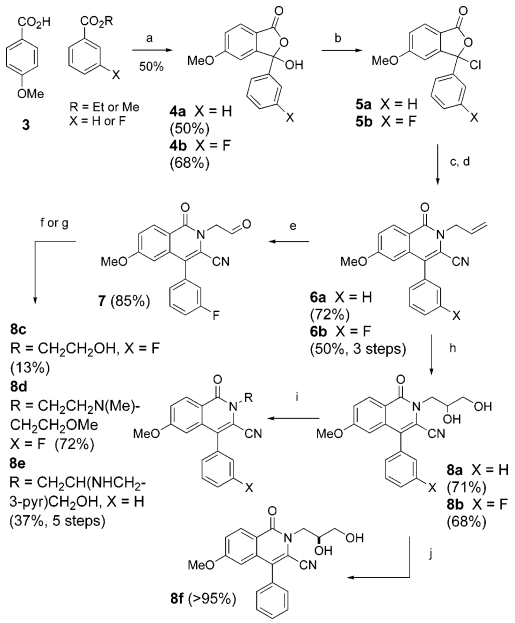
Scheme 1[a]



[a] Conditions: (a) *sec*-butyllithium, tetramethylethlyenediamine, tetrahydrofuran, −78 °C; (b) thionyl chloride, catalytic dimethylformamide, 1,2-dichloroethane, reflux; (c) allylaminoacetonitrile, 2,6-lutidine, toluene, reflux; (d) sodium methoxide, methanol; (e) osmium tetroxide, *N*-methylmorpholine *N*-oxide, acetone/water, then sodium periodate; (f) sodium borohydride, methanol to give **8c**; (g) *N*-methyl-2-methoxyethylamine, sodium triacetoxyborohydride, 1,2-dichloroethane to give **8d**; (h) osmium tetroxide, *N*-methylmorpholine *N*-oxide, acetone/water; (i) (1) *tert*-butyldiphenylsilyl chloride, triethylamine, dimethylaminopyridine, dichloromethane, (2) methanesulfonyl chloride, triethylamine, dichloromethane, (3) sodium azide, dimethylformamide, (4) hydrogen, 10% palladium on carbon, ethyl acetate, 55 psi, (5) 3-pyridinecarboxaldehyde, sodium triacetoxyborohydride, 1,2-dichloroethane to give **8e**; (j) ChiralPak AD HPLC separation.

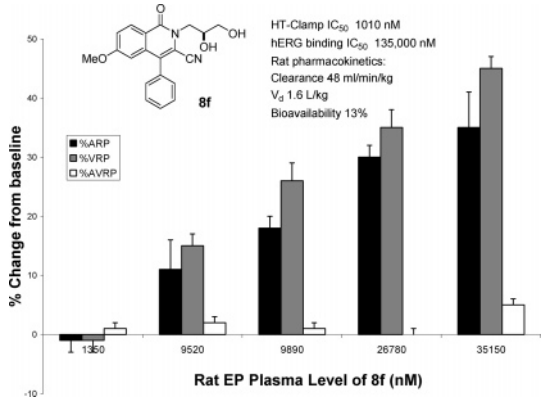


Figure 2. In vivo and in vitro profile of **8f**.

traditional patch clamp methods.[16] Data obtained using this assay[17] indicated that, while incorporation of polar groups could reduce Kv1.5 antagonist potency significantly, a variety of compounds retained appreciable potency and good selectivity versus hERG (Table 1). Hydroxyl groups proved particularly advantageous as a means of incorporating polarity without increasing undesirable hERG binding activity (in contrast to basic amines such as **8d**).

Rat pharmacokinetic profiling identified enantiomerically pure diol **8f** as a compound with moderate plasma clearance (Figure 2) and suitable physical properties for evaluation in a rat in vivo experiment. Anesthetized rat electrophysiology[18] was employed to characterize the cardiac electrophysiological effects of **8f**. Infusion of the compound to surgically instrumented rats provided measurements of effects on the atrial refractory period

Table 1. Heteroatom-Containing Isoquinolinone N-Substituents

	Ar	R	HT-Clamp IC_{50} (nM)[a,b]	hERG IC_{50} (nM)[c]
8b			870	33,500
8c			690	62,000
8d			790	1,650
8e			120	10,000
8f			1010	135,000
8g			1050	76,000
8h			250	16,000

[a] Determined according to ref 16. Values were determined from 10-point dose response curves, $n = 3-4$ cells per point. See Supporting Information for assay protocol and precision assessment. [b] Compound **1** was employed as a positive control; HT-clamp IC_{50} = 190 nM. [c] See ref 12.

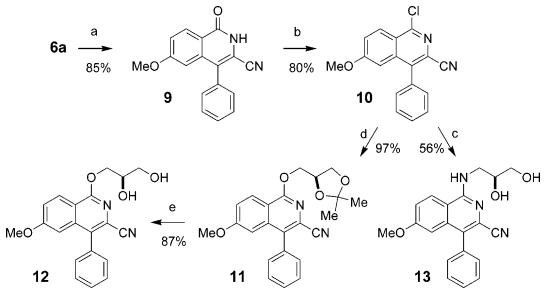
(ARP), ventricular refractory period (VRP), and a variety of other cardiac intervals. Importantly, I_{Kur} is known to function in rat atrium *and* ventricle; thus, the rat model provided a measure of in vivo efficacy but no direct measure of selectivity. Infusion of **8f** produced a distinct increase in ARP and VRP with no effects on cardiac conduction (Figure 2). At the highest dose administered ([plasma] > 35 μM), the compound did not affect AV node refractoriness (AVRP), a parameter that is significantly increased in this model by I_{Kr} blockers.[18]

Given this desirable in vivo profile, we sought to identify more potent Kv1.5 antagonists based on the dihydroxypropyl structural motif. A significant advance with regard to potency and pharmacokinetic properties was realized with the synthesis of isoquinoline **12** (Scheme 2), the result of a formal migration of the dihydroxypropyl group. Isoquinolines could be synthesized in a straightforward manner from isoquinolinone **9**, prepared by palladium-catalyzed reductive deallylation of **6a**.[19] Treatment with phosphorus oxychloride provided chloroisoquinoline **10**, which served as a precursor to alkoxy- and amino-substituted isoquinolines. Reaction of **10** and the sodium alkoxide of (*S*)-solketal provided adduct **11**. Brief exposure of **11** to acid gave diol **12**.[20] Addition of aminopropane-2,3-diol to **10** proceeded under microwave-assisted conditions to afford **13**. Both **12** and **13** were potent Kv1.5 antagonists with good selectivity versus hERG. We were pleased to find that **12** and **13** each exhibited substantially reduced plasma clearance and markedly improved bioavailability relative to **8f** when dosed to rats (Figure 3).

Rat electrophysiology studies with **12** and **13** revealed unexpected and undesirable effects on cardiac parameters (Figure 3). Infusion of each compound *decreased* ARP. Prolongation of VRP was observed, and in the case of **13**, AVRP was also increased significantly at high plasma levels. While the cause of this anomalous cardiac profile was unknown, this undesirable effect was consistently observed in the rat model for a variety of additional analogues containing the vicinal dihydroxypropyl moiety. Subsequent optimization efforts therefore focused on replacement of the dihydroxypropyl functionality.

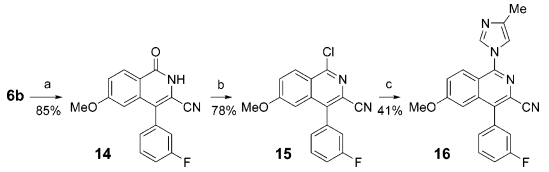
Chloroisoquinoline **15** was therefore prepared from **6b** via the established deallylation and chlorination procedures (Scheme 3). Addition of nitrogen-containing heterocycles to **15** using the

Scheme 2[a]



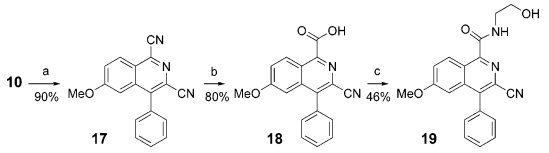
[a] Conditions: (a) triethylamine, formic acid, tetrakis(triphenylphosphine)-palladium(0), dioxane, 100 °C; (b) phosphorus oxychloride, 90 °C; (c) (*S*)-3-amino-1,2-propanediol, *n*-butanol, microwave, 210 °C, 1 h; (d) (*S*)-solketal, sodium hydride, THF, 66 °C; (e) concentrated hydrochloric acid, tetrahydrofuran, 0 °C, 10 min.

Scheme 3[a]



[a] Conditions: (a) triethylamine, formic acid, tetrakis(triphenylphosphine)-palladium(0), dioxane, 100 °C; (b) phosphorus oxychloride, 90 °C; (c) 4-methylimidazole, *n*-butanol, microwave, 200 °C, 3 h.

Scheme 4[a]



[a] Conditions: (a) zinc cyanide, zinc powder, tris(dibenzylideneacetone)-dipalladium(0), dimethylacetamide, 120 °C; (b) sodium hydroxide, ethanol/water, 40 °C, then HCl, 100 °C; (c) ethanolamine, *N*-(3-dimethylamino-propyl)-*N*′-ethylcarbodiimide hydrochloride, 7-aza-1-hydroxybenzotriazole, diisopropylethylamine, dimethylformamide.

aforementioned microwave-assisted conditions was employed to access a variety of isoquinolines with improved in vivo profiles. Imidazole **16** was typical of this class, exhibiting the desired ARP prolongation upon infusion in the rat EP model (Figure 4). No effects on other cardiac parameters were observed.

Extensive variation of the nitrogen-containing heterocycle component of **16** did not identify compounds with improved potencies or pharmacokinetic profiles. Improvements were eventually realized by substitution of the isoquinoline 1-position with carboxyl derivatives. Palladium-catalyzed cyanation[21] of **10** (Scheme 4) could be employed to obtain bis-cyano intermediate **17**. Key to further elaboration was the discovery that **17** could be regioselectively hydrolyzed to provide carboxylic acid **18**. Peptide coupling chemistry then produced carboxamido-substituted isoquinolines typified by ethanolamide **19**.

Ethanolamide **19** exhibited improved potency, excellent selectivity versus hERG, and good pharmacokinetic properties (Figure 5). Rat EP experiments confirmed that the compound potently increased ARP without significant effects on AVRP (Supporting Information).

To more definitively assess the potential for atrial selectivity in this series, **19** was evaluated in a canine electrophysiological model.[22] Kv1.5 and its associated I_{Kur} current figure prominently in canine atrial repolarization,[23] and anesthetized canine electrophysiology experiments with Kv1.5 blockers have demonstrated

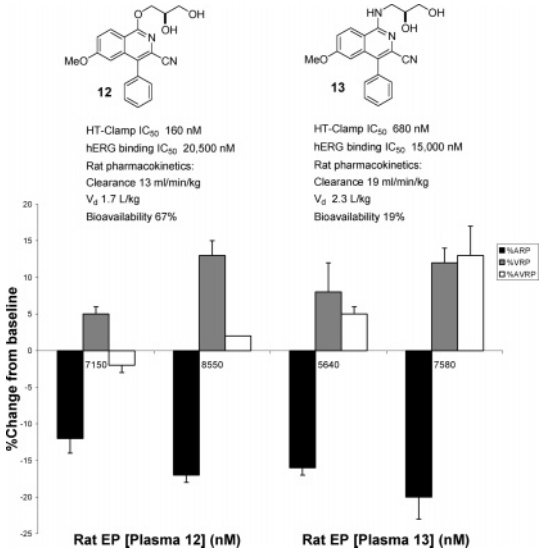


Figure 3. In vivo and in vitro profiles of **12** and **13**.

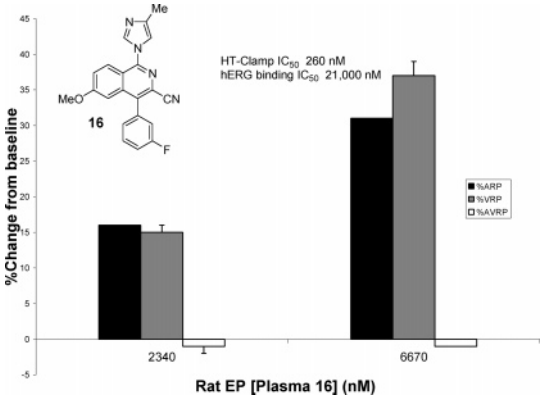


Figure 4. In vitro and in vivo profile of **16**.

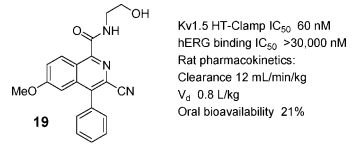


Figure 5. In vitro and in vivo characterization of **19**.

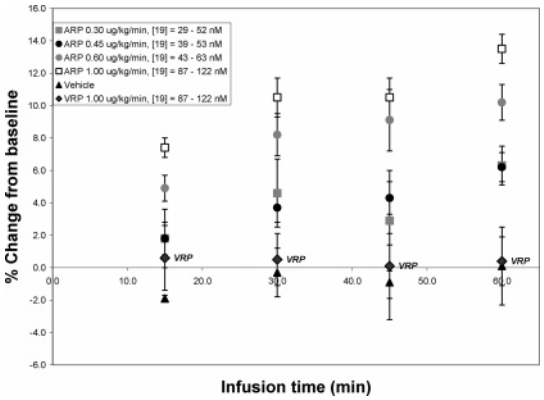


Figure 6. Effects on ARP and VRP upon infusion of **19** to anesthetized dogs. Infusion regimens and associated plasma levels are noted in the inset.

ARP prolongation without concomitant VRP prolongation.[24] Consistent with these observations, administration of compound **19** to anesthetized dogs (Figure 6) selectively prolonged ARP (plasma $EC_{10} \approx 110$ nM). No effect on VRP was observed at

any dose (data for highest dose shown, Figure 6). The combination of pharmacokinetic properties and in vivo effects observed establish **19** as a promising atrial-selective agent.

In conclusion, when a high-throughput patch clamp assay was used to guide compound design, lead optimization provided compounds that were efficacious in rat EP experiments. These rodent experiments evaluated multiple cardiac parameters and exposed undesirable in vivo effects of key lead compounds. Given its low compound requirements and fast turnaround times, the rat EP model served as an effective method for identifying off-target effects and eliminating these effects without resort to extensive in vitro counterscreening. Guidance of structural optimization by rat EP provided compounds with good potency, selectivity, pharmacokinetic properties, and in vivo efficacy. Compound **19** represents a potent Kv1.5 antagonist with a promising pharmacokinetic profile that has demonstrated the ability to selectively increase ARP in canine electrophysiological experiments.

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Supporting Information Available: Spectral and analytical data for new compounds, one scheme detailing the preparation of **2**, rat EP data for **19**, and the HT-clamp assay protocol. This material is available free of charge via the Internet at http://pubs.acs.org.

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