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Potent antagonists of the Kv1.5 potassium channel: Synthesis and evaluation of analogous *N*,*N*-diisopropyl-2-(pyridine-3-yl)acetamides

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Abstract—This letter describes the discovery of a novel series of potent Kv1.5 ion channel antagonists based on a diisopropyl amide scaffold. Structure–activity relationships of functionalized analogs are discussed. Key compound 1-(3-(diisopropylcarbamoyl)-2-phenyl-3-(pyridin-3-yl)propyl)-3-(2-fluorobenzyl)urea (**10**) exhibits significant atrial-selective effects in an in vivo model.

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia in clinical practice, and is implicated in approximately 15% (>80,000 per year) of all strokes in the United States.[1] Intervention with non-selective ion channel blockers can be effective in treating and preventing arrhythmias, but at present, available drug therapies carry a significant risk of potentially lethal ventricular proarrhythmia.[2] In an effort to minimize the risk of proarrhythmia, more selective treatments are being pursued which do not act on the ventricle.

A promising therapeutic approach is to selectively block the ultra-rapid delayed rectifier potassium ion current (I_{Kur}), thereby delaying atrial repolarization and converting AF to normal sinus rhythm. The I_{Kur} current has been observed in human atrial, but not ventricular myocytes. This difference presents the possibility of selectively targeting atria.[3]

Herein, we describe our efforts to identify a selective antagonist of Kv1.5, the molecular ion channel correlate of I_{Kur}, that delays atrial repolarization in vivo. Other members of the Kv1.x subfamily of potassium channels are expressed in the central nervous system (CNS), where their function is believed to be unrelated to cardiac currents. In an effort to reduce brain exposure and minimize the possibility of antagonizing CNS Kv1.x channels, we sought inhibitors which were substrates for human P-glycoprotein (P-gp), the most well-characterized transporter that pumps xenobiotics out of the CNS across the blood/brain barrier.[4]

High-throughput screening of the Merck sample collection identified methyl 4-(diisopropylcarbamoyl)-3,4-diphenylbutanoate (**1**)[5] as a potent inhibitor of Kv1.5 (IC_{50} = 252 nM),[6] although in vivo clearance was high in dog (43 ml/min/kg). This compound is structurally distinct from two recently disclosed cardiac ion channel antagonists currently under investigation (Fig. 1).[7]

The general synthesis of compounds in the diisopropyl amide series is represented in Scheme 1. After

Keywords: Arrhythmia; Atrial fibrillation; Potassium channel; Kv1.5; I_{Kur}.
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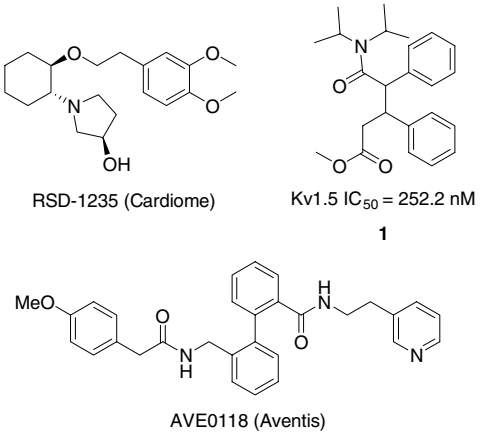


Figure 1. Cardiac ion channel antagonists.

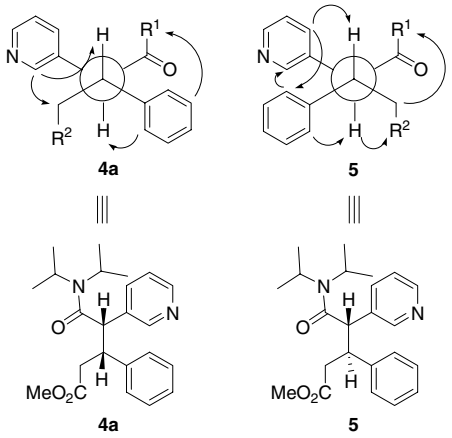


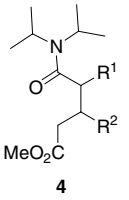
Figure 2. Relative stereochemistry of 1,4-addition reaction products; arrows indicate diagnostic NOE correlations. R^1 = diisopropyl amine, $R^2 = CO_2CH_3$. The reaction temperature for the 1,4-addition was −78 °C for **4a** and 0 °C for structure **5**.

straightforward preparation of **3** via an amide coupling of an arylacetic acid with diisopropyl amine, deprotonation and treatment with β-aryl vinyl esters generated 1,4-addition products **4**. Competing 1,2-addition products were not observed.[8]

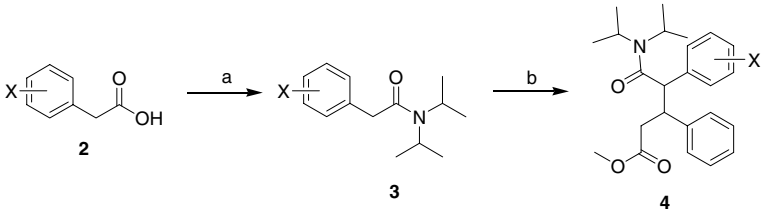
It is noteworthy that the 1,4-addition exhibits temperature-dependent diastereoselectivity in the absence of additional cation solvating agents.[9] At −78 °C, a single racemic diastereomer of **4** is generated while at higher temperatures (0 °C) the alternative isomer is formed selectively. It has been reported that E-enolates give rise to *syn* conjugate addition products, while the Z-enolates give *anti* conjugate addition products.[10,11] Assignment of relative stereochemistry was carried out spectroscopically for **4a** and **5** (Fig. 2) on the basis of vicinal benzylic proton coupling constants and ROESY correlations.

The selective 1,4-addition provided a convenient route to the diastereomeric racemates **4a** and **5**, and high selectivity was also observed across a range of arylacetamide and cinnamate substrates. Generally, isomers formed at low temperature showed increased Kv1.5 potency compared with their analogs formed at 0 °C. For example, **4a** (Kv1.5 IC_{50} 265 nM) exhibits 10-fold higher potency than **5** (Kv1.5 IC_{50} 3698.0 nM). Although the magnitude of this difference in potency varied across the series, generally the low-temperature diastereomers were more potent.

Utilizing this methodology, attention was focused on exploring the SAR of the two aryl rings in **4** (Table 1; data for the more potent diastereomer are presented). Replacement of either of the phenyl rings with a 3-pyridine was tolerated (see **4a** and **4b**), and incorporation of other pyridine isomers resulted in a ca. 20-fold decrease in potency (**4c**–**4f**). Compounds containing two pyridine rings, including those in which one 3-pyridine ring is retained, lost potency versus the 3-pyridyl, phenyl analogs (compare **4a** and **4g**/**4h**). Substituent effects were also briefly investigated: 3-bromophenyl analogs **4i** and **4k** exhibited 2-fold greater potency than the parent phenyls **4a** and **4b**. The corresponding nitriles, **4j** and **4l**, were more potent and less potent, respectively, than **4a** and **4b**. The 4-cyanophenyl analog **4m** was slightly more active than the parent phenyl, and the 2-cyano substituent (**4n**) was not well tolerated. Additionally, 2-biaryls such as **4o** exhibit improved potency versus Kv1.5.

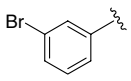
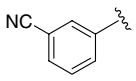
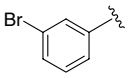
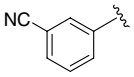
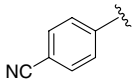
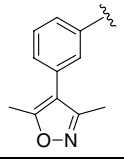


Having established that polar groups could be incorporated into the two aryl rings of **4**, a search for replacements of the diisopropyl amide was undertaken. Despite significant attempts, no potent replacement was identified, and the diisopropylamide moiety was found to be an essential component for potent Kv1.5 activity.



Scheme 1. Reagents and conditions: (a) EDC, HOBt, Et_3N, diisopropylamine, rt, 18 h; (b) methyl cinnamate, LDA, THF, 0 ° or −78 °C, 2 h.

Table 1. Kv1.5 antagonist SAR of biaryl diisopropylamides **4**

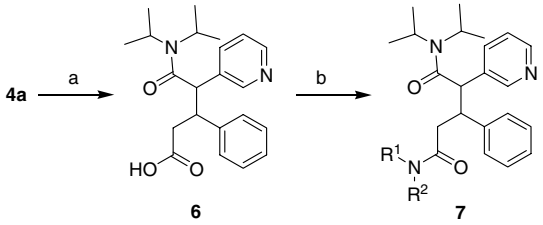
Compound	R^1	R^2	Kv1.5 IC_{50}[a] (nM)
4a			265
4b			266
4c			13760
4d			5323
4e[b]			6348
4f			6868
4g			708
4h			3836
4i			137
4j			184
4k			147
4l			326
4m			215
4n			1773
4o			113

[a] Values represent IC_{50} for more potent diastereomer, determined in quadruplicate.

[b] Compound **4e** was evaluated as a 2.5:1 mixture of diastereomers.

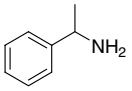
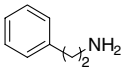
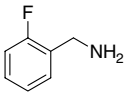
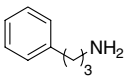
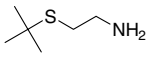
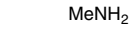
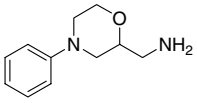
Subsequently maintaining the upper portion of the scaffold, we addressed the metabolically labile ester functionality within the 2-pyridin-3-yl series. Amide analogs were prepared via saponification (NaOH) of the methyl ester **4a** and the coupling of carboxylic acid intermediate **6** with various amines (Scheme 2).

While simple amides exhibited moderate Kv1.5 antagonist activity (**7f**, Table 2), larger groups, benzylic amines



Scheme 2. Reagents and conditions: (a) EtOH, NaOH (1 N aqueous), rt 24 h, quantitative; (b) NHR^1R^2, PS-DCC, HOBt or HOAt, Hunig's base, DCM, rt, 18 h.

Table 2. Inhibition activity of 5-amides **7**

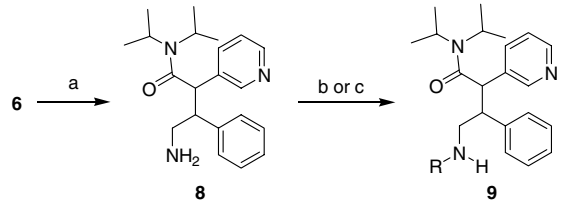
Compound	HNR^1R^2	Kv1.5 IC_{50}[a] (nM)
7a		249
7b		410
7c		432
7d		589
7e		1594
7f		2669
7g		3379

[a] Values represent IC_{50} for more potent diastereomer, determined in quadruplicate.

in particular, conferred potency equivalent to the methyl ester. The branched α-methyl benzylamine **7a** was the most potent of the series and, with the exception of **7e** and **7f**, all compounds with $IC_{50} < 5000$ nM contained one or more saturated carbon–carbon bonds linking a substituted benzene ring. While potent, the representative amides from the series did not meet the minimal P-gp susceptibility criteria (BA/AB ratio <3).

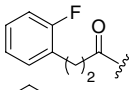
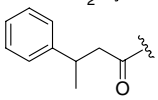
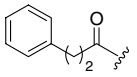
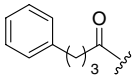
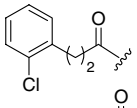
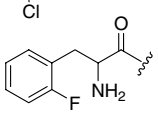
In hopes of conferring P-gp susceptibility, reversed amides were prepared via Curtius rearrangement of **6** (DPPA) in the presence of triethylamine in *tert*-butanol.[12] The *tert*-butoxycarbonyl protected Curtius rearrangement product was isolated in 81% yield, deprotected (TFA) to give **8**, and coupled with carboxylic acids to give the reversed amides **9** (Scheme 3).

The 5-reversed amides exhibited significant improvements in Kv1.5 potency (Table 3). Homologous reversal of the amide **7c** resulted in **9a**, the most potent compound identified in this series to date ($IC_{50} = 36$ nM). A comparison of **9c** and analog **7b** emphasizes the



Scheme 3. Reagents and conditions: (a) i—DPPA, Et_3N, *tert*-butyl alcohol, reflux, 24 h, 81%; ii—TFA, DCM, quantitative; (b) carboxylic acid, PS-DCC, HOBt or HOAt, DIPEA, DCM, rt, 18 h.; (c) isocyanate, Et_3N, DCM, rt, 30 min.

Table 3. Inhibition activity of 5-reversed amides **9a–f**

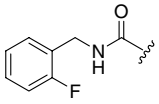
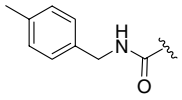
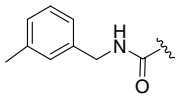
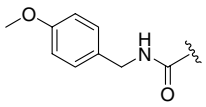
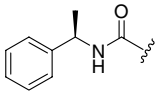
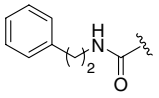
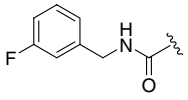
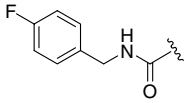
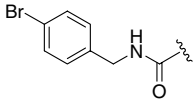
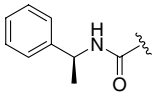
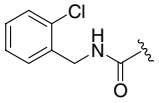
Compound	R	Kv1.5 IC_{50}[a] (nM)
9a		36
9b		94
9c		100
9d		250
9e		498
9f		1310

[a] Values represent IC_{50} for more potent diastereomer, determined in quadruplicate.

importance of the amide orientation, and reversed amides generally gain 2- to 10-fold in activity. Despite its high potency, compound **9a** was not a P-gp substrate (BA/AB ratio <3) and was not further pursued.

It has been noted that P-gp susceptibility depends on a number of factors, including increased polar functionality and/or the availability of hydrogen-bond donating groups on substrates. Because reversed amides were so well tolerated with regard to Kv1.5 activity, it was reasoned that incorporation of an additional H-bond donor would not affect potency and could generate a P-gp substrate. Preparation of these analogs was straightforward from amine **8** via reaction with the appropriate isocyanate (Scheme 3). As we had hoped, adding polar functionality did confer P-gp susceptibility in this series (for **9g**, BA/AB ratio = 6.6). Furthermore, the resulting ureas generally exhibited potent Kv1.5 antagonism, with benzylic amine-derived ureas among the most potent (Table 4). 2-Fluorobenzyl urea **9g** demonstrated the best potency with a Kv1.5 IC_{50} = 150 nM. The diastereomerically pure racemic analog **9g** was resolved via chiral chromatography (ChiralPak AD, *n*-hexanes/ethanol, 85:15) to yield a single active enantiomer, **10**, which exhibited potency equivalent to **9g**

Table 4. Inhibition activity for 5-ureas **9g–q**

Compound	R	Kv1.5 IC_{50}[a] (nM)
9g		150
9h		240
9i		252
9j		256
9k		283
9l		298
9m		341
9n		353
9o		362
9p		490
9q		757

[a] Values represent IC_{50} for more potent diastereomer, determined in quadruplicate.

and was used for all further characterization. The dog i.v. pharmacokinetic properties of this analog (Cl = 9.81 mL/min/kg) were sufficient to allow evaluation in vivo in a canine electrophysiology model.

Canine cardiac electrophysiological studies were conducted as previously described.[13] Compound **10** was infused continuously over 1 h to anesthetized dogs, and atrial refractory period (ARP), ventricular refractory period (VRP), QT interval, heart rate, and mean arterial pressure were monitored. Analog **10** elicited a significant dose-responsive increase in atrial refractory period (Fig. 3). Animals tested at all doses showed no changes in ventricular refractory period (Fig. 4) or QT interval, indicating a selective atrial effect. Plasma compound levels associated with a 10% increase in atrial refractory period were ~20 nM.

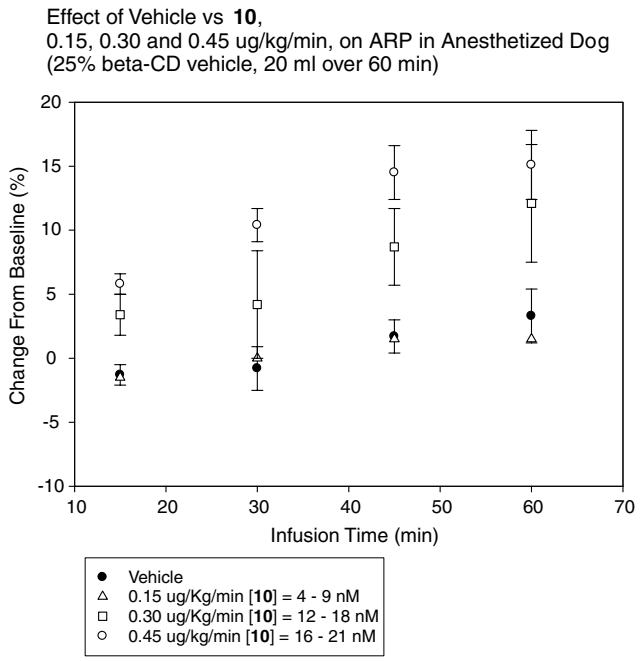


Figure 3. In vivo study of **10** and atrial refractory period.

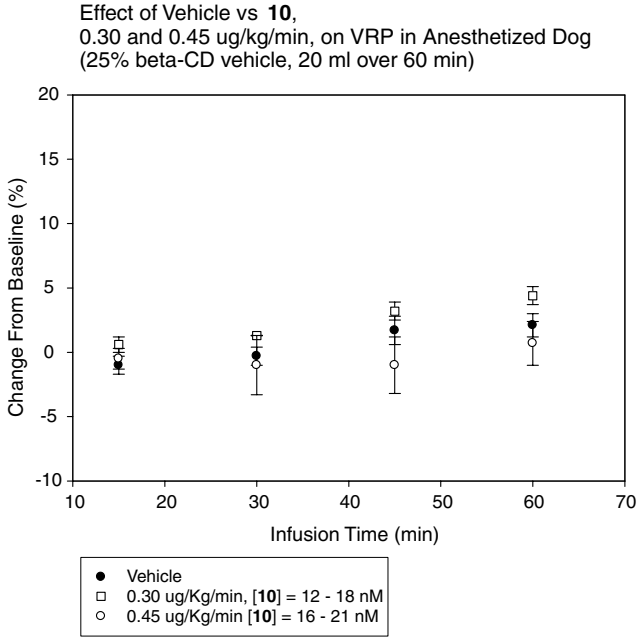


Figure 4. In vivo study of **10** and ventricular refractory period.

In summary, we have discovered a novel series of potent Kv1.5 antagonists based on the diisopropyl amide scaffold **4**. These compounds exhibit in vitro I_{Kur}/Kv1.5 antagonist activity and, notably, key compound 1-(3-(diisopropylcarbamoyl)-2-phenyl-3-(pyridin-3-yl)propyl)-3-(2-fluorobenzyl)urea (**10**) showed significant atrial-selective effects in the dog electrophysiological model. Compound **10** is also a P-gp substrate with low potential for CNS exposure.

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