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Lactam sulfonamides as potent inhibitors of the Kv1.5 potassium ion channel

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ABSTRACT

A series of lactam sulfonamides has been discovered and optimized as inhibitors of the Kv1.5 potassium ion channel for treatment of atrial fibrillation. In vitro structure–activity relationships from lead structure **C** to optimized structure **3y** are described. Compound **3y** was evaluated in a rabbit PD-model and was found to selectively prolong the atrial effective refractory period at submicromolar concentrations.

Atrial fibrillation (AF) is the most common cardiac arrhythmia and patients suffering from AF generally have a reduced quality of life with lower cardiac output and impaired ability to work and exercise. AF patients also have an increased risk of stroke and other cardiovascular diseases.[1,2] Patients with AF commonly need hospitalization, representing a considerable economic burden to society. Several drug treatment options exist but they suffer from poor efficacy or adverse effects that limit their use. Drugs that inhibit the IKr (hERG) current may affect the ventricle repolarization and cause QT prolongation and life-threatening proarrhythmias, such as Torsades de Pointes.[3]

Since a large unmet medical need still exists for AF patients, the search for new treatments and new drugs continue. Kv1.5 is a voltage gated potassium ion channel conducting the ultra rapidly activating delayed rectifier K^+ current (IKur) and has received attention since the channel protein is predominantly expressed in the human atria. Blocking IKur has the potential to selectively prolong atrium effective refractory period (ERP) over ventricular ERP and reduce the risk of ventricular proarrhythmias. A number of publications have summarized research around Kv1.5.[4–9] However, whilst a block of IKur in animal models of AF has been demonstrated to prolong the atrial refractory period, one recent study has shown that this does not readily translate into effects on human atrial refractory period in healthy volunteers.[10] A number of factors such as high vagal tone in healthy volunteers[10] and lack of electrical remodeling[11] could explain this lack of effect. Ultimately, the final proof of whether or not Kv1.5 is a suitable target for AF treatment will come from clinical studies in AF patients.

In the present paper we describe the synthesis and evaluation of a series of lactam sulfonamides with the aim to find a potent and hERG selective inhibitor of Kv1.5. The start point of the program was anthranilic amide **A** (Fig. 1), an inhibitor described in the early literature with a Kv1.5 IC_{50} potency of 0.6 μM.[12] Using compound **A** as a reference for automated shape-based molecular alignment of single compounds,[13–15] compound **B** (Fig. 1) was identified as an interesting candidate for synthesis. Towards this goal, a direct benzylation of *N*-(2-hydroxycyclohexyl)-4-methoxybenzene-sulfonamide failed, instead affording compound **C** (Fig. 1), as a racemate. As an alternative, a literature synthetic method was adapted to enable successful preparation of racemic **B**.[16]

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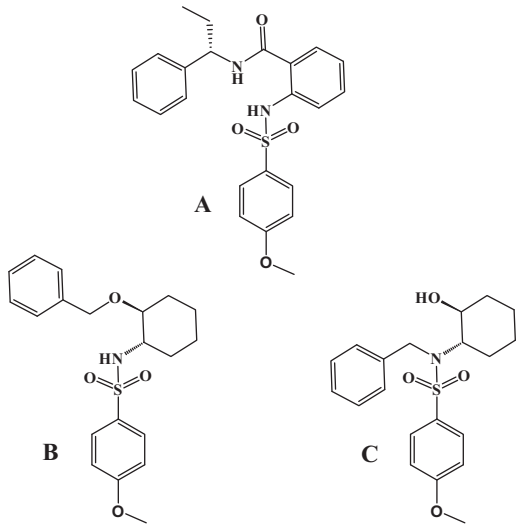


Figure 1. Structures of compounds **A**, **B** and **C**.

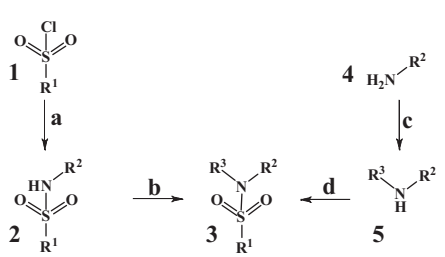
Preliminary testing of both **B** and **C** against Kv1.5, using a Rb^+-efflux assay,[17] showed that **B** had a blocking potency of 28 μM, whereas **C** had a blocking potency of 2 μM. These results made us choose the tertiary sulfonamide **C** as lead structure and a synthetic program was started to explore structure–activity relationship (SAR) around **C**.

Different routes for synthesis of analogues to compound **C** are shown in Scheme 1. Sulfonyl chloride **1** was reacted with the appropriate primary amine under basic conditions. The formed secondary sulfonamide **2** was then alkylated with the appropriate alkyl halide under basic conditions to give the desired compound **C** analogue **3**. Alternatively, sulfonamide **3** was synthesized by reacting primary amine **4** under basic alkylation or reductive amination conditions. The formed secondary amine **5** was then reacted with the appropriate sulfonyl chloride under basic conditions to give **3**.

The ion channel-blocking potency of synthesized compounds on the Kv1.5 channel was assessed in a CHO cell line stably expressing the human cardiac ion channel Kv1.5. Electrophysiological studies were performed using a high throughput planar patch clamp assay and standard extra- and intracellular solutions.[18,19] Seven different concentrations of each compound were run in triplicates in the same plate to give the dose-response data from which the IC_{50} value was determined.[20] Human hERG channel blocking potency was similarly assessed on selected compounds and most tested compounds had IC_{50} values above 20 μM.

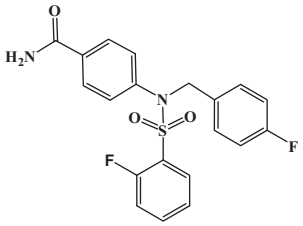
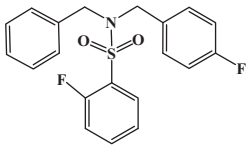
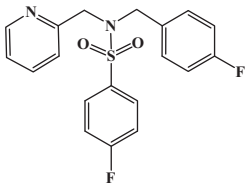
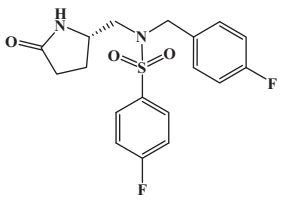
The lipophilicity of synthesized compounds was monitored since the lead compound **C** possesses a high measured chromatographic Log*D*[21] of 4.6, deemed a potential weakness and source of metabolic lability.

Compound **C** was also a racemate and trying to move away from stereoisomeric compounds we varied the cyclohexanol part of the



Scheme 1. Reagents and conditions: (a) R^2NH_2, NEt_3, DCM, 20 °C, 18 h, 49–98%; (b) R^3X, K_2CO_3, MeCN, 80 °C, 18 h, 2–73%; (c) R^3X, NEt_3, DCM, 20 °C, 18 h, 49–69%, or R^3=O, $NaBH(OAc)_3$, DCE, 18 h, 64–100%; (d) R^1SO_2Cl, NEt_3, DCM, 2 h, or R^1SO_2Cl, $NaHCO_3$, MeCN, 18 h, 7–86%.

Table 1
Potency and lipophilicity of compounds **3a–3d**

No.	Structure	Kv1.5[a]	Log*D*[b]
3a[c]		2.2	3.4
3b[c]		4.8	6.2
3c		2.0	4.7
3d		4.1	3.0

[a] IC_{50} values in μM.
[b] Chromatographic lipophilicity.
[c] Synthesized using routes c and d.

structure. The SAR of a first set of compounds is shown in Table 1. Anilinic groups, like *p*-benzamide (**3a**) were found to be active but anilinic fragments may pose a mutagenic risk.[22] A benzyl group (**3b**) was tolerated but gave a high lipophilicity. Replacing the benzyl group with a (pyridin-2-yl)methyl group (**3c**) gave a slightly higher activity with reduced lipophilicity. A (*S*)-(5-oxopyrrolidin-2-yl)methyl ((*S*)-lactam) group (**3d**) was found to be active with a Log*D* value of 3.0.

Compound **3d** had the lowest lipophilicity and was most suitable for further optimization and we proceeded to explore variations in the benzene sulfone part of the molecule, as shown in Table 2. An unsubstituted benzene sulfone ring (**3e**) gave a slight drop in potency compared to compound **3d**. A *m*-fluoro substituent (**3f**) gave a similar potency as an unsubstituted ring, but a *m*-chloro substituent (**3g**) gave a marked increase in potency. 3,5-Dichloro substitution (**3h**) did not help to improve potency further but instead gave a similar potency as the unsubstituted ring. Glutathione trapping experiments in human liver microsomes[23] also showed that fluorine and possibly chlorine substitution on the benzene sulfone ring gave rise to reactive metabolites and we wished to avoid these going forward.

A *m*-methoxy group (**3i**) was found active but linking the *para* and *meta* positions together in a six membered ring gave an inactive compound (**3j**). A *m*-methylsulfone group (**3k**) showed lower activity and a low lipophilicity. *m*-Cyano substitution (**3l**) gave an active compound with a Log*D* value of 2.7. Varying the position of the cyano group on the benzene sulfone ring showed that both *p*-cyano (**3m**) and *o*-cyano (**3n**) substituted compounds were inactive.

Compound **3l** showed a suitable combination of potency and low lipophilicity and was used to further explore SAR around the (*S*)-lactam, as shown in Table 3. Inversion of the stereochemistry

Table 2
Potency and lipophilicity of compounds **3e**–**3n**

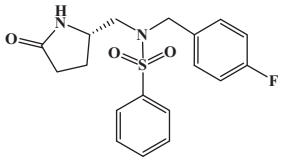
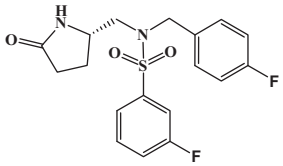
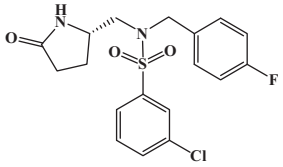
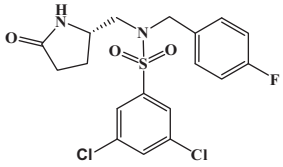
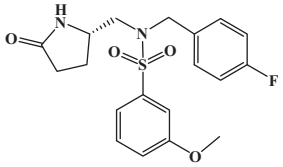
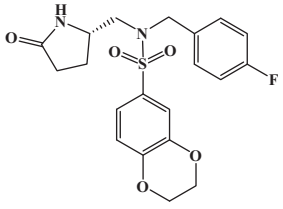
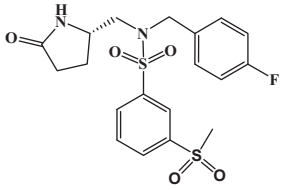
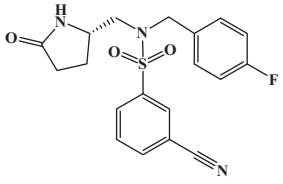
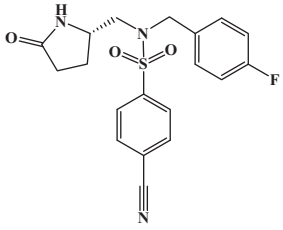
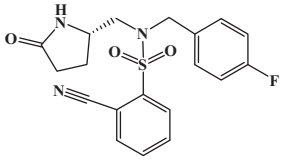
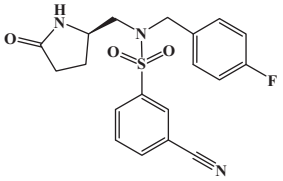
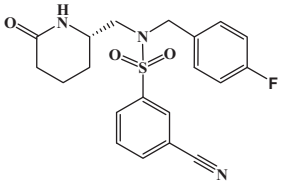
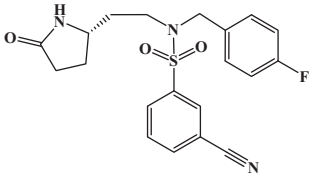
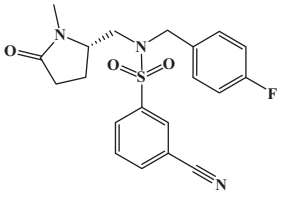
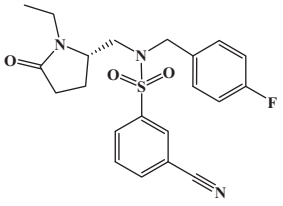
No.	Structure	Kv1.5[a]	Log *D*[b]
3e[c]		8.8	2.7
3f		7.2	3.1
3g[c]		0.8	3.6
3h[c]		8.1	4.4
3i[c]		2.0	3.1
3j		>10	3.0
3k		8.7	2.0
3l[c]		2.6	2.7
3m[c]		>30	2.6

Table 2 (*continued*)

No.	Structure	Kv1.5[a]	Log *D*[b]
3n		>30	2.5

[a] IC_{50} values in μM.
[b] Chromatographic lipophilicity.
[c] Synthesized using routes c and d.

Table 3
Potency and lipophilicity of compounds **3o**–**3s**

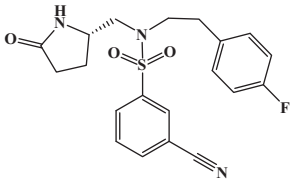
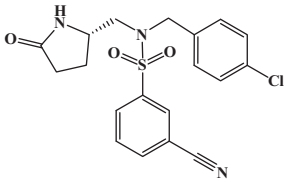
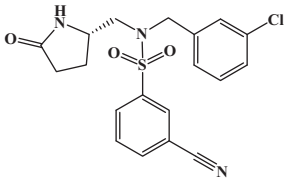
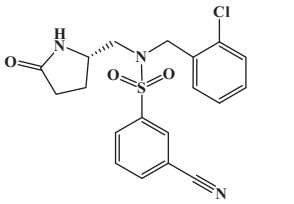
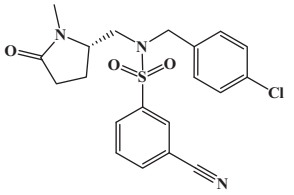
No.	Structure	Kv1.5[a]	Log *D*[b]
3o[c]		8.7	2.5
3p		7.6	2.9
3q[c]		2.7	2.9
3r[c]		0.81	3.1
3s[c]		2.8	3.5

[a] IC_{50} values in μM.
[b] Chromatographic lipophilicity.
[c] Synthesized using routes c and d.

of the (*S*)-lactam to (*R*)-configuration (**3o**) gave a drop in potency. Expanding the (*S*)-lactam to a six membered ring (**3p**) gave a lower potency. Extending the one-carbon linker of the (*S*)-lactam to a two-carbon linker[24] gave an equipotent compound (**3q**). Methylation of the (*S*)-lactam nitrogen (**3r**), using a literature method,[25] gave a 3-fold increase in potency whereas ethylation (**3s**) gave a similar potency as the unsubstituted nitrogen.

The SAR of the benzyl part of compound **3l** was further explored in a final optimization, as shown in Table 4. Extending the

Table 4
Potency and lipophilicity of compounds **3t**–**3y**

No.	Structure	Kv1.5[a]	Log*D*[b]
3t		>10	2.9
3u[c]		2.2	3.1
3v		4.3	3
3x		>10	2.7
3y		0.21	3.6

[a] IC_{50} values in μM.
[b] Chromatographic lipophilicity.
[c] Synthesized using routes c and d.

p-fluorobenzyl group of **3l** to a *p*-fluorophenethyl group gave an inactive compound (**3t**). A *p*-chlorobenzyl group (**3u**) was tolerated, whereas *m*-chloro substitution (**3v**) reduced potency and *o*-chloro substitution (**3x**) gave an inactive compound. Methylation of the (*S*)-lactam nitrogen was earlier found to increase potency in the series (compound **3l** vs **3r**). Applying the same strategy to compound **3u**, yielding compound **3y**, was found to give a 10-fold increase in potency. Overall, compound **3y** was found to be the most potent compound in the series and was selected for pharmacokinetic (PK) and pharmacodynamic (PD) evaluations.

Compound **3y** was found to have a CLint value of 116 μL/min × mg protein in human liver microsomes which indicate a high metabolism. The compound was further profiled in vivo in rats and dogs (Table 5). Clearance was found to be intermediate in both rat and dog. Terminal half-life was short, below 1 h in both species. Oral bioavailability (*F*) was 44–45% in the two species.

The pharmacodynamic effects of compound **3y** were tested in a modified rabbit PD model[26] which measured the ERP in the atrium as well as the QT-interval. Male, New Zealand white rabbits were administered with 3 increasing doses given as consecutive 15 min infusions, followed by a wash-out period. The doses ranged between 0.1 and 1 μmol/kg/min. PK/PD modeling using link models[27] on effect-plasma concentration data was applied to estimate the unbound steady-state plasma concentration (C_{eu20}) giving a 20% change in the atrium ERP. The maximum observed change in the QT-interval during the drug exposure period was also modeled to give a measure of the effects on ventricular repolarization.

Table 5
Selected PK-parameters of **3y** in rat and dog

	Rat[a]	Dog[b]
CL (mL/min/kg)	45	31
$t_{1/2}$ (h)	0.53	0.87
F (%)	45	44

[a] Sprague Dawley, dosed at 2 μmol/kg iv and 6 μmol/kg p.o.
[b] Beagle, dosed at 1 μmol/kg iv and 2 μmol/kg p.o.

Table 6
Selected in vitro and in vivo PD data of **3y** in human and rabbit

Measure	**3y**
Rabbit atrium ERP C_{eu20} (μM)	0.35
Human Kv1.5 IC_{50} (μM)	0.21
Human hERG IC_{50} (μM)	30
Rabbit QT-interval (ms)	<10

Compound **3y** caused a marked increase in the atrium ERP with a C_{eu20} of 0.35 μM (Table 6), that is, at the same order of magnitude as the IC_{50} value from the human cellular assay. The human hERG channel was blocked by compound **3y** with an IC_{50} value of 30 μM, indicating a 140-fold margin of the hERG and Kv1.5 in vitro values. No measurable change was noted in the QT-interval in the rabbit experiments, which also indicates a good margin to block of the hERG channel. The compound was well tolerated in rabbits with no signs of the CNS-like side effects observed for other Kv1.5 blockers.[28] No other compound in the series was tested in the rabbit model.

Overall, compound **3y** was judged to have too short half-life to be selected as a clinical candidate but was used as a tool compound for further pharmacological evaluation, which will be reported in a future publication.

In conclusion, we have discovered a series of lactam sulfonamides that are potent inhibitors of the Kv1.5 channel. The optimized compound **3y** was evaluated in a rabbit PD model and showed a marked prolongation of ERP in the atrium at submicromolar concentrations.

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