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Pyrrolidine amides of pyrazolodihydropyrimidines as potent and selective $K_V1.5$ blockers

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ABSTRACT

Design and synthesis of pyrazolodihydropyrimidines as $K_V1.5$ blockers led to the discovery of **7d** as a potent and selective antagonist. This compound showed atrial selective prolongation of effective refractory period in rabbits and was selected for clinical development.

Atrial fibrillation (AF) is the most common cardiac arrhythmia and affects over 2.2 million patients in the United States alone.[1] In this condition the atria beat rapidly and inefficiently which allows for blood stasis that can lead to clot formation. A higher incidence of clot formation puts AF patients at increased risk of stroke and 15% of strokes are in patients with AF.[2] Treatment options for patients with AF include surgical procedures, radiofrequency ablation, implanted devises and drug treatments. One approach to drug treatment is agents that prolong refractory period in atrial tissue. Such drugs currently available include sodium channel blockers, potassium channel blockers or some combination of these activities either in a single drug or in multiple drugs.[3] All the current drugs target ion channels that are expressed in both atrium and ventricle. Prolongation of refractory period in the ventricle has been shown to increase the incidence of the potentially life threatening arrhythmia torsades de points.[4] The ultra rapid potassium current (I_{Kur}) is a current conducted by the $K_V1.5$ potassium channel that in humans is only expressed in the atrium.[5] Block of I_{Kur} would therefore be a way to treat AF without the risk of ventricular arrhythmia.

In our previous publications we disclosed $K_V1.5$ blockers based on a pyrazolodihydropyrimidine template.[6] We noted the pyrrolidine amides (**1** and **2**) and how substitution of the appropriate stereochemistry enhanced potency (Fig. 1). This paper will describe our efforts to further optimize this series of pyrrolidine amides with regard to $K_V1.5$ potency, pharmacokinetic properties and pharmacodynamic effects.

The synthesis of pyrazolodihydropyrimidines has been described previously. The pyrazolodihydropyrimidine intermediate (**4**) was available from the Biginelli reaction of 3-aminopyrazole, 3,4-dichlorobenzaldehyde and *t*-butylacetoacetate. The ester was hydrolyzed to provide the acid (**5**). Amide coupling under standard conditions with substituted pyrrolidines provided compounds **6–23** (Scheme 1). The final compounds were initially obtained as mixtures of diastereomers with the stereochemistry uncontrolled at the 7-position of the dihydropyrimidine. In some cases the diastereomers where separated into isomers a and b.

The pyrrolidines used in the synthesis of compounds **6**, **8**, **9**, and **11** were commercially available. Several aryl and heteroaryl substituted pyrrolidines, including the 2-(4-fluorophenyl)pyrrolidine, the 2-pyridylpyrrolidine and the 4-pyridyl pyrrolidine, were synthesized using known methods[7] (Scheme 2).

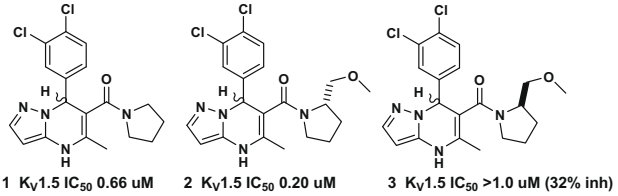

Figure 1. Pyrazolodihydropyrimidine $K_V1.5$ antagonists.

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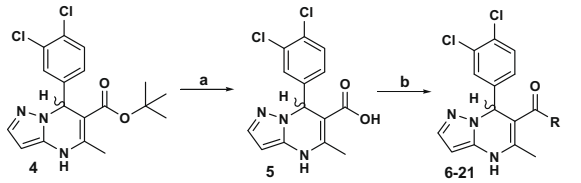

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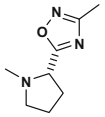
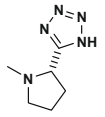
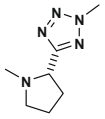
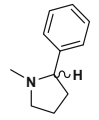
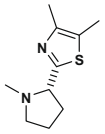
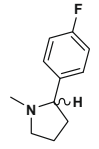
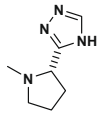
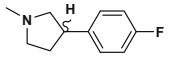
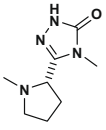
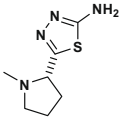
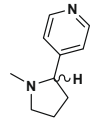
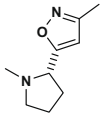
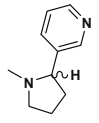
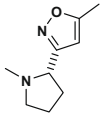
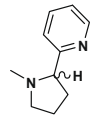
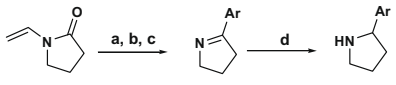
1		13a 13b	
2		14a 14b	
3		15	
6		16	
7		17a 17b	
8		18	
9		19	
10		20a 20b	
11		21a 21b	
12			

Scheme 1. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 0–25 °C, 2 h, 88%; (b) pyrrolidine, EDC, HOBt, CH_2Cl_2, 14–68%.



Scheme 2. Reagents and conditions: (a) NaH, $ArCO_2Me$ THF, 65 °C, 1 h; (b) HCl, reflux, 12 h; (c) NaOH, 64% over 3 steps; (d) $NaBH_4$, EtOH, 40%.

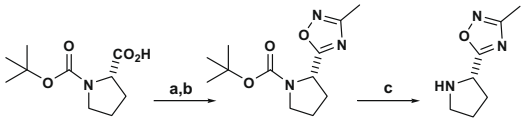
The remaining heterocyclic pyrrolidines were synthesized from L-proline. The 1,2,4-oxadiazole pyrrolidine was synthesized by reaction of Boc-L-proline with hydroxyacetamidine followed by dehydration and deprotection (Scheme 3).

The tetrazoles were synthesized by dehydration of the Cbz-L-proline amide to the nitrile followed by reaction with sodium azide. The methyl tetrazoles were then formed by alkylation with methyl iodide then deprotection (Scheme 4).

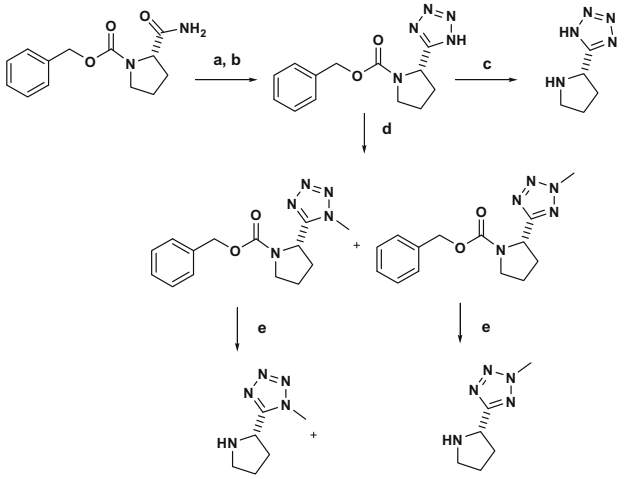
The thiazoles were synthesized by formation of the thioamide from the Cbz-L-proline amide followed by reaction with 3-bromobutane or chloroacetaldehyde, then deprotection (Scheme 5).

The triazole was synthesized by reaction of Cbz-L-proline amide with DMF-dimethylacetal followed by reaction with hydrazine. Deprotection was performed with HBr in acetic acid. The triazolone was synthesized by reaction of *N*-benzyl-L-proline ethyl ester with hydrazine to form the acylhydrazide. Reaction of the hydrazide with methylisocyanate provided the acylsemicarbazide that could be cyclized under basic conditions. Hydrogenation provided the substituted pyrrolidine (Scheme 6.).

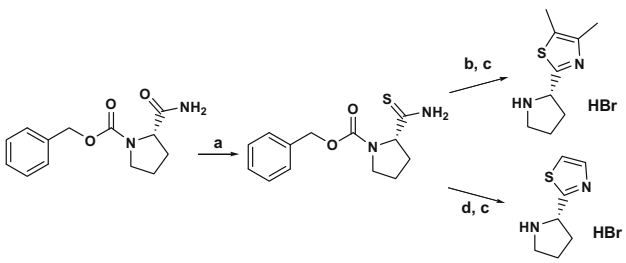
The aminothiadiazole was synthesized by coupling of Fmoc-L-proline with thiosemicarbazide followed by dehydration. The Fmoc group was removed using piperidine (Scheme 7).



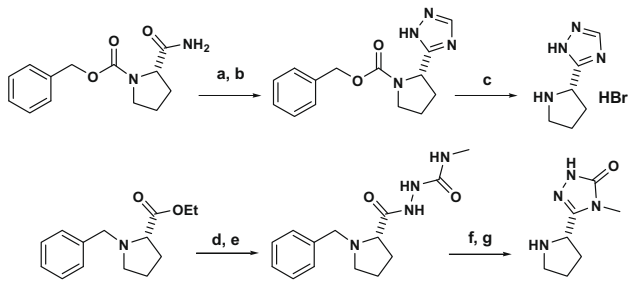
Scheme 3. Reagents and conditions: (a) $CH_3C(NOH)NH_2$, EDC, HOBt, CH_2Cl_2; (b) Cs_2CO_3, THF, 89% (over 2 steps); (c) TFA, CH_2Cl_2, 70%.



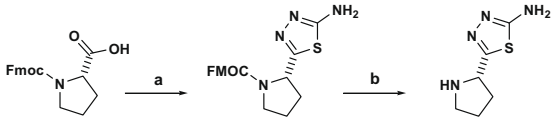
Scheme 4. Reagents and conditions: (a) $POCl_3$, pyridine, −10 °C, 75%; (b) NaN_3, AcOH, NH_4OAc, 95 °C, 88%; (c) H_2/Pd/C, AcOH, H_2O, 20%; (d) MeI, K_2CO_3, DCM, 57% (1:1 endo: exo); (e) 5%Pd/C Degussa, EtOH, H_2, 100%.



Scheme 5. Reagents and conditions: (a) Lawesson's reagent, THF, 65 °C, 100%; (b) 3-bromobutan-2-one EtOH, rt to 80 °C, 100%, (c) 30% HBr, AcOH, ether trituration, 70–80%; (d) 2-chloroacetaldehyde, $CHCl_3$, 75 °C, 45%.



Scheme 6. Reagents and conditions: (a) 2,2-dimethoxy-*N*,*N*-dimethylacetamide, 90 °C, 100%; (b) NH_2NH_2, AcOH, 95 °C, 74%; (c) 30% HBr, AcOH, ether trituration, 94%; (d) NH_2NH_2, MeOH, 65 °C, 16 h, 95%; (e) MeNCO, THF, 24 h, 86%; (f) NaOH (1.0 M, aq), 100 °C, 1 h, 95%; (g) HCO_2NH_4, 10% Pd-C, MeOH, 80 °C, 1 h, 88%.

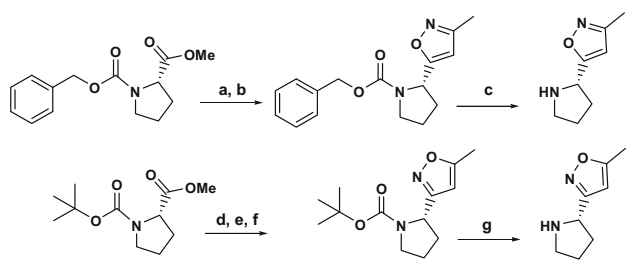


Scheme 7. Reagents and conditions: (a) $NH_2NHCSNH_2$, $POCl_3$, 100 °C, 5 min; (b) 20% piperidine, CH_2Cl_2, 20% (over 2 steps).

The isoxazole was synthesized from Cbz-L-proline methyl ester by reaction with the anion of acetone oxime followed by cyclization and deprotection of the amine in hydrochloric acid and TFA. The isomeric isoxazole was synthesized by a known route[8] from the L-prolinal (Scheme 8).

The compounds **1–3** and **6–21** were tested for block of potassium current in mouse fibroblast L929 cells expressing human $K_V1.5$.[9] As previously published, our initial survey of pyrrolidine amides revealed that the unsubstituted pyrrolidine (**1**) had moderate activity that could be enhanced with substitution on the pyrrolidine ring.[9] The 2-methoxymethyl compound (**2**) was threefold more active although the stereochemistry of the substitution was important with the *S*-isomer (**2**) favored over the *R*-isomer (**3**). The 2-phenyl substituted compound (**6**) also had significantly enhanced activity over the unsubstituted pyrrolidine amide (**1**) and was similar to the methoxymethyl substituted compound (**2**). Herein, we report the investigation of substituted pyrrolidine amides. Several 2-arylpyrrolidines with substituents on the phenyl ring were synthesized with the possibility of enhancing potency and blocking potential sites of metabolism. Among the most potent of these was the 4-fluorophenyl compound (**7**). Substitution at the 2-position of the pyrrolidine was favored over substitution at the 3-position as in compound **8**. The L-proline methyl ester amide (**9**) also had significant potency (Table 1).

With the observation that both aryl and ester substituents in the 2-position showed acceptable activity, we became interested



Scheme 8. Reagents and conditions: (a) acetone oxime, *n*-BuLi, THF, 5 h; (b) H_2SO_4, 0 °C, 1.5 h, 51% (over 2 steps); (c) triflic acid, CH_2Cl_2, 15 min, 42%; (d) $NH_2OH{\cdot}HCl$, pyridine, 100%; (e) NCS, DCE, 0–20 °C; (f) 2-bromopropene, Et_3N, DCE, 46% (over 2 steps); (g) 50% TFA, CH_2Cl_2, 77%.

Table 1
Inhibition of $K_V1.5$ of substituted pyrrolidine amides

Compds	$K_V1.5$ inhibition IC_{50}[a] (μM)
1	0.66
2	0.20
3	>1.0 (32% inh)
6	0.15
7	0.07
8	0.16
9	0.15

[a] Values are means of 2–4 experiments.

in 2-heterocyclo-substituted pyrrolidine amides. In an attempt to add an ionizable group to increase solubility, we investigated a series of pyridyl substituted pyrrolidines. The 4-pyridyl (**10**) and 2-pyridyl (**12**) compounds were three–sixfold less active than the phenyl substituted compound. The 3-pyridyl compound (**11**) was even less active. The oxadiazole (**13b**) synthesized from L-proline showed similar activity as the ester (**9**). The corresponding oxadiazole from D-proline was >6-fold less active confirming the preference for the *S*-isomer seen with compounds **2** and **3** (Table 2).

The tetrazole compounds (**14a** and **14b**) had very little activity probably due to the charged functionality. The methyl tetrazole (**15**) recovered some activity but was still about threefold less active than the ester (**9**). The thiazole (**16**) had similar potency to the methyl tetrazole. The triazoles (**17a** and **17b**) and triazolone (**18**) had very little potency as did the aminothiadiazole (**19**). Some of the most potent heterocycles were the isoxazoles (Table 3). The more active diastereomers (**20b** and **21b**) had activity similar but not superior to the 4-fluorophenyl compound (**7**) as a mixture of diastereomers.

Of the compounds synthesized in this study, the 2-(4-fluorophenyl)pyrrolidine amide (**7**) was one of the most active against $K_V1.5$ and was one of the first compounds indentified that met our ion channel selectivity criteria for progression to further studies. It was resynthesized and the four isomers separated by a combination of silica gel chromatography (eluting with 75% ethyl

Table 2
Inhibition of $K_V1.5$ of pyridyl- and oxadiazoyl-substituted pyrrolidine amides

Compds	$K_V1.5$ inhibition IC_{50}[a] (μM)
10	0.31
11	>1.0 (35% inh)
12	0.66
13a	>1.0 (22% inh)
13b	0.16

[a] Values are means of 2–4 experiments.

Table 3
Inhibition of $K_V1.5$ of heterocyclo-substituted pyrrolidine amides

Compds	$K_V1.5$ inhibition IC_{50}[a] (μM)
14a	>1.0 (4% inh)
14b	>1.0 (8% inh)
15	0.48
16	0.30
17a	>1.0 (19% inh)
17b	>1.0 (3% inh)
18	>1.0 (11% inh)
19	>1.0 (4% inh)
20a	>1.0 (21% inh)
20b	0.11
21a	>1.0 (38% inh)
21b	0.09

[a] Values are means of 2–4 experiments.

Table 4
Inhibition of $K_V1.5$ of 2-(4-fluorophenyl)pyrrolidine amides

Compds	$K_V1.5$ inhibition IC_{50}[a] (μM)
7a	2.1
7b	1.8
7c	0.15
7d	0.05

[a] Values are means of 2–4 experiments.

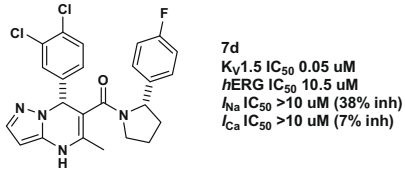


Figure 2. Stereochemistry and activity of **7d**.

Table 5
Pharmacokinetic parameters of compound **7d** in rats and dogs

	Rat[a]	Bog[a]
Dose (μmol/kg)	10 (inf)[b] 20 (po)[c]	10 (inf)[b] 20 (po)[c]
F (%)	51 ± 6	37 ± 7
$t_{1/2}$ (h)	0.57 ± 0.17	1.5 ± 0.6
Clearance (mL/min/kg)	35 ± 9	42 ± 4.3
V_{dss} (L/kg)	1.6 ± 0.6	2.1 ± 0.08

[a] Values are means from three animals.
[b] inf = intra-arterial infusion for 10 min.
[c] po = oral gavage.

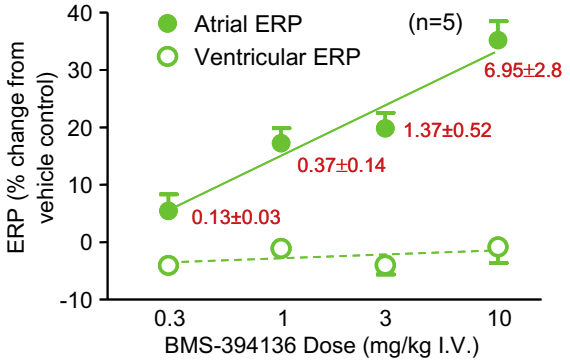


Figure 3. Pharmacodymanic effect of **7d** on prolongation of atrial and ventricular effective refractory period in rabbits (n = 5). Plasma concentrations (μM) are noted at each point on the AERP versus dose plot.

acetate and hexane) to separate the diastereomers and chiral HPLC (Chiracel AD, 13% isopropanol, heptane with 0.1% TFA) to separate the enantiomers (Table 4). The most active isomer (**7d**) showed excellent activity in blocking $K_V1.5$ and very good selectivity over *h*ERG,[10] sodium[11] and L-type calcium[12] ion channels. This compound was crystallized and the single crystal X-ray analysis showed the *R* stereochemistry in the dihydropyrimidine ring and *S* stereochemistry in the pyrrolidine ring (Fig. 2).

The pharmacokinetics of **7d** was investigated in rats and dogs (Table 5).[13] Compound **7d** has intermediate systemic clearance in rats. Steady-state volume of distribution was greater than total body water, indicating significant extravascular distribution. Terminal half-life was 0.57 h in rats. Oral bioavailability (F) was 51%. Half-life was longer in dogs (1.5 h) with slightly lower bioavailability.

The pharmacodynamic activity of **7d** was tested in a rabbit model which measured the effective refractory period (ERP) in both atrium and ventricle.[14] Like humans, rabbits express the I_{Kur} current in atrium but not ventricle. The compound was dosed at 0.3, 1.0, 3.0, and 10 mg/kg and prolonged atrial ERP by >20% at a dose of 3 mg/kg. Reflecting the selectivity for $K_V1.5$ over ventricular ion channels, **7d** showed no effect on ventricular ERP up to the highest dose of 10 mg/kg with plasma concentration of nearly 7 μM (Fig. 3). Plasma free fraction in rabbits for **7d** was 3.0%.

In conclusion, pyrrolidine amides of pyrazolodihydropyrimidines were discovered as potent and selective $K_V1.5$ blockers. A substituent at the 2-position significantly enhanced activity and the *S*-configuration is favored over the *R*-configuration. Aryl and some uncharged heteroaryl substituents were well tolerated. Compound **7d** was chosen for more complete in vitro and in vivo evaluation and found to be potent in a pharmacodynamic model measuring effective refractory period. This compound was chosen for further pre-clinical toxicology studies and development as a clinical candidate.

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13. Compound **7d** was administered to rats and dogs, as a solution in polyethylene glycol 200: ethanol/water (1:1:1). Plasma was prepared from each blood sample by centrifugation and analyzed by LC/MS. Plasma concentrations versus time data were analyzed by non-compartmental methods. The total plasma clearance, terminal half-life ($t_{1/2}$), and the steady state volume of distribution (V_{dss}) were calculated after intra-arterial administration. The absolute oral bioavailability (F, expressed as %) was estimated by taking the ratio of dose-normalized AUC value after an oral dose to that after an intra-arterial dose.
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