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Dihydropyrazolopyrimidine Inhibitors of $K_V1.5$ (I_{Kur})

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ABSTRACT

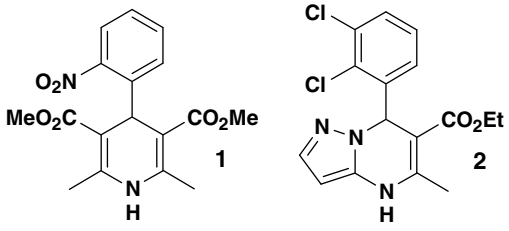
A series of dihydropyrazolopyrimidine inhibitors of $K_V1.5$ (I_{Kur}) have been identified. The synthesis, structure–activity relationships and selectivity against several other ion channels are described.

Atrial fibrillation and flutter are the most common cardiac arrhythmias encountered in clinical practice.[1] Although some presently available antiarrhythmic drugs such as ibutilide and dofetilide can convert sustained fibrillation to sinus rhythm, these drugs prolong the ventricular effective refractory period and thereby increase the risk for life threatening ventricular arrhythmias. Thus, there is presently an unmet medical need for safe and efficacious treatment of atrial fibrillation and other supraventricular arrhythmias. The ultrarapid delayed rectifier potassium current (I_{Kur}) is a rapidly activating sustained current that has been identified in human atrial myocytes, but is not functionally expressed in human ventricular myocytes.[2] I_{Kur} plays a significant role in the repolarization of the atrial action potential and selective inhibition of this current in human atrial myocytes prolongs action potential duration.[3] Prolongation of the action potential would consequently prolong the atrial effective refractory period; that is, inhibition of I_{Kur} would produce a class III antiarrhythmic effect. Importantly, an inhibitor of I_{Kur} may have utility against atrial fibrillation without having the attendant ventricular proarrhythmic risk associated with other class III agents that have potassium channel targets that are expressed in both the atrium and the ventricle.

I_{Kur} is most likely encoded by the potassium channel gene $K_V1.5$.[4] Since screening compounds against I_{Kur} in human atrial cells is impractical, recombinant human $K_V1.5$ expressed in Xenopus oocytes or mouse fibroblast L929 cells were used to screen for inhibitors of I_{Kur} ($K_V1.5$).[5]

It had been reported that the L-type calcium antagonist nifedipine **1** was a weak inhibitor of $K_V1.5$ (Table 1).[6] Therefore, a collection of compounds assembled from Bristol–Myers Squibb ion channel programs was evaluated for $K_V1.5$ inhibitory activity using EP voltage clamp techniques. Compounds that inhibited human $K_V1.5$ in a higher throughput Xenopus oocyte assay were further evaluated against human $K_V1.5$ in the lower throughput but more sensitive mammalian cell (mouse fibroblast L929) assay. These efforts identified **2**, a compound with modest $K_V1.5$ activity, but encouraging $K_V1.5$ versus L-type calcium channel selectivity. A study was undertaken to determine if the $K_V1.5$ activity and selectivity of **2** could be improved by structural modification.

Table 1
Ion channel activity of compounds **1** and **2**.[9]



Compound	L-type calcium HEK293, IC_{50}, μM	$K_V1.5$ L929, IC_{50}, μM
1	0.06	9.38
2	6.1	1.1

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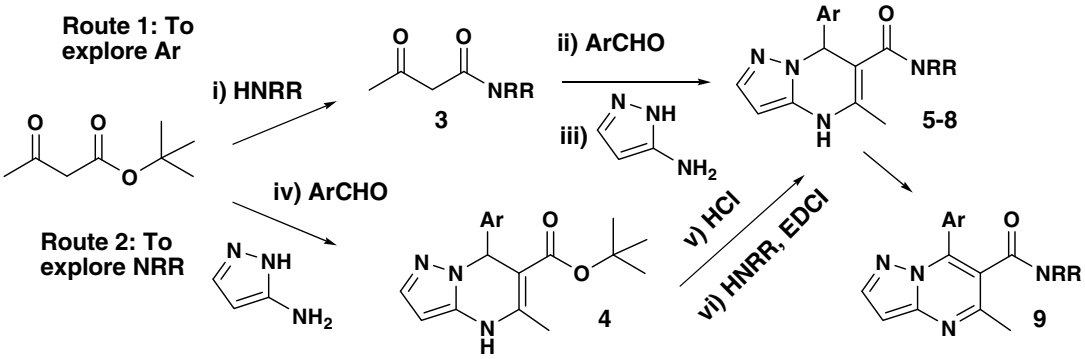
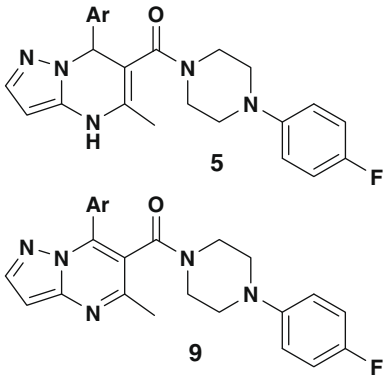
The requisite dihydropyrazolopyrimidines **5-8** were readily assembled by modification of the Knovenegal reaction as shown in Scheme 1.[5,7,11,12] The ester/amide exchange reaction to prepare β-ketoamides **3** as shown in Route 1 works well with secondary amines but gives mixtures requiring purification with primary amines.[8] Thus, mono-substituted amide analogs **5-8** were most often prepared by Route 2. The seemingly straight forward conversion of dihydropyrazolopyrimidine esters such as **4** to the corresponding acids proved to be anything but straight forward. Attempts to convert either the ethyl, 2-(cyano)ethyl or benzyl ester analogs of **4** to the corresponding carboxylic acid under a variety of conditions often resulted in multi-component mixtures. Eventually we found that **4** could be smoothly converted to the corresponding acid by treatment with 4 M HCl in dioxane followed by stirring overnight at room temperature. Concentration of the resulting slurry provides the carboxylic acids which were used without further purification. Attempts to purify the acids resulted in complex mixture formation. In general, Route 1 was used to explore the aryl group, and Route 2 was used to explore the amide group of **4**. Occasionally the prepared dihydropyrazolopyrimidines **5–8** would oxidize to the corresponding pyrazolopyrimidines **9**. Oxidation was not predictable and seemed to be dependent on the nature of the aryl and amide groups. For most compounds, oxidation was not an issue and could be avoided by careful handling of dihydropyrazolopyrimidines **5–8**.

Initial SAR studies identified piperazinyl amides such as **5** as submicromolar inhibitors of $K_V1.5$ (Table 2). Exploration of the C-7 position of **5** found that unsubstituted aryl analogs had little



Scheme 1. Reagents and conditions: (i) HNRR, Toluene, reflux, overnight. (ii) ArCHO, piperidine, acetic acid, toluene, azeotrope, overnight. (iii) 3-Aminopyrazole, NaOAc, DMF, 70 °C, overnight. (iv) ArCHO, 3-aminopyrrazole, $NaHCO_3$, DMF, 70 °C, overnight. (v) 4 M HCl in dioxane, overnight, rt. (vi) HNRR, EDCI, DMAP, CH_2Cl_2

Table 2
$K_V1.5$ inhibition of dihydropyrazolopyrimidines **5** and pyrazolopyrimidines **9**.[9]

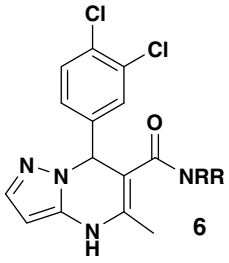


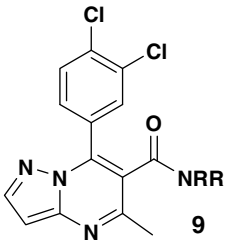
Compound	Ar	$K_V1.5$ Oocytes % Inh, 3 μM	$K_V1.5$ L929 cells IC_{50}, μM
5a	2-Thiophene	7	
5b	2-(5-Me-thiophene)	1	
5c	2-(5-Cl-thiophene)	51	0.23
5d	2-(3-Me-thiophene)	29	
5e	2-(3-Me-benzthiophene)	56	0.20
5f	2-Furan	1	
5g	2-(5-Me-Furan)	7	
5h	2-Benzofuran	33	0.16
5i	Ph	24	
5j	2-ClPh	30	
5k	3-ClPh	68	0.19
5l	4-ClPh	42	0.44
5m	2,3-diClPh	72	0.16
5n	2,4-diClPh	50	0.28
5o	2,5-diClPh	41	0.82
5p	3,4-diClPh	65	0.12
5q	3,5-diClPh	62	0.14

Table 2 (*continued*)

Compound	Ar	$K_V1.5$ Oocytes % Inh, 3 μM	$K_V1.5$ L929 cells IC_{50}, μM
5r	2-MePh	37	48% (1 μM)
5s	3-MePh	57	0.54
5t	4-MePh	27	
5u	3,4-diMePh	34	0.75
5v	3,5-diMePh	58	0.42
5w	2-MeOPh	2	
5x	3-MeOPh	30	
5y	4-MeOPh	11	
5z	2,3-diMeOPh	7	
5aa	3,4-diMeOPh	1	
5bb	3-CNPh	11	
5cc	4-CNPh	14	
5dd	2-NO_2Ph	4	
5ee	3-NO_2Ph	15	
5ff	4-NO_2Ph	24	
5gg	2-Pyrridine	3	
5hh	2-Quinoline	29	
5ii	3-Pyridine	3	
5jj	3-Quinoline	17	
5kk	2-Napthalene	39	0.65
5ll	2,3-(Methylenedioxy)Ph	18	
5mm	3,4-(Methylenedioxy)Ph	26	
9a	2,3-diClPh	17	

Table 3
$K_V1.5$ inhibition of dihydropyrrolopyrimidines **6** and pyrazolopyrimidines **9**.[9]

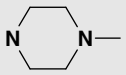
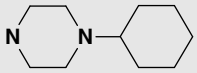
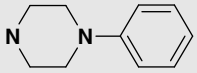
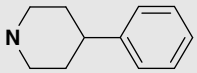
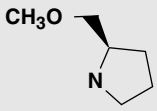
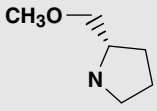




Compound	NRR	$K_V1.5$ Oocytes % Inh at 3 μM	$K_V1.5$ L929 cells IC_{50} (μM)
6a	NH-Propyl	88 (10 μM)	0.49
6b	NH-Butyl	56	0.44
6c	NH-Pentyl	48	0.59
6d	*N*-$(Ethyl)_2$	61	0.44
6e	*N*-$(Propyl)_2$	72	0.07
6f	*N*-$(Butyl)_2$	45	0.31
6g	*N*-$(Pentyl)_2$	69	0.58
6h	NH-Ph	83 (10 μM)	0.17
6i	NH-CH_2Ph	62	0.16
6j	NH-$(CH_2)_2$Ph	84 (10 μM)	0.10
6k	NH-(3-Pyridyl)	9 (10 μM)	
6l		52	0.97
6m		67	0.66
6n		77	0.09
6o		90	0.19
6p		17	

(*continued on next page*)

Table 3 (*continued*)

Compound	NRR	$K_V1.5$ Oocytes % Inh at 3 μM	$K_V1.5$ L929 cells IC_{50} (μM)
6q		5	
6r		17	
6s		82 (10 μM)	0.085
6t		59	0.159
6u		39	32% (1 μM)
6v		88	0.20
9b		17	

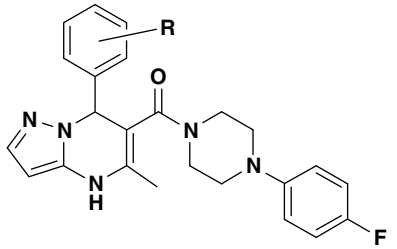
$K_V1.5$ inhibitory activity at the concentrations tested. However, certain substituted or fused aryl analogs of **5** were found to be potent inhibitors of $K_V1.5$. For example, thiophene **5a** and 5-methylthiophene **5b** have little $K_V1.5$ inhibitory activity while 5-chlorothiophene **5c** is a potent inhibitor. Fusion of a phenyl ring on to the weakly active thiophene **5d** or furan **5f** provides benzothiophene **5e** and benzofuran **5h**, both potent inhibitors of $K_V1.5$. The phenyl derivative **5i** was also found to be a weak inhibitor $K_V1.5$, however phenyl analogs with 3-chloro substitution such as **5k, 5m, 5p,** and **5q** were among the most potent inhibitors of $K_V1.5$ identified in this study. Fusion of an additional phenyl ring onto **5i** provides the napthalene **5kk**, and as was seen in the corresponding thiophene (**5a, 5e**) and furan (**5f, 5h**) series, improves activity. As was observed for chloro substituted analogs, substitution of a methyl group at the 3-position improves activity in the phenyl series, compare **5i** with **5s, 5u–v**. Aryl analogs substituted with more polar functionality (**5w–5ff**) or heteroaryls (**5gg–5ii**) were weak inhibitors of $K_V1.5$. We found that in general 2,3- and 3,4-dichlorophenyl were the preferred 7-position substituents for $K_V1.5$ inhibitory activity. It was also observed that pyrazolopyrimidines **9** were less active inhibitors of $K_V1.5$ than the corresponding dihydropyrazolo-pyrimidines **5**, compare **5m** and **9a**.

Holding the 3,4-dichlorophenyl group constant, we next turned our attention to the exploration of the amide fragment. Amide SAR for dihydropyrazolopyrimidine **6** is summarized in Table 3.

Dihydropyrazolopyrimidine **6** was found to be quite tolerant of variation of the amide group. A variety of mono- and disubstituted amides are inhibitors of $K_V1.5$. Diisopropylamide **6e** was the most potent analog identified in the acyclic alkylamide series (**6a–g**). A series of phenyl substituted amides (**6h–j**) were also quite active. However, replacement of the phenyl of **6h** with 3-pyridyl, as in **6k**, resulted in a reduction in $K_V1.5$ activity. In the cyclic amide series (**6l–o**), piperidine **6n** was preferred. Morpholine **6p** and piperazines **6q** and **6r** were much less active. $K_V1.5$ activity may be restored in the piperazine series (**6q–s**) by the addition of a 4-aryl group as in **6s**. The distal nitrogen of piperazine **6s** is not required for $K_V1.5$ activity as demonstrated by the 4-phenylpiperidine analog **6t**. $K_V1.5$ activity in the pyrrolidine series (**6m, 6u–v**) suggests that the nature of the substituent and stereochemistry are important determinates of $K_V1.5$ activity. The wide variety of amides that are tolerated should assist in the fine tuning of specificity for $K_V1.5$, as well as, the ADMET properties for evaluation of in vivo efficacy.

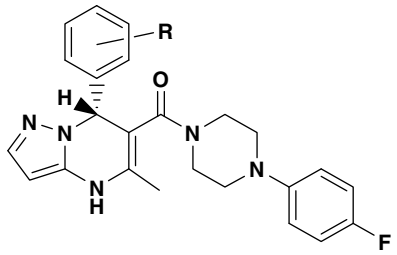
Both the 2,3- and 3,4-dichlorosubstituted analogs **5m** and **5p** were resolved by chiral HPLC to provide the corresponding pure enantiomers **7a,b** and **8a,b** (Table 4). Most of the $K_V1.5$ activity was found to reside in a single enantiomer. The absolute stereochemistry of the more active enantiomer was determined by X-ray crystallography and circular dichroism. The priority rules designate

Table 4
The effect of stereochemistry on $K_V1.5$ inhibition.[9]



Compound	Stereo	R	$K_V1.5$ Oocytes % Inh, 3 μM	$K_V1.5$ L929 cells IC_{50}, μM
5m	Racemic	2,3-Cl	72	0.16
7a	(R)	2,3-Cl	2	
7b	(S)	2,3-Cl	67	0.16
5p	Racemic	3,4-Cl	65	0.12
8a	(S)	3,4-Cl	19	
8b	(R)	3,4-Cl	72	0.07

Table 5
Ion channel selectivity of **7b** and **8b**.[9]



Compound	7b	8b
R	2,3-Cl	3,4-Cl
$K_V1.5$	IC_{50} 0.16 μM	IC_{50} 0.07 μM
HERG	41% inh (10 μM)	69% inh (10 μM)
I_{Na}[10]	14% inh (10 μM)	42% inh (10 μM)
$I_{Ca\ (L)}$	55% inh (10 μM)	59% inh (10 μM)
I_{KS}	7% inh (10 μM)	21% inh (10 μM)
I_{K1}	1% inh (10 μM)	3% inh (10 μM)

an (S)-configuration for 2,3-dichloro analog **7b** and an (R)-configuration for the 3,4-dichloro analog **8b**. For both compounds **7b** and **8b**, the C-4 aryl group is oriented back behind the plane of the paper.

Compounds **7b** and **8b** were evaluated for selectivity versus a panel of ion channels as reported in Table 5. Compounds **7b** and **8b** are both greater than 50 fold selective for $K_V1.5$ versus HERG, I_{Na}, I_{Ca} (L-type), I_{Ks}, and I_{K1} ion channels. The ion channel selectivity of these compounds suggests that they may be useful for the treatment of atrial fibrillation without the risk of ventricular proarrhythmia.

In summary, we have reported the discovery, initial SAR and optimization studies of a novel series of dihydropyrazolopyrimidine inhibitors of $K_V1.5$ (I_{Kur}). Future reports from these labs will describe our efforts to further improve the potency, selectivity and ADMET properties that will permit in vivo evaluation of the described dihydropyrazolopyrimidine class of $K_V1.5$ (I_{Kur}) inhibitors.

Acknowledgment

We thank Ruth Wexler and Michael Poss for their suggestions in the preparation of this manuscript.

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7. All compounds gave suitable NMR and LC/MS data.
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9. All data is an average of 2–4 determinations.
10. Sodium channel patch clamp experiments were performed at 0.2, 1 and 4 Hz. 1 Hz results are reported in Table 5.
11. *Preparation of 5–8 via Route 1: Step 1A:* A mixture of *t*-butoxyacetoacetate (6.8 mL, 45 mmol) and the appropriate disubstituted amine (41 mmol) in toluene (50 mL) was refluxed overnight. The mixture was cooled to room temperature, transferred to a separatory funnel, diluted with ethyl ether and extracted with aqueous HCl (1 M). The HCl extracts were combined and washed with ethyl ether, made basic (pH 9) with aqueous NaOH (50% w/w) and extracted with ethyl acetate. The ethyl acetate extracts were combined, washed with water and brine, dried over anhydrous sodium sulfate, filtered and concentrated to provide compound **3**. *Step 1B:* A mixture of compound **3** (40 mmol), the appropriate arylaldehyde (45 mmol), piperidine (1.0 ml, 10 mmol), acetic acid (0.59 mL, 10 mmol) in toluene (100 mL) were refluxed overnight with azeotropic removal of water via a Dean-Stark trap. The mixture was cooled to room temperature and concentrated in vacuo. *Step 1C:* The product of Step 1B (40 mmol) was dissolved in dimethylformamide (100 mL). 3-aminopyrazole (5.1 g, 62 mmol) and sodium acetate (10.1 g, 123 mmol) were added, and the mixture was stirred at 70 °C overnight (17 h). The reaction was cooled to room temperature, transferred to a separatory funnel, diluted with water and ethyl acetate, washed with water (a small amount of methanol was sometimes added to breakup emulsions that may form) and brine, dried over anhydrous sodium sulfate and concentrated. The resulting residue was purified by silica gel chromatography to provide compounds **5–8**.
12. *Preparation of 5–8 via Route 2: Step 2A:* A mixture of t-butoxyacetoacetate (23.4 mL, 141 mmol), the appropriate arylaldehyde (141 mmol), piperidine (3.5 ml, 35.3 mmol), and acetic acid (2.01 mL, 35.3 mmol) in toluene (300 mL) was refluxed overnight with azeotropic removal of water via a Dean-Stark trap. The mixture was cooled to room temperature and concentrated in vacuo. *Step 2B:* A mixture of the product of Step 2A (141 mmol), 3-aminopyrazole (17.6 g 212 mmol) and sodium acetate (46.3 g, 564 mmol) in dimethylformamide (300 mL) was stirred at 70 °C overnight (17 h). The mixture was cooled to room temperature, transferred to a separatory funnel, diluted with water and ethyl acetate, washed with water (a small amount of methanol was sometimes added to breakup emulsions that may form) and brine, dried over anhydrous sodium sulfate and concentrated. The resulting residue was purified by silica gel chromatography to provide compound **4**. *Step 2C:* HCl (4 M in dioxane) was added to compound **4** (2.97 mmol) at room temperature. The resulting thick reaction mixture was allowed to stir overnight. The mixture was concentrated in vacuo and used without further purification. *Step 2D:* The appropriate amine (0.47 mmol) was added to a suspension of the product of **Step 2C** (0.32 mmol), EDCI (0.09 g, 0.47 mmol), DMAP (0.004 g, 0.03 mmol) in dichloromethane (1 mL). When LC/MS analysis indicated the reaction was complete the mixture was loaded directly onto a silica cartridge (Worldwide Monitoring Clean-up cartridge, CUSIL12M6) which had been equilibrated with 100% hexanes. Elution with 100% hexanes (40 mL), followed by 50% Ethyl acetate/hexanes (40 mL) and 100% ethyl acetate (70 mL). The purest fractions (TLC analysis) were combined to give compounds **5–8**.