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Dihydropyrazolopyrimidines containing benzimidazoles as $K_V1.5$ potassium channel antagonists

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ABSTRACT

Dihydropyrazolopyrimidines with a C6 heterocycle substituent were found to have high potency for block of $K_V1.5$. Investigation of the substitution in the benzimidazole ring and the substituent in the 5-position of the dihydropyrazolopyrimidine ring produced **31a** with an IC_{50} for $K_V1.5$ block of 0.030 μM without significant block of other cardiac ion channels. This compound also showed good bioavailability in rats and robust pharmacodynamic effects in a rabbit model.

Atrial fibrillation (AF) is a cardiac arrhythmia that affects a large and growing population. In 1999, over 2 million cases of AF were reported in the United States and that number increased 2–3-fold over the previous 15 years.[1] The number of cases will likely grow in the future because the incidence of AF increases with age. It is predicted that 5.6 million adults in the US will have AF by 2050.[2] Although AF is not a fatal condition, the primary mortality risk is from stroke.[3] The inefficient emptying of the atrium caused by AF results in stasis of blood in the atrium and can lead to thrombus formation.

Re-polarization of membrane potential in cardiac myocytes by the outward flow of potassium ions is essential for regulating cardiac rhythm. The net potassium current is conducted through several different potassium channels, including the ultra-rapid (I_{Kur}), rapid (I_{Kr}), and slow (I_{Ks}) delayed rectifier potassium currents. The ultra-rapid potassium current (I_{Kur}) in humans is functionally expressed in the atrium but not ventricle.[4] Therefore, selectively blocking this current may lead to a method of treating atrial arrhythmia without increasing the risk of arrhythmias caused by prolonging ventricular refractory period.[5] I_{Kur} is conducted by the voltage gated potassium channel encoded by $K_V1.5$.[6] Drugs that block this channel have the potential to increase atrial action potential duration and prevent and/or terminate atrial fibrillation without the risk of ventricular effects.

In our previous paper, we described the discovery of dihydropyrazolopyrimidines as potent and selective blockers of $K_V1.5$.[7] We learned that amide substitution in the C6-position improved potency and that a wide variety of substituents were tolerated. The simple phenyl amide (**1**) and dipropylamide (**2**) were some of the more potent compounds in this series. This information suggested replacement of the amide with a heterocycle could provide potent and novel blockers of $K_V1.5$ (Fig. 1).

The dihydropyrazolopyrimidine template was synthesized as recently described.[7] The oxazole (**4**) was synthesized from the acid (**3**) by coupling to 2-aminoacetophenone followed by treatment of the resulting amide with phosphorus oxychloride at 120 °C (Scheme 1).

The 1,3,4-oxadiazoles (**5a,b**) were synthesized by coupling of the appropriate acyl hydrazines to the acid (**3**) followed by treatment with phosphorus oxychloride (Scheme 2).

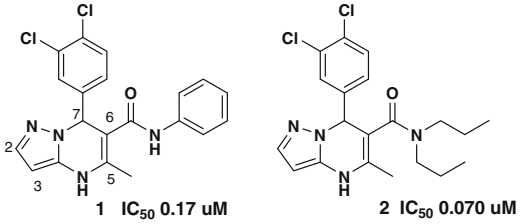

Figure 1. Potent pyrazolodihydropyrimidine $K_V1.5$ blockers.

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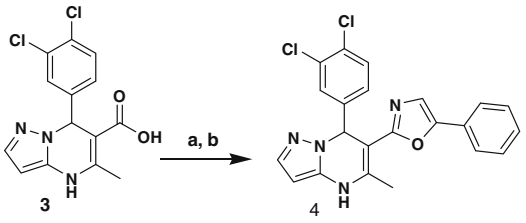
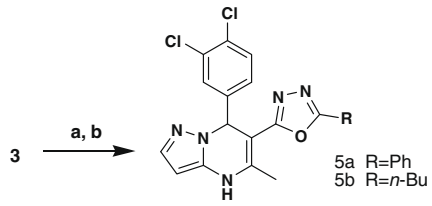


Scheme 1. Reagents and conditions: (a) $PhCOCH_2NH_2$, PyBroP, CH_2Cl_2, 39%; (b) $POCl_3$, 120 °C, 52%.



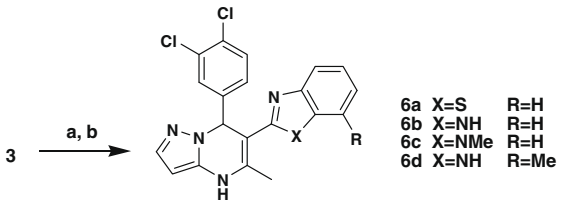
Scheme 2. Reagents and conditions: (a) $RCONHNH_2$, PyBroP, CH_2Cl_2 (30–50%); (b) $POCl_3$, 110 °C (20–50%).

Synthesis of the benzothiazole (**6a**) and the benzimidazoles (**6b–d**) were similarly accomplished by coupling of either o-phenylenediamine or 2-aminothiophenol to the acid (**3**) and cyclization with phosphorus oxychloride (Scheme 3).

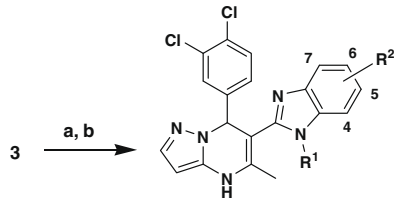
These reactions were quite general and the acid (**3**) could be coupled to various phenylenediamines (**26**) with substituents on either the aromatic ring or nitrogen (Scheme 4). In most cases a mixture of the two regioisomeric amides was formed. The mixtures of amides were cyclized to the substituted benzimidazoles using phosphorous oxychloride at 80–100 °C in 1–4 h.

The requisite phenylenediamines (**26**) were either commercially available or were synthesized by displacement of the appropriately substituted 2-fluoronitrobenzene with an amine. Reduction of the nitro group with zinc and acetic acid in methanol provided the phenylenediamines (Scheme 5).

In an effort to introduce polar substituents, we also synthesized compounds with a methoxymethyl group at the 5-position of the dihydropyrimidine ring system. These compounds were made using the Biginelli reaction in the same way as the previous compounds (Scheme 6). 4-Keto-5-methoxy ethyl butanoate (**28**) was synthesized from condensation of the methoxy acetyl chloride with Meldrum's acid (**27**) followed by decarboxylation with *tert*-butanol. The intermediate *t*-butyl ester (**29**) was condensed with 3,4-dichlorobenzaldehyde and 3-aminopyrazole using sodium bicarbonate in DMF to form the dihydropyrazolopyrimidine and subsequently deprotected to the carboxylic acid (**30**) using TMSOTf. This intermediate was condensed with phenylenediamines, then cyclized to form benzimidazoles. In this way we synthesized the fluorobenzimidazole (**31**). The racemic benzimidazole was purified by chiral HPLC (Chiracel AD column, 25% isopropanol, hexane, 0.1% TEA to give the individual enantiomers (**31a,b**).

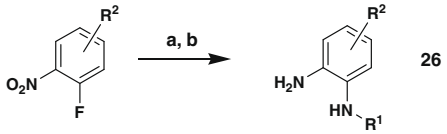


Scheme 3. Reagents and conditions: (a) 2-HX-Ph-NH_2, PyBroP, CH_2Cl_2 (25–70%); (b) $POCl_3$, 110 °C (20–40%).

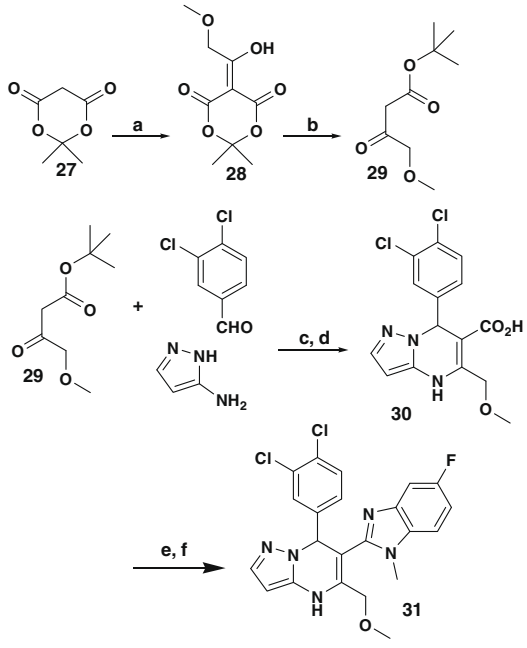


Compd	R^1	R^2	Compd	R^1	R^2
7	CH_3	4-F	**17**	CH_3	6-CN
8	CH_3	5-F	**18**	CH_3	6-CO_2CH_3
9	CH_3	6-F	**19**	CH_3	6-CF_3
10	CH_3	7-F	**20**	CH_3	6-SO_2CH_3
11	CH_3	4-Cl	**21**	CH_3	6-SO_2NHPh
12	CH_3	5-Cl	**22**	CH_3	6-$NHCOCH_3$
13	CH_3	6-Cl	**23**	Et	6-Cl
14	CH_3	4-CH_3	**24**	*i*-Pr	6-Cl
15	CH_3	5-CH_3	**25**	*n*-Pr	6-F
16	CH_3	6-CH_3			

Scheme 4. Reagents and conditions: (a) **26**, PyBroP, CH_2Cl_2 (30–70%); (b) $POCl_3$, 80–100 °C (25–70%).



Scheme 5. Reagents and conditions: (a) R^1NH_2, K_2CO_3, DMF, 45 °C (30–90%); (b) Zn, HOAc, MeOH (70–90%).



Scheme 6. Reagents and conditions: (a) $MeOCH_2COCl$, pyridine, DCM, 5 °C; (b) *t*-butanol, toluene, reflux; (c) $NaHCO_3$, DMF, 70 °C (99%, 3 steps); (d) TMSOTf, DCM, hexane, (99%); (e) **26**, EDCI, CH_2Cl_2; (f) AcOH, 100 °C (34%, 2 steps).

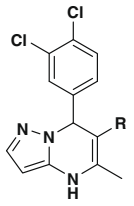
All the compounds described were tested for their ability to block the potassium current in mouse fibroblast L929 cells expressing human $K_V1.5$.[8] Our strategy was to investigate heterocyclic amide replacements, particularly, analogs of **1** and **2** with improved potency or physicochemical properties. Our initial amide

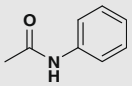
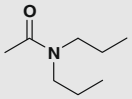
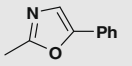
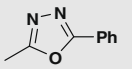
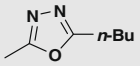
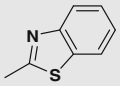
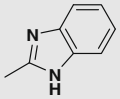
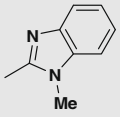
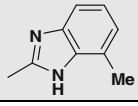
replacements were the oxazole (**4**) and 1,3,4-oxadiazoles (**5a,b**). Although these compounds contained the phenyl or alkyl functionality found in the amides (**1**, **2**), they lacked potency. We developed the hypothesis that the conformation of these inactive compounds was such that the heterocycle and the phenyl substituent would be orthogonal. This conformation would be in contrast to the conformation of the phenyl amide where the phenyl and the amide would be expected to be closer to coplanar. We hypothesized that the solution would be to fuse the phenyl ring to the heterocycle to force co-planarity. We first synthesized the benzothiazole (**6a**) and the benzimidazole (**6b**) to test this hypothesis. Both compounds were significantly more potent than the non-fused compounds. We next replaced the hydrogen on the amine of the benzimidazole with a methyl (**6c**) and the potency improved by eightfold. It was found that the methyl group did not have to be attached to the nitrogen to improve potency. The benzimidazole with the methyl substitution on the aromatic ring (**6d**) was over threefold more potent than the unsubstituted compound (**6b**) (Table 1). This may indicate the presence of a hydrophobic binding region that can be accessed from both positions. It also indicated that the presence of a hydrogen bond donor in the benzimidazole is not detrimental.

Substitution on the benzimidazole template was easily varied at either the nitrogen substituent or the ring substituent. We initially studied substitution on the aromatic ring and synthesized compounds with a halogen (F, Cl) or methyl substituent (Table 2). In all three cases, substitution in the 5 position was less favored than other positions. In the case of fluorine substitution, the 6-fluoro compound (**9**) was 10-fold more potent than the 5-fluoro analog (**8**) and threefold more potent than the unsubstituted compound (**6c**).

Because substitution in the 6-position was well tolerated, we synthesized compounds with a broader range of functional groups in this position (Table 3). As already mentioned, the fluorine and chlorine substituents showed similar or improved potency over the *N*-methylbenzimidazole (**6c**). The methyl (**16**), cyano (**17**), and ester (**18**) substituted compounds resulted in a 2–3-fold loss

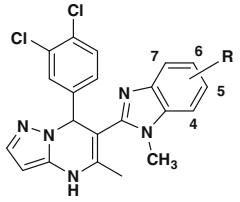
Table 1
Dihydropyrazolopyrimidine $K_V1.5$ blockers



Compd	R	$K_V1.5$ inhibition IC_{50}[a] (μM)
1		0.17
2		0.070
4		>1.0 (25% inh)
5a		>1.0 (18% inh)
5b		>1.0 (29% inh)
6a		0.74
6b		0.51
6c		0.075
6d		0.150

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

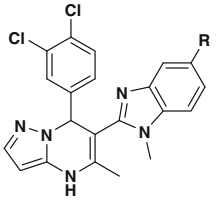
Table 2
Aromatic substitution



Compd	R	$K_V1.5$ inhibition IC_{50}[a] (μM)
6c	H	0.075
7	4-F	0.15
8	5-F	0.29
9	6-F	0.024
10	7-F	0.064
11	4-Cl	0.071
12	5-Cl	0.66
13	6-Cl	0.068
14	4-Me	0.11
15	5-Me	0.98
16	6-Me	0.20

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

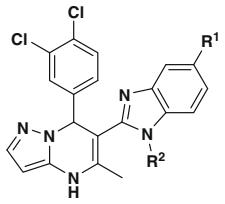
Table 3
6-substituted benzimidazoles



Compd	R	$K_V1.5$ inhibition IC_{50}[a] (μM)
6c	H	0.075
9	F	0.024
13	Cl	0.068
16	Me	0.20
17	CN	0.22
18	CO_2Me	0.20
19	CF_3	0.54
20	SO_2Me	>1.0 (29% inh)
21	SO_2NHPh	>1.0 (30% inh)
22	NHCOMe	>1.0 (8% inh)

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

Table 4
N-Substitution



Compd	R^1	R^2	$K_V1.5$ inhibition IC_{50}[a] (μM)
13	Cl	Me	0.068
23	Cl	Et	0.057
24	Cl	*i*-Pr	0.094
9	F	Me	0.024
25	F	*n*-Pr	0.048

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

in potency. The trifluoromethyl containing compound (**19**) showed a fivefold loss of potency and the sulfone (**20**), sulfonamide (**21**), and acetamide (**22**) were significantly less potent indicating that polar groups are not well tolerated in the binding site.

With both the chlorine and fluorine substituents in the 6-position, we varied the nitrogen substituent. In the 6-chlorine series, the methyl (**13**), ethyl (**23**), and isopropyl (**24**) substituents were of similar potency. Likewise in the 6-fluoro series the methyl (**13**) and *n*-propyl (**25**) compounds were equipotent (Table 4).

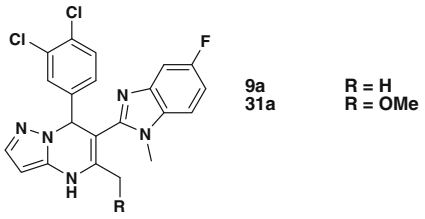
Compound **9** with the 6-fluoro substituent on the benzimidazole was one of the most potent so we synthesized and tested the 5-methoxymethyl analog (**31**). We made this change with the hope of introducing polar functionality that would improve physical properties. This compound had similar potency to the methyl analog (**9**). The individual enantiomers of both compounds were separated using chiral chromatography (Chiracel AD column, 1% *i*-propanol/hexane eluent). The active enantiomers (**9a** and **31a**) were very potent blockers of $K_V1.5$. Both compounds were tested for block of *h*ERG, sodium and calcium channels and **31a** was found to have >200-fold selectivity over these other ion channels (Table 5).

The pharmacokinetics of compounds **9a** and **31a** were investigated in rats (Table 6).[9] Compound **9a** 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%. Compound **31a** showed a longer half-life (1.5 h) with similar clearance and bioavailability.

Because of the acceptable pharmacokinetic profile and ion channel selectivity, the methoxymethyl compound (**31a**) was chosen for further in vivo characterization. The pharmacodynamic activity was tested in a rabbit model which measured the effective refractory period (ERP) in both atrium and ventricle (Fig. 2).[10] 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. There was no effect on ventricular ERP reflecting the selectivity for $K_V1.5$ over ventricular ion channels.

In conclusion, we have successfully replaced the amides of known I_{Kur} blockers (**1**, **2**) with benzimidazole. We have partially optimized the substituents on the aromatic ring and nitrogen of the benzimidazole and the substituent at the 5-position of the dihydropyrazolopyrimidine ring. We discovered compound **31a** to be a very potent and selective blocker of $K_V1.5$. This compound also had good oral bioavailability in rats and showed a significant pharmacodynamic effect in rabbits. For these reasons it was chosen for further preclinical development.

Table 5
Ion channel selectivity of **9a** and **31a**



Compd	$K_V1.5$ inh IC_{50}[a] (μM)	*h*ERG % inh 10 μM[a]	I_{Na} %inh 10 μM[a]	I_{Ca} % inh 10 μM[a]
9a	0.015	83	37	NT
31a	0.030	47	27	13

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

Table 6
Pharmacokinetic parameters of compound **9a** and **31a** in rats

	9a[a]	**31a**[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 3 animals.
[b] inf = intra-arterial infusion for 10 min.
[c] *po* = oral gavage.

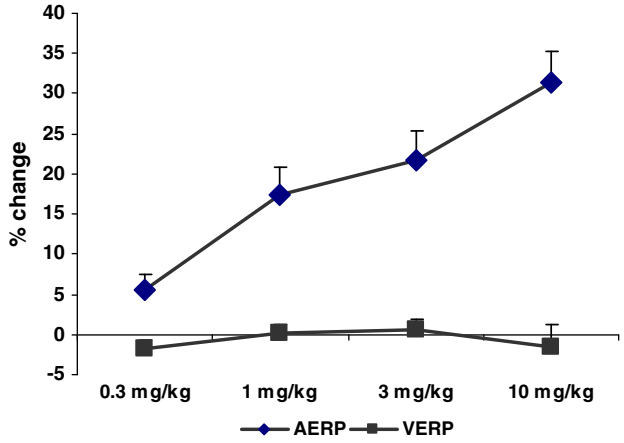


Figure 2. Pharmacodymanic effects of **31a** in rabbit.

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9. Compound **9a** was administered to rats, 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 concentration 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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