Discovery of 5-Phenyl-*N*-(pyridin-2-ylmethyl)-2-(pyrimidin-5-yl)quinazolin-4-amine as a Potent I_{Kur} Inhibitor

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Supporting Information

ABSTRACT: A new series of phenylquinazoline inhibitors of $K_v 1.5$ is disclosed. The series was optimized for $K_v 1.5$ potency, selectivity versus *h*ERG, pharmacokinetic exposure, and pharmacodynamic potency. 5-Phenyl-*N*-(pyridin-2-ylmethyl)-2-(pyrimidin-5-yl)-quinazolin-4-amine (**13k**) was identified as a potent and ion channel selective inhibitor with robust efficacy in the preclinical rat ventricular effective refractory period (VERP) model and the rabbit atrial effective refractory period (AERP) model.

Positional isomers and substituents optimized for potency

Optimized for potency

Optimized for potency, selectivity and PK

Compound 13k
 $K_v 1.5$ IC_{50} = 0.09 μ M

KEYWORDS: $K_v 1.5$, phenylquinazoline, ventricular effective refractory period, atrial effective refractory period

Atrial fibrillation (AF) is the most common form of sustained cardiac arrhythmia, and in addition to significantly affecting quality of life, AF is directly associated with increased risk of stroke (5-fold) and increased mortality (2-fold).¹ The prevalence of AF increases significantly with age and affects an estimated 34 million patients worldwide.² Recent approval of novel anticoagulants indicated for the treatment of AF have been successful in risk reduction with respect to stroke.³ These agents and rate control antiarrhythmics, however, do not restore normal sinus rhythm (NSR) in patients, and there is a potential additional benefit to maintaining NSR concomitant with anticoagulation therapy.⁴

The most widely used antiarrhythmic drugs, for example, amiodarone/dronedarone⁵ are efficacious but are nonselective and inhibit ion channels, which are expressed in the human atrium and ventricle. Agents that inhibit ion channels that prolong ventricular effective refractory period have the potentially life threatening arrhythmia torsades de pointes,⁶ and therefore, administration of nonselective drugs is often limited to a hospital setting with monitoring. There is currently an unmet medical need for agents that effectively restore NSR and have an increased margin for safety for the treatment of AF.

I_{Kur} is a delayed rectifier repolarization potassium current encoded by the *hKv 1.5* gene in humans,⁷ which is functionally expressed in the human atrium and not in the ventricle. Selective inhibition of I_{Kur} leads to a prolongation in effective refractory period and should terminate AF without being pro-

arrhythmic in the ventricle leading to a potentially safer treatment for patients with AF.⁸

We have recently disclosed optimized dihydro-pyrazolopyrimidines, **1**, and phenylcyclohexane heterocycles, **2** and **3**, as potent and selective blockers of I_{Kur} (Figure 1).^{9–11}

Figure 1. Recently disclosed $K_v 1.5$ inhibitors.

Concomitant with these efforts, we sought to identify and advance a structurally distinct back up series. Disclosed in the literature were thienylpyridine and furanopyrimidine series; however, we were concerned about the potential for reactive intermediate formation and modified the template to replace the thienyl/furano group.^{12,13} The resulting quinazoline chemotype is described extensively in the kinase literature.¹⁴ However, from analysis of the literature, we noted that

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substituents were generally required at the C6 and C7 position for compounds described with kinase activity.¹⁵ We synthesized regioisomers **6a–d** as described in Scheme 1 and were gratified

Scheme 1^a

Example	Phenyl position	K _v 1.5 potency (% inhibition at 0.3 μM, average of n=2,3)
6a	5	86 (IC ₅₀ 0.07 μM)
6b	6	14
6c	7	9
6d	8	9

^aReagents and conditions: (a) NaOH; (b) H₂O₂, crude yield range, 2 steps 50–88%; (c) NaOCN, H₂O, yield range, 50–88%; (d) PhB(OH)₂, Pd(PPh₃)₄, K₂CO₃, dioxane, H₂O, yield range, 44–50%; (e) POCl₃, DIPEA, yield range, 36–59%; (f) amino-methyl-2-pyridine, 65 °C, 14 h, yield range, 75–91%.

to see that only the C5 phenyl analogue, **6a** had K_v 1.5 potency¹⁶ and selectivity for hERG^{17,18} (hERG flux IC₅₀ > 80 μM). In addition, compound **6a** was assayed against an in house kinase panel and found to be devoid of kinase activity for those kinases tested.

Despite low rat liver microsomal stability, Compound **6a** demonstrated sufficient rat *i.v.* pharmacokinetic exposure (Table 1) to test in the rat hemodynamic and VERP

Table 1. Rat Pharmacokinetic Profile for Compounds **6a** and **13k**^a

	compound 6a (<i>i.v.</i> 2 mg/kg p.o. 5 mg/kg)		compound 13k (<i>i.v.</i> 2 mg/kg p.o. 5 mg/kg)	
clearance (mL/min/kg)	8.2 ± 2.6	NC	4.6 ± 1.8	NC
V _{ss} (L/kg)	2.3 ± 1.7	NC	5.2 ± 3.2	NC
T _{1/2} (h)	7.3 ± 0.6	1.9 ± 1.3	15 ± 3.9	3.5 ± 1.3
bioavailability (%)	NC	10	NC	6.3

^aSprague–Dawley rats, male, approximate weight 250 g, isofluorene anesthesia, *i.v.* dosed in PEG-400/PG/ethanol (50/40/10), collection from the jugular vein at eight timepoints, *n* = 3. NC = not calculated.

model.¹¹ In human and rabbit, I_{Kur} is functionally expressed in atrium and not ventricle; however, in rats, it is expressed in both. Therefore, in the rat model, changes in VERP were measured using a multielectrode catheter inserted into the left ventricle for pharmacodynamic effect; and in the rabbit model, changes in AERP were measured. The VERP increase in the rat model at plasma concentration 26 ± 4 μM (10 mg/kg *i.v.* infusion over 10 min, *n* = 2) was 98 ± 3%, and the compound was subsequently also tested in the rabbit AERP model.¹⁹ A modest 9.0 ± 2.4% AERP increase was observed (3 mg/kg *i.v.* infusion over 30 min, plasma concentration at the end of the infusion of 9.1 ± 1.1 μM, *n* = 4). We, therefore, focused our subsequent efforts on analogues with the C5 phenyl group and further optimized the substituent at the C2 position with the goal of increasing both the *in vitro* potency and pharmacodynamic potency. Additional groups at C2 were explored (synthetic routes are shown in Schemes 2 and 3), and structure–activity relationships (SARs) indicated that electron

rich substituents at C2 were not as potent for K_v 1.5 and did not maintain selectivity versus hERG.

Scheme 2^a

Example	R	Synthetic steps	K _v 1.5 potency (IC ₅₀ μM)	hERG potency (Flux, IC ₅₀ μM)
7	N(CH ₃) ₂	a	59% ^b	2.5
8	CN	b	0.05	> 80
9	CH ₃	c	72% ^b	4.6
10	Cyclopropyl	d	0.17	ND
11	CO ₂ NH ₂	b, e	9% ^b	ND
12	CF ₃	Scheme 3	0.08	10

^aReagents and conditions: (a) Dimethylaniline in MeOH, 2M, 150 °C, microwave irradiation, 10 min, quantitative yield; (b) Zn(CN)₂, Zn, Pd₂(dba)₃·dppf, DMA, 150 °C, microwave irradiation, 30 min, 21% yield; (c) Al(CH₃)₃, THF, Pd(PPh₃)₄, 50 °C, 90 min, 75 °C 14 h, 100 °C, microwave irradiation, 5 min, 21% yield; (d) ZnBr₂, Pd(dppf)Cl₂·DCM, THF, cyclopropylmagnesium bromide, −78 °C 1 h to RT 18 h, 7% yield; (e) KOH, THF, H₂O, 100 °C, microwave irradiation, 30 min, 120 °C, microwave irradiation, 30 min, 48% yield. ^b% inhibition at 0.3 μM, average of *n* = 2,3.

Scheme 3^a

^aReagents and conditions: (a) pyridine, trifluoroacetic anhydride, chloroform, RT addition; reflux 2 h, RT addition of NH₃(g), 37% yield; (b) Pd(OAc)₂, KF, 2-(di-*tert*-butylphosphino)biphenyl, phenylboronic acid, microwave irradiation, 100 °C, 10 min; (c) POCl₃, *N,N*-dimethylaniline, 100 °C, 10 min then DCM, 1.5 M KH₂PO₄, 83% yield for 2 steps; (d) pyridin-2-ylmethanamine, TEA, RT, 46% yield.

Compound **8** had good potency and selectivity versus hERG, thus combining the sp² character at C2 with incorporation of additional heteroatoms led to the synthesis of targeted heterocycles. Various C2 heterocycle analogues were prepared from **6a** as shown in Scheme 4. Multiple heterocycles including **13a**, **13c**, **13e**, and **13g–13k** were potent inhibitors of K_v 1.5, however, all had low selectivity versus hERG with the exception of **13f**. On further profiling, compound **13f** had low microsomal metabolic stability (7% remaining at 10 min in rat and 22% remaining in mouse) and was a potent inhibitor of CYP2C9 (1 μM) and CYP2C19 (1 μM). Compound **13k** demonstrated potency, selectivity versus hERG, acceptable metabolic stability (58% remaining at 10 min in rat and 75% remaining in mouse) and CYP profile CYP2C9 (4 μM) and CYP2C19 (3 μM). Structurally close analogues to **13k**, compounds **13l** and **13m**, indicated a narrow SAR for potency and hERG selectivity with substitution. The C2 pyrimidine was therefore selected as the optimal heterocycle to further explore SAR in the series.

We also surveyed the C4 position extensively keeping the C2 pyrimidine constant, including positional isomers of the pyridine, as well as extensive library synthesis at C2 (not

Scheme 4^a

Example	R	Synthetic steps	K _v 1.5 potency (IC ₅₀ , μM)	hERG potency (Flux IC ₅₀ , μM)
13a		a	0.05	6.2
13b		a	41% ^b	2.7
13c		b	0.09	3.1
13d		b	20% ^b	ND
13e		b	0.16	88% ^c at 1 μM
13f		b	0.06	20
13g		b	0.12	8.4
13h		b	0.04	6.7
13i		b	0.13	51% ^c at 1 μM
13j		b,c	0.11	91% ^c at 0.3 μM
13k		b	0.09	43% ^c at 10 μM
13l		b	0.10	1.9
13m		b	20% ^b	ND

^aReagents and conditions: (a) RSnBu₃, Pd(PPh₃)₂Cl₂, DMF, THF, 100 °C, microwave irradiation, 60 min, 46 and 50% yield; (b) RB(OH)₂, K₂CO₃, Pd(PPh₃)₂Cl₂, DME, EtOH, H₂O, 100 °C, microwave irradiation, 10 min, yield range 16–86%; (c) BBr₃, DCM, 0 °C, 18 h, 77% yield. ^b% inhibition at 0.3 μM, average of *n* = 2,3. ^c% inhibition at given concentration, average of *n* = 2,3.

shown); however, we did not identify compounds with increased K_v 1.5 potency. We subsequently combined substitution on the phenyl group at the C5 position keeping the C4 aminomethylpyridine and the C2 pyrimidine in place using the chemistry described in Schemes 5 (compounds 14a–14d). Modification at C5 did not result in compounds with improved potency or selectivity profiles; thus, compound 13k was selected as the lead compound for further *in vivo* evaluation.

Pharmacokinetic exposure of compound 13k was measured in rats dosed *p.o.* at 5 mg/kg and was comparable to exposure

Scheme 5^a

Example	X	Synthetic steps	K _v 1.5 potency (IC ₅₀ , μM or % inhibition at 0.3 μM)
14a	4-F	a, b, c, d	0.25
14b	3-F	a, b, c, d	37%
14c	4-CF ₃	a, b, c, d	0.15
14d	4-CONH ₂	a, b, c, d, e, f	8%

^aReagents and conditions: (a) Ph(X)B(OH)₂, Na₂CO₃, Pd(dppf)-Cl₂DCM complex, DMF, H₂O, 110 °C, microwave irradiation, 14 h, yield quantitative; (b) POCl₃, DIPEA, yield range, 35–50%; (c) aminomethyl-2-pyridine, DIPEA, THF yield range, 46–80%; (d) pyrimidin-5-ylboronic acid, Pd(dppf)Cl₂DCM complex, DMF, H₂O, 110 °C, microwave irradiation, 14 h, yield range, 29–99%; (e) LiOH, MeOH, H₂O, yield 48%; (f) EDC.HCl, HOBT, DMF, DIPEA, NH₄OAc, RT, 14 h, yield 56%.

observed for compound 6a (Table 1). Compound 13k was screened in the rat VERP model¹¹ at 3 and 10 mg/kg and demonstrated robust effects in the absence of seizures at plasma concentrations of 7.7 μM (10 mg/kg). Compound 13k was subsequently dosed at 3 mg/kg, infusion over 30 min in the anesthetized rabbit pharmacodynamic model, and a robust increase of 21 ± 2.8% in AERP was observed without significant effect on VERP, QTc (Delta QTc cf, 13 ± 4.2 ms), or BP (Table 2).¹⁷

Table 2. Rabbit Pharmacodynamic Profile for Compounds 6a and 13k (3 mg/kg Infusion over 30 min, Parameters at 20 min, *n* = 3 or *n* = 4)

	compound 6a	compound 13k
vehicle	DMF	DMF
% AERP increase	9 ± 2.4	21.4 ± 2.8
% change in BP (systolic, mean BP)	0.1 ± 5.4, −1.0 ± 4.9	−3.4 ± 3.1, −3.8 ± 4.0
% change in heart rate	−3.2 ± 4.1	−3.1 ± 2.0
plasma exposure (μM)	9.1 ± 1.1	2.7 ± 1.5

We were encouraged by the robust increase in AERP in this model; however, in the rat VERP studies, a significant level of brain penetration was observed (3 mg/kg *i.v.* 5 min infusion, 10 min post-end of infusion C_{plasma} 3.1 ± 0.3 μM, brain exposure 8.8 ± 1.6 μM; 10 mg/kg *i.v.* 5 min infusion, 10 min post-end of infusion C_{plasma} 7.7 μM, brain exposure 25 μM; *n* = 3). Due to the multiple potassium ion channels expressed in the brain, differential distribution in species,²⁰ and the difficulty in screening compounds broadly for ion channel activity, we focused our efforts on significantly reducing brain penetration while maintaining good efficacy in the rabbit PD model. Our efforts to identify and further optimize this series to our current clinical candidate will be disclosed in a subsequent manuscript.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.6b00117.

Experimental procedures and analytical data for compounds 4 to 14d (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

VERP, ventricular effective refractory period; AERP, atrial effective refractory period; AF, atrial fibrillation; NSR, normal sinus rhythm

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