Letter

Discovery of 5-phenyl-N-(pyridin-2-ylmethyl)-2-(pyrimidin-5-yl)quinazolin-4-amine as a potent *I* inhibitor

Heather J. Finlay, James A. Johnson, John L. Lloyd, Ji Jiang, James Neels, Prashantha Gunaga, Abhisek Banerjee, Naveen Dhondi, Anjaneya Chimalakonda, Sandhya Mandlekar, MaryLee Conder, Harinath Sale, Dezhi Xing, Paul C. Levesque, and Ruth R. Wexler

ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.6b00117 • Publication Date (Web): 09 Jun 2016

Discovery of 5-phenyl-N-(pyridin-2-ylmethyl)-2-(pyrimidin-5-yl)quinazolin-4-amine as a potent I_{Kur} inhibitor

Heather J. Finlay,[*,†] James A. Johnson,[†] John L. Lloyd,[†] Ji Jiang,[†] James Neels,[†], Prashantha Gunaga, [† †] Abhisek Banerjee, [† †] Naveen Dhondi, [† †] Anjaneya Chimalakonda, [#] Sandhya Mandlekar, [##] Mary Lee Conder, [‡] Harinath Sale[##] Dezhi Xing, [‡] Paul Levesque, [‡] Ruth R. Wexler. [†]

[†]Departments of Discovery Chemistry, [‡]Biology and [#]Preclinical Candidate Optimization, Bristol-Myers Squibb, Research and Development, P.O. Box 5400, Princeton, New Jersey 08543-5400.

[† †]Departments of Discovery Chemistry, [‡‡]Biology and [##]Preclinical Candidate Optimization, BBRC, Bangalore, India

[*]To whom correspondence should be addressed. Heather Finlay Phone: 609-818-3734. Fax: 609-818-3550. E-mail: heather.finlay@bms.com.

KEYWORDS (Word Style "BG_Keywords"). If you are submitting your paper to a journal that requires keywords, provide significant keywords to aid the reader in literature retrieval.

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 pre-clinical rat ventricular effective refractory period (VERP) model and the rabbit atrial effective refractory period (AERP) model.

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 anti-coagulants indicated for the treatment of AF have been successful in risk reduction with respect to stroke.[3] These agents and rate control anti-arrhythmics, however, do not restore normal sinus rhythm (NSR) in patients and there is a potential additional benefit to maintaining NSR concomitant with anti-coagulation therapy.[4]

The most widely used anti-arrhythmic drugs, for example amiodarone/dronedarone[5] are efficacious, but are non-selective and inhibit ion channels which are expressed in the human atrium and ventricle. Agents which inhibit ion channels that prolong ventricular effective refractory period have the potentially life threatening arrhythmia torsades de pointe[6] and, therefore, administration of non-selective drugs is often limited to a hospital setting with monitoring. There is currently an unmet medical need for agents which 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 *h*Kv 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 dihydropyrazolopyrimidines, 1 and phenylcyclohexane heterocycles 2 and 3 as potent and selective blockers of I_{Kur} (Figure 1).[9,10,11]

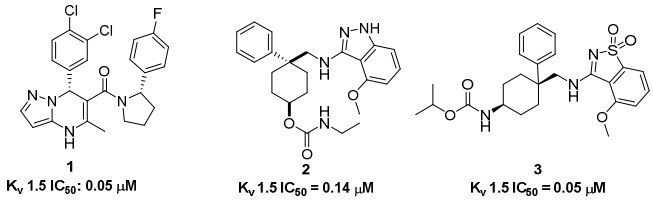
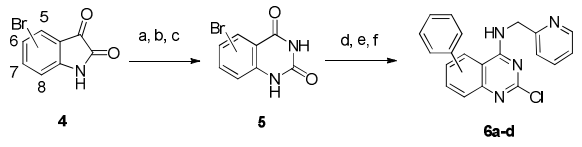


Figure 1. Recently disclosed K_v 1.5 inhibitors

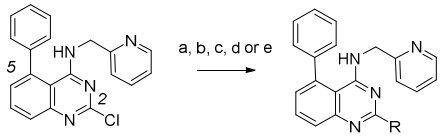
Concomitant with these efforts, we sought to identify and advance a structurally distinct back up series. Disclosed in the literature were thienylpyridine and furanopyrimidine series, however, we were concerned about the potential for reactive intermediate formation and modified the template to replace 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 substitutents 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 to see that only the C5 phenyl analog, 6a had K_v 1.5 potency [16] and selectivity for *h*ERG[17,18] (*h*ERG flux IC_{50} > 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.



Example	Phenyl position	K_V1.5 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

Scheme 1. Reagents and conditions: a) NaOH; b) H_2O_2, crude yield range, 2 steps 50-88%; c) NaOCN, H_2O, yield range, 50% - 88%; d) $PhB(OH)_2$, $Pd(PPh_3)_4$, K_2CO_3, dioxane, H_2O, yield range, 44% - 50%; e) $POCl_3$, DIPEA, yield range, 36% - 59%; f) amino-methyl-2-pyridine, 65 °C, 14h, yield range, 75% - 91%.

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 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 minutes, 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 mins, plasma concentration at the end of the infusion of 9.1 ± 1.1μM, n = 4). We, therefore, focused our subsequent efforts on analogs 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 SAR indicated that electron rich substituents at C2 were not as potent for K_v 1.5 and did not maintain selectivity versus *h*ERG.

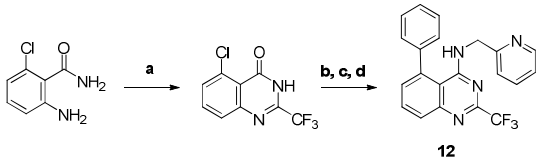


Example	R	Synthetic steps	K_V1.5 potency (IC_{50}, μM)	*h*ERG potency (Flux, IC_{50}, μM)
7	$N(CH_3)_2$	a	59%*	2.5
8	CN	b	0.05	> 80
9	CH_3	c	72%*	4.6
10	Cyclopropyl	d	0.17	ND
11	CO_2NH_2	b, e	9%*	ND
12	CF_3	Scheme 3	0.08	10

3

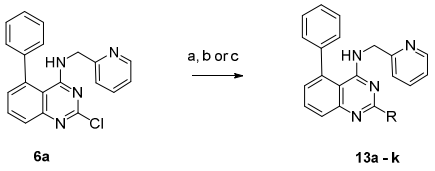
*** % inhibition at 0.3 μM, average of n=2,3**

Scheme 2. Reagents and conditions: a) Dimethylaniline in MeOH, 2M, 150 °C, microwave irradiation, 10 min, quantitative yield; b) $Zn(CN)_2$, Zn, $Pd_2(dba)_3$.dppf, DMA, 150 °C, microwave irradiation, 30 min, 21% yield; c) $Al(CH_3)_3$, THF, $Pd(PPh_3)_4$, 50 °C, 90 min, 75 °C 14 h, 100 °C, microwave irradiation, 5 min, 21% yield; d) $ZnBr_2$, $Pd(dppf)Cl_2$.DCM, THF, cyclopropylmagnesium bromide, -78 °C, 1h to RT 18h, 7% yield; e) KOH, THF, H_2O, 100 °C, microwave irradiation, 30 min, 120 °C, microwave irradiation, 30 min, 48% yield.



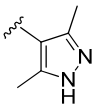
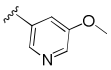
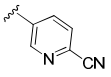
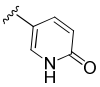
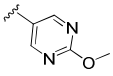
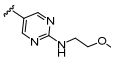
Scheme 3. Reagents and conditions: a) Pyridine, trifluoroacetic anhydride, chloroform, RT addition; reflux 2 h, RT addition of NH_3(g), 37% yield; b) $Pd(OAc)_2$, KF, 2-(di-*tert*-butylphosphino)biphenyl, phenylboronic acid, microwave irradiation, 100 °C, 10 min; c) $POCl_3$, N,N-dimethylaniline, 100 °C, 10 min then DCM, 1.5 M KH_2PO_4, 83% yield for 2 steps; d) Pyridin-2-ylmethanamine, TEA, RT, 46% yield.

Compound 8 had good potency and selectivity versus *h*ERG, thus combining the sp^2 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, 13g, 13h, 13i, **13j, 13k were potent inhibitors of Kv1.5, however, all had low selectivity versus *h*ERG 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 *h*ERG, 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 *h*ERG selectivity with substitution. The C2 pyrimidine was therefore selected as the optimal heterocycle to further explore SAR in the series.**



Scheme 4. Reagents and conditions: a) $RSnBu_3$, $Pd(PPh_3)_2Cl_2$, DMF, THF, 100 °C, microwave irradiation, 60 min, 46 and 50% yield; b) $RB(OH)_2$, K_2CO_3, $Pd(PPh_3)_2Cl_2$, DME, EtOH, H_2O, 100 °C, microwave irradiation, 10 min, yield range 16 – 86%; c) BBr_3, DCM, 0 °C, 18h,77% yield.

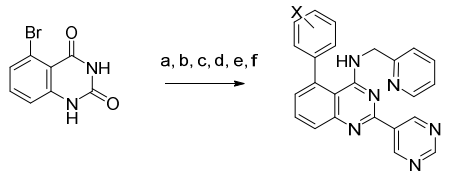
Example	R	Synthetic steps	K_V1.5 potency (IC_{50}, μM)	*h*ERG potency (Flux IC_{50}, μM)
13a		a	0.05	6.2

13b		**a**	**41%***	**2.7**
13c		**b**	**0.09**	**3.1**
13d		**b**	**20%***	**ND**
13e		**b**	**0.16**	**88%** at 1μM**
13f		**b**	**0.06**	**20**
13g		**b**	**0.12**	**8.4**
13h		**b**	**0.04**	**6.7**
13i		**b**	**0.13**	**51%** at 1μM**
13j		**b,c**	**0.11**	**91%** at 0.3μM**
13k		**b**	**0.09**	**43%** at 10μM**
13l		**b**	**0.10**	**1.9**
13m		**b**	**20%***	**ND**

*** % inhibition at 0.3 μM, average of n=2,3**

**** % inhibition at concentration, average of n=2,3**

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 shown) however, we did not identify compounds with increased Kv 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.



5a **14a-d**

Scheme 5. Reagents and conditions: Yields a) $Ph(X)B(OH)_2$, Na_2CO_3, $Pd(dppf)Cl_2DCM$ complex, DMF, H_2O, 110 °C, microwave irradiation, 14 h, yield quantitative; b) $POCl_3$, DIPEA, yield range, 35% - 50%; c) aminomethyl-2-pyridine, DIPEA, THF yield range, 46% - 80%; d) pyrimidin-5-ylboronic acid, $Pd(dppf)Cl_2DCM$ complex, DMF, H_2O, 110 °C, microwave irradiation, 14 h, yield range, 29% - 99%; e) LiOH, MeOH, H_2O, yield 48%; f) EDC.HCl, HOBt, DMF, DIPEA, NH_4OAc, RT, 14 h, yield 56%.

Example	X	Synthetic steps	K_V1.5 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%**

Pharmacokinetic exposure of compound 13k was measured in rats dosed *p.o.* at 5 mg/Kg and was comparable to exposure observed for Compound 6a (Table 1). Compound 13k was screened in the rat VERP model[11] 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]

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 minute infusion, 10 minute post end of infusion C_{plasma} 3.1 ± 0.3 μM, brain exposure 8.8 ± 1.6 μM. 10 mg/Kg *i.v.* 5 minute infusion, 10 minute 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.

	Compound 6a (*i.v.* 2 mg/Kg *p.o.* 5 mg/Kg)		Compound 13k (*i.v.* 2 mg/Kg *p.o.* 5 mg/Kg)	
Clearance (mL/min/Kg)	8.2±2.6	NC	4.6±1.8	NC

Vss (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

TABLE 1. Rat pharmacokinetic profile for Compounds 6a and 13k. Sprague Dawley rats, male, approximate weight 250g, isofluorene anesthesia, *i.v.* dosed in PEG-400/PG/ethanol (50/40/10), collection from the jugular vein at 8 timepoints, n = 3. NC = not calculated.

	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

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)

ASSOCIATED CONTENT

Supporting Information. Experimental procedures and analytical data for Compounds 4 to 14d is supplied as Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

ACKNOWLEDGMENT

The authors would like to acknowledge with thanks, Robert Languish for running the high resolution mass spectral analysis of all final compounds.

ABBREVIATIONS

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

REFERENCES

1. Zimetbaum, P. Antiarrhythmic drug therapy for atrial fibrillation *Circulation* **2012**, *125*, 381-389.
2. Chugh, S.S.; Havmoeller, R.; Narayanan, K.; Singh, D.; Rienstra, M.; Benjamin, E.J.; Gillum, R.F.; Kim, Y.; McAnulty, J.H.; Zheng, Z.; Forouzanfar, M.H.; Naghavi, M.; Mensah, G.A.; Ezzati, M.; Murray, C.J.L. Worldwide epidemiology of atrial fibrillation: a Global Burden of Disease 2010 Study *Circulation* **2014**, *129*, 837-847.
3. Bassand, J. Review of atrial fibrillation outcome trials of oral anticoagulant and antiplatelet agents *Europace,* **2012**, *14,* 312-324.
4. Hohnloser, S.H.; Crijns, H.J.G.M.; van Eickels, M.; Gaudin, C.; Page, R.L.; Torp-Pedersen, C.; Connolly, S.J. Effect of dronedarone on cardiovascular events in atrial fibrillation *N. Engl. .J Med.,* **2009**, *360*, 668-678.
5. Sun, W.; Sarma, J.S.; Singh, B.N. Electrophysiological Effects of Dronedarone (SR33589), a Noniodinated Benzofuran Derivative, in the Rabbit Heart: Comparison With Amiodarone *Circulation,* **1999***, 100 (22)*, 2276-2281.
6. De Bruin, M. L.; Hoes, A. W.; Leufkens, H. G. QTc-prolonging drugs and hospitalizations for cardiac arrhythmias *Am. J. Cardiol.* **2003**, *91*, 59-62.
7. Ford, J. W.; Milnes, J. T. New Drugs Targeting the Cardiac Ultra-Rapid Delayed-Rectifier Current (IKur): Rationale, Pharmacology and Evidence for Potential Therapeutic Value *J. Cardiovasc. Pharmacol.* **2008**, *52* (2), 105-120.
8. Nattel, S. *Nature* New ideas about atrial fibrillation 50 years on **2002**, *415*, 219-226.
9. Lloyd, J; Finlay, H. J.; Vacarro, W; Hyunh, T; Kover, A; Bhandaru, R; Yan, L; Atwal, K; Conder, M; Jenkins-West, T; Shi, Hong; Huang, C; Li, D; Sun, H; Levesque, P. Pyrrolidine amides of pyrazolodihydropyrimidines as potent and selective KV1.5 blockers *Bioorg. Med. Chem. Lett.* **2010***, 20 (4),* 1436-1439.
10. Johnson, James A.; Xu, Ningning; Jeon, Yoon; Finlay, Heather J.; Kover, Alexander; Conder, Mary L.; Sun, Huabin; Li, Danshi; Levesque, Paul; Hsueh, Mei-Mann. Design, synthesis and evaluation of phenethylaminoheterocycles as Kv1.5 inhibitors *Bioorg. Med. Chem. Lett.* **2014***, 24 (14),* 3018-3022.
11. Lloyd, John; Finlay, Heather J.; Kover, Alexander; Johnson, James; Pi, Zulan; Jiang, Ji; Neels, James; Cavallaro, Cullen; Wexler, Ruth; Conder, Mary Lee. Pseudosaccharin amines as potent and selective K_V1.5 blockers *Bioorg. Med. Chem. Lett.* **2015**, 25(21), 4983-4986.
12. Palmer, N.J.; Madge, John, D., Ford, J., Atherall, J.F. Preparation of thienopyridine derivatives as potassium channel inhibitors for the treatment of arrhythmia and diabetes WO 2006061642 A1.
13. Atherall, F.J., Ford, J., Madge, D.; Palmer, N.J. Preparation of furanopyrimidine derivatives effective as potassium channel inhibitors WO 2005121149 A1.
14. Harris, C.S.; Hennequin, L.; Morgentin, R.; Pasquet, G. Synthesis and functionalization of 4-substituted quinazolines as kinase templates *Targets in Heterocyclic Systems* **2010**, *14*, 315-350.
15. Stauffer, F.; Furet, P. Patent Application Preparation of 2,4-substituted quinazolines as lipid kinase inhibitors WO 2008012326.
16. Synders, D.J.; Tamakun, M.N.; Bennett, P.B. A rapidly activating and slowly inactivating potassium channel cloned from human heart: functional analysis after stable mammalian cell culture expression. *J. Gen. Physiol.* 1993, 101, 513-543.
17. Weaver CD, Harden D, Dworetzky SI, Robertson B, Knox RJ. A thallium-sensitive, fluorescence-based assay for detecting and characterizing potassium channel modulators in mammalian cells. *J. Biomol Screen.* **2004**, *9(8)*, 671-7.
18. Zhou, Z.; Vorperian, V.R.; Gong, Q.; Zhang, S. and January, C.T. Block of HERG potassium channels by the antihistamine astemizole and its metabolites desmethylastemizole and norastemizole. *J. Cardiovasc. Electrophysiol*., 1999, 10, (6), 836-843.
19. Carlsson, L. The anaesthetised methoxamine-sensitised rabbit model of torsades de pointes *Pharmacol. Ther.*, **2008**, *119(2)*, 160-167.
20. Vacher, H. Structure, Function, and Modulation of Neuronal Voltage-Gated Ion Channels (2009), 127. Publisher: (John Wiley & Sons, Inc., Hoboken, N. J)

For Table of Contents Only