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Pseudosaccharin amines as potent and selective $K_V1.5$ blockers

John Lloyd *, Heather J. Finlay, Alexander Kover, James Johnson, Zulan Pi, Ji Jiang, James Neels, Cullen Cavallaro, Ruth Wexler, Mary Lee Conder, Hong Shi, Danshi Li, Huabin Sun, Anjaneya Chimalakonda, Christine Huang, Mark Salvati, Paul Levesque

Bristol-Myers Squibb Research and Development, PO Box 5400, Princeton, NJ 08534-5400, USA

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ABSTRACT

Phenethyl aminoheterocycles like compound **1** were known to be potent I_{Kur} blockers although they lacked potency in vivo. Modification of the heterocycle led to the design and synthesis of pseudosaccharin amines. Compounds such as **14**, **17d** and **21c** were found to be potent $K_V1.5$ blockers and selective over other cardiac ion channels. These compounds had potent pharmacodynamic activity, however, they also showed off-target activities such as hemodynamic effects.

Atrial fibrillation (AF) is the most common cardiac arrhythmia seen in clinical practice and effects over 2.2 million patients in the United States.[1] The prevalence of AF increases with age and with an aging population it is estimated by 2050 there will be 5.6 million cases of AF in the US.[2] Although some AF is asymptomatic, many patients suffer from fatigue, syncope, palpitations and decreased quality of life. In addition to these symptoms, AF is associated with a 5-fold increase in the incidence of strokes and a 2-fold increase in overall mortality.[3] One approach to drug treatment has been to restore and maintain normal sinus rhythm. This could be achieved by prolongation of the refractory period in cardiac myocytes by blocking the ion currents responsible for repolarization. Existing drug treatments such as sodium channel blockers (flecainide and propafenone) and I_{Kr} potassium channel blockers (sotalol and dofetilide) are effective at prolonging refractory period but have proven to be pro-arrhythmic because they affect repolarizing currents in both the 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] More recently, targets for drug therapy that are specific to the atrium have been sought as a potentially safer and more effective way to treat AF.[5] The ultra-rapid potassium current (I_{Kur}) is a current conducted by the $K_V1.5$ potassium channel that in humans is expressed only in the atrium.[6] Therefore, block of I_{Kur} could potentially provide a way to treat AF without the risk of ventricular arrhythmia.

In our previous publication, we disclosed $K_V1.5$ blockers based on an aminoheterocycle with a phenethyl substituent.[7] Among those, the aminobenzisoxazole (**1**) and aminobenzoisothiazole (**2**) showed significant block of $K_V1.5$ in isolated cells, however, they did not show any increase of atrial effective refractory period (AERP) in rabbits. The isoindolone (**3**) showed short lived effects on AERP and chemical instability. This Letter will describe our efforts to extend the SAR to other heterocyclic structures, specifically for pseudosaccharin amines (A = SO_2), and to further optimize these compounds with regard to chemical stability, pharmacokinetic properties and pharmacodynamic effects (Fig. 1).

Synthesis of the pseudosaccharin core structure started with 3-fluorobenzene sulfonyl chloride (**4**) that reacted with *t*-butylamine to form the *t*-butyl sulfonamide (**5**). With the benefit of two directing groups, the metalation followed by reaction with carbon dioxide proceeded regioselectively to provide the benzoic acid (**6**). The benzoic acid cyclized to the 4-fluoro saccharine (**7**) in good yield under acidic conditions. The fluorine was replaced by methoxy with microwave heating and the saccharin was converted to the thioethyl pseudosaccharin (**9**)[8] that was used in the subsequent syntheses (Scheme 1).

We synthesized several compounds with a phenethylamino substituent on the pseudosaccharin ring system. The most potent contained a spirocyclic cyclohexane ring substituent on the benzylic carbon. The compounds with substituents on the cyclohexane ring that were linked through oxygen at the 4-position were synthesized from 4-cyano-4-phenylcyclohexanone (**10**) which was protected as the ethylene glycol ketal (**11**) followed by reduction

* Corresponding author.

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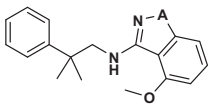


compd	A	$K_V1.5$ IC_{50} (uM)
1	O	0.043
2	S	0.024
3	CO	0.034

Figure 1. Phenethylamino heterocycle $K_V1.5$ antagonists.

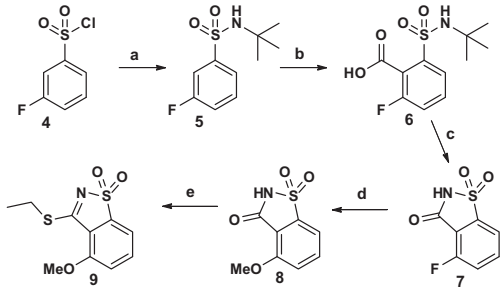
of the nitrile to the amine (**12**) (Scheme 2). Reaction with thioethylpseudosaccharin (**9**) followed by deprotection provided the ketone (**13**). Reduction with sodium borohydride gave a mixture (2:1) of the *cis* (**14**) and *trans* (**15**) isomers that could be separated by column chromatography. The pure *cis* alcohol (**14**) could be synthesized by simultaneous reduction of the ketone and the nitrile of **10** with Red-Al® to provide the *cis*-amino alcohol (**16**). Reaction **16** with the thioethylpseudosaccharin (**9**) provided the pure *cis*-**14.**

Reaction of the alcohol intermediate (**14**) with isocyanates or activation as the *p*-nitrophenyl carbonate and reaction with amines provided a variety of analogs (**17a–i**) (Scheme 3).

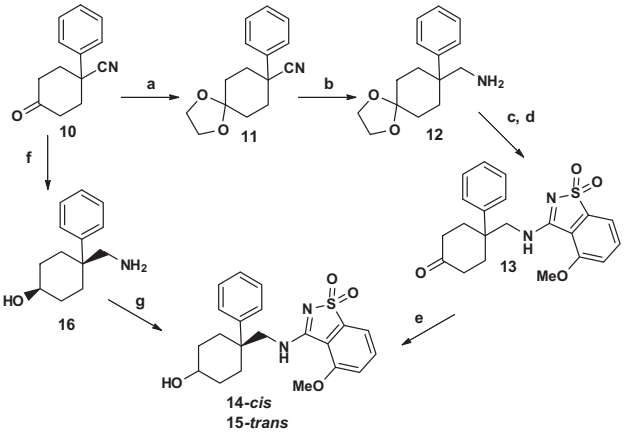
Compounds with substituents on the cyclohexane ring that were linked through nitrogen at the 4-position were synthesized stereoselectively in the *cis* configuration from 4-cyano-4-phenylcyclohexanone (**10**) (Scheme 4). Formation of the imine with (*s*)-*t*-butylsulfinamine followed by reduction with LAH at low temperature provided the *cis*-4-amino-1-cyanocyclohexane (**18**). Removal of the sulfinamine and reprotection with a *t*-butyloxycarbonyl group followed by reduction of the nitrile provided the *cis*-aminomethylcyclohexane (**19**). Reaction of this amine with the thioethylpseudosaccharin (**9**) followed by removal of the BOC group provided the aminocyclohexane (**20**). Reaction of this intermediate with chloroformates, isocyanates and sulfonyl chlorides was used to synthesize a variety of analogs (**21a–g**).

We were also interested in compounds with a heterocycle substituent at the 4 position of the cyclohexane ring. The synthesis of the pyrimidine compound (**22**) was accomplished through palladium catalyzed coupling of the amine (**20**) with 2-chloropyrimidine. The tetrazole (**23**) was synthesized from the amine (**20**) using sodium azide and trimethylorthoformate in acetic acid. (Scheme 5).

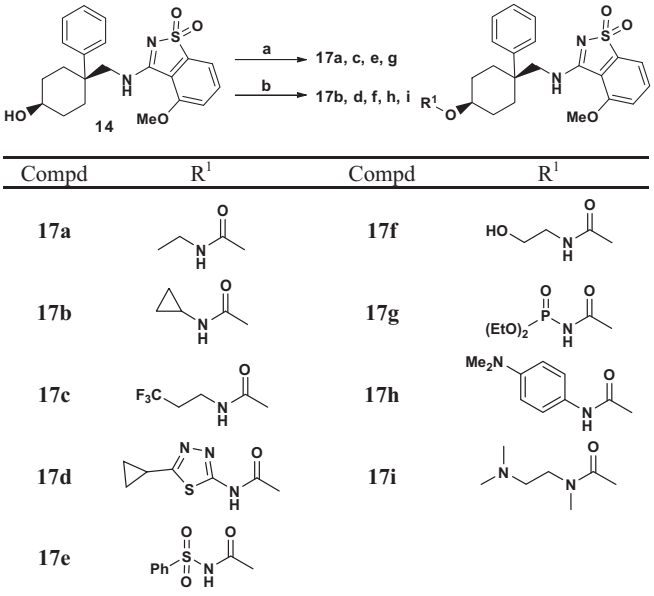
The compounds **14**, **15**, **17a–i**, **21a–g**, **22** and **23** were tested for block of potassium current in mouse fibroblast L929 cells expressing human $K_V1.5$.[9] We also evaluated these compounds for in vitro metabolic stability in liver microsomes to select the more metabolically stable analogs for progression to additional ion channel selectivity assays, in vivo pharmacokinetic and pharmacodynamic studies. Our hypothesis was that replacement of the carbonyl in compound **3** with a sulfonyl would lead to compounds that were more chemically stable. We also replaced the dimethyl substituents on the phenethyl chain with a spirocyclohexane which we knew from previous work was tolerant of a wide range of substitution and was associated with greater pharmacodynamic potency.[7] The



Scheme 1. Reagents and conditions: (a) *t*-$BuNH_2$, $CHCl_3$, 0 °C, 20 h, 89%; (b) *n*-BuLi, CO_2, THF, 0 °C, 86%; (c) H_2SO_4, 25 °C, 18 h then 55 °C, 3 h, 73%; (d) NaOMe, MeOH, 150 °C, 20 min, 86%; (e) $(EtO)_2P(S)SH$, 150 °C, 1 h, 71%.



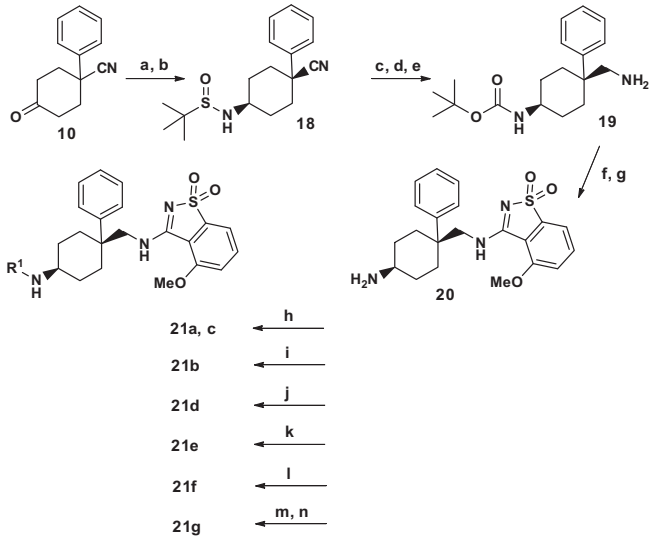
Scheme 2. Reagents and conditions: (a) ethylene glycol, pTSA, benzene, 90 °C, 98%; (b) LAH, THF, 70 °C, 90%; (c) 9, *n*-BuOH, 70 °C, 82%; (d) 1.0 M HCl, THF, 60 °C, 95%; (e) $NaBH_4$, MeOH, 25 °C, 86% (2:1 mixture of **14** and **15**); (f) Red-Al®, toluene, 100 °C, 3 h, 50%; g. 9, *n*-BuOH, 70 °C, 69%.

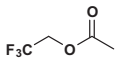


Scheme 3. Reagents and conditions: (a) RNCO, dibutyl tin diluarate (0.1 equiv), toluene, MeCN, 85 °C, 60–87%; (b) (i) *p*-nitrophenylchloroformate, Et_3N, CH_2Cl_2, 16 h, 25 °C, 71%; (ii) RNH_2, Et_3N, CH_2Cl_2, 16 h, 25 °C, 40–77%.

initial compound (**14**) contained a 4-*cis*-hydroxycyclohexane substituent on the phenethyl chain and was very potent in blocking $K_V1.5$ and was chemically stable during purification and handling. We compared the *cis* (**14**) to the *trans* (**15**) analogs and found the *cis* compound to be greater than 10-fold more potent. Therefore, in subsequent studies we synthesized and tested only the *cis* isomers.

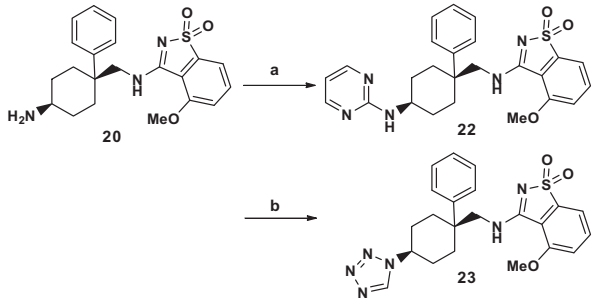
We explored elaboration of the hydroxyl group to carbamates and found that a wide range of small non-polar alkyl groups were well tolerated (Table 1). We were particularly interested in branched or fluorinated groups as a way to potentially block oxidative metabolism. Unfortunately this was not the case. Although the alkyl carbamates (**17a–c**) were potent they were also readily degraded in our liver microsome assay. However, the carbamate with the aminothiadiazole substituent (**17d)** showed good potency and metabolic stability. We also synthesized carbamates that contained small polar substituents such as hydroxyl, amino, acyl sulfonamide and acyl phosphoramide in an effort to improve the



Compd	R^1	Compd	R^1
21a		**21e**	
21b		**21f**	
21c		**21g**	
21d			

Scheme 4. Reagents and conditions: (a) (*S*)-*t*BuSONH$_2$, |Ti(OEt)$_4$, THF, rt, 4 h, 65%; (b) LAH, THF, −78 °C, 0.2 h; (c) 4.0 M HCl, dioxane, rt, 0.5 h, 50% over 2 steps; (d) BOC$_2$O, NaOH, THF, rt, 1 h, 100%; (e) NaBH$_4$, CoCl$_2$·6H$_2$O, MeOH, 0 °C to rt, 14 h, 74%; (f) **9**, *n*-BuOH, 70 °C, 81%; (g) TFA, CH$_2$Cl$_2$, 25 °C, 1 h, 92%; (h) ROCOCl, Et$_3$N, CH$_2$Cl$_2$, 25 °C, 3 h, 58–67%; (i) EtNCO, CH$_2$Cl$_2$, 25 °C, 16 h, 81%; (j) (i) triphosgene, CH$_2$Cl$_2$, 25 °C, 2 h, 95%; (ii) CF$_3$CH$_2$OH, dibutyl tin diluarate (0.1 equiv), toluene, 25 °C, 48 h, 40%; (k) EtSO$_2$Cl, Et$_3$N, CH$_2$Cl$_2$, 25 °C, 16 h, 60%; (l) NH$_2$SO$_2$NH$_2$, dioxane, 150 °C, 4 min, 62%; (m) chlorosulfonyl isocyanate, 2-bromothanol, 0 °C, 30 min, then Et$_3$N, 25 °C, 20 h, 35%; (n) Et$_2$NH, MeCN, 160 °C, 6 min, 80%.

physical properties of the compounds. Unfortunately, these polar, acidic or basic substituents were not well tolerated for K_V1.5 potency. Only when the amino group was less basic as in the aniline (**17h**) was the potency restored. Unfortunately, this compound had low metabolic stability. Both compounds **14** and **17d** showed sufficient potency and in vitro metabolic stability that they progressed to additional in vitro and in vivo characterization.



Scheme 5. Reagents and conditions: (a) 2-chloropyrimidine, *i*-PrOH, 140 °C, microwave 5 min then 160 °C, microwave 20 min, 25%; (b) NaN$_3$, trimethylorthoformate, acetic acid, 0–25 °C over 12 h followed by 100 °C, mw 10 min, 56%.

Table 1
Inhibition of K_V1.5 of 4-hydroxycyclohexane analogs

Compd	K_V1.5 inhibition[a] IC_{50}, μM	Metabolic stability[b] (% remaining in human, rat, mouse)
1	0.043	7, 13, 11
2	0.024	13, ND, ND[c]
3	0.034	99, 43, ND
14	0.034	90, 68, 53
15	>0.3 (14% inh)	98, 73, 73
17a	0.065	2, <1, <1
17b	0.17	9, 5, <1
17c	0.089	<1, <1, 3
17d	0.092	75, 70, 66
17e	>>10 (1% inh)	NT[d]
17f	>10 (27% inh)	NT
17g	>10 (13% inh)	NT
17h	0.030	2, <1, <1
17i	>10 (4% inh)	NT

[a] Values are means of 2–4 experiments.
[b] Percent of parent compound (3 μM) remaining following a 10 min incubation in liver microsomes containing appropriate co-factors was determined.
[c] ND = not determines.
[d] NT = not tested.

Table 2
Inhibition of K_V1.5 of 4-aminocyclohexane analogs

Compd	K_V1.5 inhibition[a] IC_{50}, μM	Metabolic stability[b] (% remaining in human, rat, mouse)
20	>10 (5% inh)	NT
21a	0.055	55, 19, NT
21b	>10 (20% inh)	NT
21c	0.052	85, 29, <1
21d	0.028	95, 57, 48
21e	0.060	75, 67, 30
21f	>10 (18% inh)	NT
21g	0.052	<1, <1, <1
22	0.047	6, <1, <1
23	0.032	56, 19, 56

[a] Values are means of 2–4 experiments.
[b] Percent of parent compound (3 μM) remaining following a 10 min incubation in liver microsomes containing appropriate co-factors was determined.

The 4-amino intermediate (**20**) gave us access to a range of analogs containing carbamate, amide, urea and heterocycle. Although the 4-amino compound (**20**) was not potent, non-basic analogs were very potent in blocking K_V1.5 (Table 2). Similar to the 4-hydroxyl analogs, carbamates with small alkyl substituents showed good potency, however, ureas (**21b**) were less potent. The ethyl carbamate (**21a**), isopropyl carbamate (**21c**), trifluoroethyl carbamate (**21d**) and the ethyl sulfonamide (**21e**) all showed good potency and higher levels of metabolic stability. The primary sulfamide (**21f**) showed very little potency, however, the diethyl sulfamide (**21g**) was quite potent although it lacked metabolic stability. Heteroaryl substituents linked through the amine like pyrimidine (**22**) or directly attached to the cyclohexane like tetrazole (**23**) were very potent but lacked sufficient metabolic stability. Based on these data, the isopropyl carbamate (**21c**), trifluoroethyl carbamate (**21d**) and sulfonamide (**21e**) were chosen for further in vitro and in vivo evaluation.

The compounds which were chosen for additional characterization were first assayed for ion channel selectivity. Most of these compounds showed >100-fold selectivity over block of sodium, calcium and *h*ERG channels (Table 3).

The pharmacokinetics of selected analogs with a combination of good potency, selectivity and higher metabolic stability (**14**, **17d** and **21c**) were characterized for pharmacokinetic properties in rats (Table 4). Compounds **14** and **21c** showed good exposure (C_{max} and AUC) and moderate clearance. Compound **17d** showed significantly

Table 3
Ion channel selectivity[a]

Compd	IC_{50} (μM)	% inhibition at 10 μM		
	I_{Kur}	I_{Ca}	I_{Na}	*h*ERG
14	0.034	14	27	54
17d	0.092	8	21	17
21c	0.052	41	14	68
21d	0.028	51	23	47
21e	0.060	14	7	59

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

Table 4
Pharmacokinetic parameters in rats

Compd	**14**[a]	**17d**[a]	**21c**[a]
Dose *inf* (mg/kg)[b,d]	2.0	1.6	2.0
Dose *po* (mg/kg)[c,d]	5.0	3.6	5.0
C_{max} (μM)	1.7 ± 1.6	0.011 ± 0.006	0.93 ± 0.33
AUC *po* (μM h)	2.7 ± 2.1	0.20	1.8 ± 0.5
Half-life (h)	5.1	2.3 ± 0.8	0.9 ± 0.1
Cl (mL/min/kg)	27 ± 7.3	6.8 ± 1.2	9.4 ± 4.7
Vss (L/kg)	3.2	0.9 ± 0.15	0.3 ± 0.2
F (%)	34	1.0	7.2

[a] Values are means from 3 animals.
[b] *inf* = intra-arterial infusion for 10 min.
[c] *po* = oral gavage.
[d] Vehicle 40% PEG 400, 40% water, 20% ethanol for both *iv* and *po* doses.

Table 5
Pharmacodynamic effects in rats[a]

Compd	QT interval (% change)	Mean BP (% change)	Plasma concn (μM)
14	66 ± 11	13 ± 11	153 ± 39
17d	23 ± 3	19 ± 4	72 ± 12
21c	68 ± 8	44 ± 11	38 ± 4

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

Table 6
Pharmacodynamic effects in rabbits[11] of compound **14**, **17d**, **21c**

Compd	EC_{20} (μM)	Mean BP (%change)	Max plasma conc. (μM)
14	0.6	19	6.1
17d	23	6	23 ± 9.6
21c	<0.7	17	4.7 ± 1.6

lower exposure despite similar microsomal stability. This would suggest other degradation and elimination pathways in addition to oxidative metabolism.

The pharmacodynamic activities of the selected compounds were first evaluated at 3 mg/Kg in rats.[10] Since rats express $K_V1.5$ in both the atrium and ventricle, the QT interval in the ECG was used to evaluate block of I_{Kur}. All the compounds tested (**14**, **17d**, **21c**) were effective at increasing the QT interval as predicted by their in vitro activity (Table 5). However, in addition to high level of activity in prolonging QT, these compounds were also found to significantly increase blood pressure.

To check if this increase in blood pressure was specific to rats, the pharmacodynamic activities of **14**, **17d**, and **21c** were also tested in a rabbit model.[11] Like humans, rabbits express the I_{Kur} current in atrium but not ventricle and the effective refractory period (ERP) was determined in both atrium and ventricle to measure atrial selectivity. The compounds were dosed at 0.3, 1.0 and 3.0 mg/Kg and the plasma concentration to prolong AERP by 20% (EC_{20}) was calculated (Table 6). There was no effect on ventricular ERP reflecting the selectivity for $K_V1.5$ over ventricular ion channels. Compounds **14** and **17d** showed a robust pharmacodynamic effect with 20% prolongation of AERP at less than 1 μM plasma concentration. However, like the rat pharmacodynamic study, significant increases in blood pressure were also observed in the rabbits. In addition, a significant increase in gastrointestinal motility was observed with compound **14**. We attributed these effects to off-target activity since other $K_V1.5$ blockers have been reported that do not show these liabilities.[12] In an effort to determine the nature of the off-target activities we screened compounds **14** and **21c** at a concentration of 10 μM for over 37 receptors and enzymes that could potentially cause cardiovascular effects or toxicity (Supplemental material). Both compounds were inactive against most of the receptors with only modest activity against the opiate receptor (64%), acetylcholine esterase (55%) and adenosine A3 receptor (64%). Both **14** and **21c** had maximum plasma concentrations less than 10 μM at the highest dose so it seemed unlikely these activities were the source of the off-target toxicities.

In conclusion, pseudosaccharin amines were discovered as potent and selective $K_V1.5$ blockers. Variation of the substituent at the 4-position of the spirocyclohexane ring was shown to modulate potency and metabolic stability. Several compounds showed very high potency, ion channel selectivity, and in vitro metabolic stability. Compounds **14, 17d** and **21c** increased AERP in the rabbit pharmacodynamic model, however, they also showed increases in blood pressure. This type of off target activity precluded further advancement of this series.

Supplementary data

Supplementary data (Supplemental information includes off-target liability screening for **14** and **21c**, the details of the rat AERP experiments and experimental details for the synthesis of **21c**.) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.02.066.

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