Design and Synthesis of 4-Substituted Benzamides as Potent, Selective, and Orally Bioavailable I_{Ks} Blockers

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Abstract: Multiple delayed rectifier potassium currents, including I_{Ks}, are responsible for the repolarization and termination of the cardiac action potential, and blockers of these currents may be useful as antiarrhythmic agents. Modification of compound **5** produced **19(S)** that is the most potent I_{Ks} blocker reported to date with >5000-fold selectivity over other cardiac ion channels. Further modification produced **24A** with 23% oral bioavailability.

Multiple delayed rectifier potassium currents are responsible for the repolarization and termination of the cardiac action potential. Blockade of these potassium currents delays repolarization, prolongs action potential duration, and thus produces an antiarrhythmic (class III) effect.[1] The delayed rectifier potassium current is carried by at least two different potassium channels, I_{Kr} and I_{Ks}.[2] The pore forming α-subunits of I_{Kr} and I_{Ks} channels are encoded by *HERG*[3] and K_vLQT1[4] potassium channel genes, respectively. I_{Kr} is characterized by rapid activation and deactivation kinetics whereas I_{Ks} exhibits slow activation and deactivation kinetics. Blockade of I_{Kr} and action potential prolongation at resting heart rates can cause severe arrhythmias.[5] Because of the slow deactivation kinetics of I_{Ks}, this current may accumulate at fast heart rates and thus selective block may lengthen action potential duration more at fast heart rates than at slow rates. Consequently, blockers of I_{Ks} may not show the negative rate dependence that is exhibited by agents that block I_{Kr}.[6] In this Letter we report the discovery of orally bioavailable benzamides as potent and selective blockers of I_{Ks}.[7]

Directed screening for inhibitors of the I_{Ks} current was carried out using voltage clamp techniques. Initially, compounds were chosen for testing based on similarity to the known I_{Ks} blockers azimilide (**1**) and clofilium (**2**) (Figure 1). In most cases, compounds were first screened in Xenopus oocytes expressing the cloned I_{Ks} β-subunit, minK.[8] MinK associates with an endogenous I_{Ks} α-subunit in Xenopus oocytes to form the I_{Ks} current.[9] Compounds showing greater than 30% inhibition of this current at 10 μM were further tested for inhibition of the native I_{Ks} current in guinea pig ventricular myocytes. Using this protocol, we discovered a tetralone analogue **3** that blocked I_{Ks} with moderate potency (IC_{50} = 5 μM). Although a substantial effort to optimize the activity of these analogues led to compounds with much greater potency against I_{Ks}, they also showed significant block of I_{Kr} as well as affinity for other receptors.

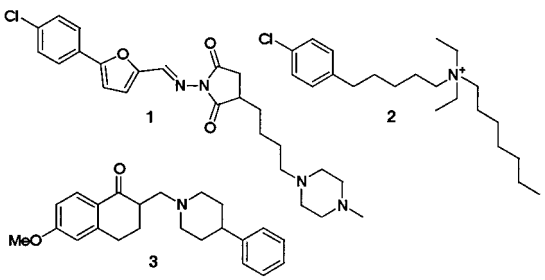

Figure 1. Known I_{Ks} blockers and the screening hit **3**.

Further directed screening using the tetralone **3** as a template identified the phenyl ether **4** and the ketone **6**, both of which had moderate activity against I_{Ks} (Table 1). In our initial studies we found that the basic amine was not necessary, as the amide **5** was equipotent to the amine **4**. We also found that we could replace the ketone in **6** with an amide. Benzamides such as **7** then became our primary target.

Using high throughput automated synthetic procedures, we initially varied the amine component of the amide while keeping the 4-hexyloxybenzoic acid portion of the molecule constant. After the synthesis and testing of over 100 benzamide analogues from amines chosen for structural diversity, it became clear that amides derived from small, branched alkylamines were the most potent. The amide derived from 3,3-dimethylbutylamine (**8**) showed a >10-fold enhancement in potency over the amide **7** (Table 2). It was interesting to note the sharply defined structural requirements of the alkyl chain as changing the length of the alkyl chain or deletion of one or both of the terminal methyl groups from compound **8** resulted in significantly decreased potency (see Supporting Information). We were also encouraged since compound **8** showed only weak (<50%) block of I_{Kr} at 10 μM.

Constraint of a conformationally mobile alkyl group in some cases can increase potency. Therefore, we replaced the 3,3-dimethylbutylamine with cyclized amines (Table 2). Bridging from the nitrogen to the β-carbon with a two-carbon link as in compound **9** resulted in a large loss of activity. Constraint from the α-carbon to the terminal methyl with a one-carbon link resulted in compound **10** of equal potency to compound **8**. Constraint of the β-carbon to the terminal methyl group with a two-carbon link as in compound **11** resulted in a slight improvement in potency for the more active enantiomer. Decreasing the distance from the nitrogen to the cyclopentane in compound **12** resulted in substantial loss of potency, as did increasing the ring size from five to six carbons in compound **13**. However, moving the methyl groups to the adjacent carbon (**14**) resulted in recovery of some I_{Ks} blocking potency.

While studying the constrained amines, we simultaneously investigated the replacement of the 4-hexyloxyphenyl group. Automated synthesis was used to produce amides of 3,3-dimethylbutylamine with a diverse set of

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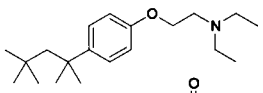
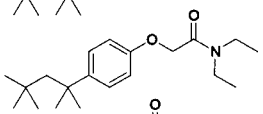
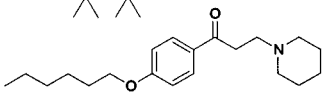
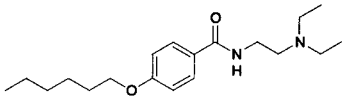
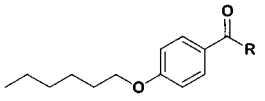
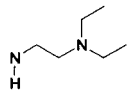
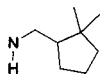
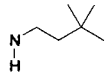
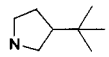
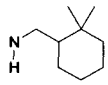
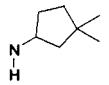
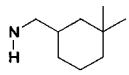
Table 1. Screening Hits and Initial Modifications

compd	Structure	IC_{50} (μM)[b]
4		5.0
5[a]		5.0
6		4.0
7[a]		3.5

[a] Compounds gave satisfactory spectral and analytical data (C, H, N; ±0.4% of theoretical values). [b] Molar concentration required to inhibit 50% of the I_{Ks} current in isolated guinea pig ventricular myocytes $n = 2-5$ at two to four concentrations (see Supporting Information for protocol).

Table 2. Inhibition of I_{Ks} with 4-Hexyloxybenzamides



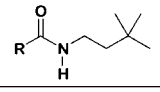
compd[a]	R	IC_{50} (μM)[b]	compd[a]	R	IC_{50} (μM)[b]
7		3.5	11A[c] 11B[c]		0.71 0.16
8		0.25	12		>30
9		>3	13A[c] 13B[c]		1.4 >3
10		0.36	14		0.37

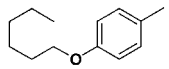
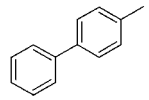
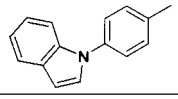
[a] All compounds gave satisfactory spectral and analytical data (C, H, N; ±0.4% of theoretical values). [b] Molar concentration required to inhibit 50% of the I_{Ks} current in isolated guinea pig ventricular myocytes $n = 2-5$ at two to four concentrations (see Supporting Information for protocol). [c] Enantiomers separated by chiral HPLC; the absolute stereochemistry was not determined.

acids. Again, the requirements for the substituent on the aromatic ring were quite specific (see Supporting Information). Shortening of the alkyl chain or substitution of a carbon for the oxygen of the hexyloxy substituent in compound **8** resulted in >10-fold decreases in potency. Ortho substitution or ring fusion also was not tolerated. However, we found that the biphenyl analogue **15** was nearly as potent as compound **8** (Table 3). We also found that the distal phenyl ring in the biphenyl compound **15** could be replaced by a heterocycle such as indole (**16**) and maintain equal potency to compound **8**.

Substitution of a heterocycle for the distal phenyl ring of the biphenyl compound **15** was interesting in light of the highly lipophilic character of these compounds and their anticipated low aqueous solubility. This led us to synthesize the phenyl oxadiazole **17** that had similar potency to the indole **16** (Table 4). Based on our earlier observations that an alkyl substituent of the proper length enhanced the activity of the 4-alkoxybenzamides,

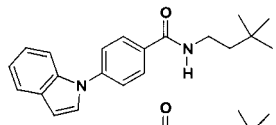
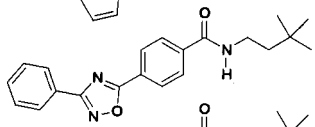
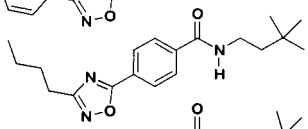
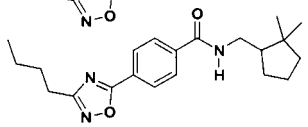
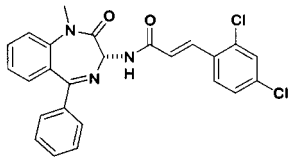
Table 3. Acid Modifications of Benzamides



compd[a]	R	IC_{50} (μM)[b]
8		0.25
15		0.54
16		0.26

[a] All compounds gave satisfactory spectral and analytical data (C, H, N; ±0.4% of theoretical values). [b] Molar concentration required to inhibit 50% of the I_{Ks} current in isolated guinea pig ventricular myocytes $n = 2-5$ at two to four concentrations (see Supporting Information for protocol).

Table 4. Heterocycle Substituted Benzamides

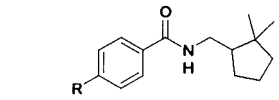
compd[a]	structure	IC_{50} (μM)[b]
16		0.26
17		0.13
18		0.023
19(S)[c] 19(R)[c]		0.002 0.009
20(R)		0.031

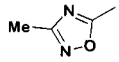
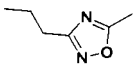
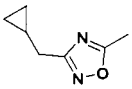
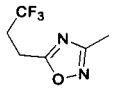
[a] All compounds gave satisfactory spectral and analytical data (C, H, N; ±0.4% of theoretical values). [b] Molar concentration required to inhibit 50% of the I_{Ks} current in isolated guinea pig ventricular myocytes $n = 2-5$ at two to four concentrations (see Supporting Information for protocol). [c] Enantiomers separated by chiral HPLC.

we synthesized the butyloxadiazole analogue **18** that was 10-fold more potent than the indole **16**. When this modification was combined with the constrained amine used in compound **11**, the resulting compound **19(S)** showed 10-fold enhancement in potency over the 3,3-dimethylbutyl analogue **18** and was 4-fold more potent than its enantiomer **19(R)**. In our assay system, these compounds were both more potent than the benzodiazepine **20** that is one of the most potent compounds previously reported.[7] Both **19(S)** and **19(R)** had little block of I_{Kr} (IC_{50} >30 μM), sodium, or calcium currents (IC_{50} >10 μM). Thus we had achieved our objective of identifying a potent and selective blocker of the I_{Ks} current.

Our next goal was to identify an orally active agent. The oral bioavailability of compound **19(S)** was <1% in rats. Analysis by LC/MS of the bile of the orally dosed

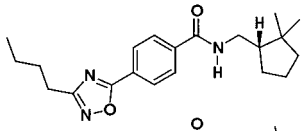
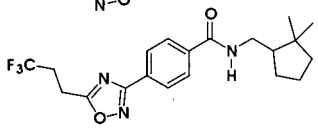
Table 5. Side Chain and Heterocycle Replacements



compd[a]	R	IC_{50} (μM)[b]
21A[c,d]		0.22
21B[c,d]		0.58
22A[c]		0.010
22B[c]		0.027
23A[c]		0.018
23B[c]		0.023
24A[c]		0.040
24B[c]		0.027

[a] All compounds gave satisfactory spectral and analytical data (C, H, N; ±0.4% of theoretical values). [b] Molar concentration required to inhibit 50% of the I_{Ks} current in isolated guinea pig ventricular myocytes $n = 2-5$ at two to four concentrations (see Supporting Information for protocol). [c] Enantiomers separated by chiral HPLC; the absolute stereochemistry was not determined. [d] C, H, N; ±0.5% of theoretical values.

Table 6. Oral Bioavailability and iv Half-Life in Rats

compd	structure	bioavailability[a]	*iv* $t_{1/2}$[a]
19(S)		<1%	4.2±0.3 h
24A		23±5%	3.2±0.7 h

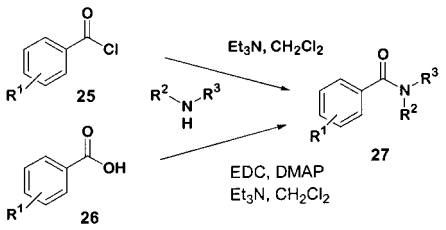
[a] $n = 4-6$ rats both iv and po.

animals showed that the amide and cyclopentane remained intact; however, the butyl chain was extensively metabolized. These studies suggested the need to block degradation of the butyl group, and we achieved this by modification of the butyl chain and the heterocycle (Table 5). Substantially reducing the size of the butyl group was not a useful option since the methyl analogue **21** was 100-fold less potent than **19(S)**. The propyl analogue **22** and cyclopropylmethyl analogue **23** were both slightly less potent than compound **19(S)**. We also synthesized the isomeric oxadiazole **24** which contained a trifluoromethyl substituent on the alkyl chain to block metabolism.

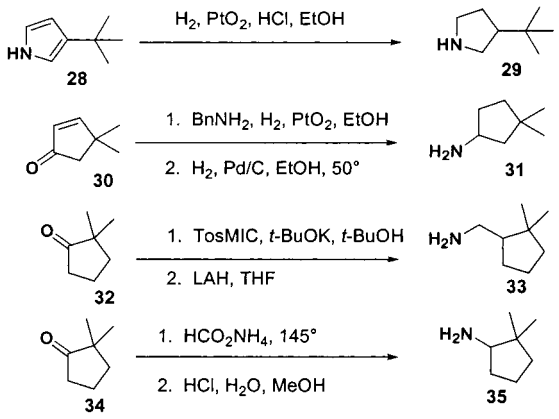
Several of the more potent compounds were screened for maximal plasma levels after oral dosing in rats. Oral bioavailability was determined for compounds that showed the highest plasma levels. The compound with the greatest oral bioavailability was compound **24A** which was 23% orally bioavailable and had a half-life after iv administration of 3.2 h in rats (Table 6). Although **24A** had lower potency (IC_{50} = 40 nM) compared to compound **19(S)**, it showed little block (<30%) of I_{Kr}, I_{Na}, or I_{Ca} at concentrations up to 10 μM.

We have shown that amides of benzoic acids are potent and selective blockers of I_{Ks}. The most potent

Scheme 1



Scheme 2



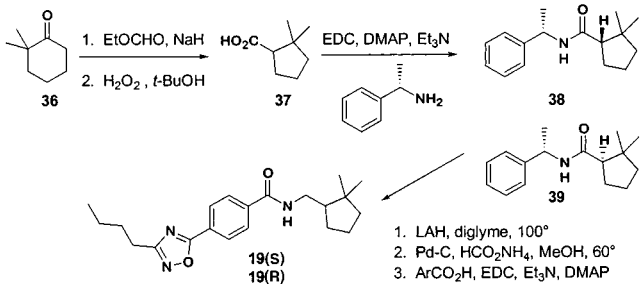
member of this series, **19(S)**, has an IC_{50} of 2 nM with no significant block of I_{Kr}, I_{Na}, or I_{Ca} up to 10 μM. Compound **19(S)** is the most potent and selective I_{Ks} blocker reported to date. We have identified compound **24A** with 23% oral bioavailability. Future studies will include further optimization of the heterocycle and the trifluoropropyl chain of compound **24A** to improve potency while preserving this level of oral activity.

Chemistry. Most of the benzamides were synthesized by either reaction of 4-hexyloxybenzoyl chloride with various amines or the reaction of an amine with various acids using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) as the coupling agent (Scheme 1).

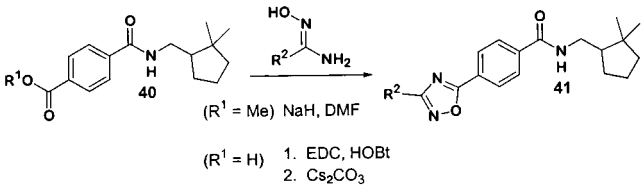
A variety of methods were used to synthesize the cycloalkylamines (Scheme 2). The 3-*tert*-butylpyrrolidine (**29**) was synthesized from 3-*tert*-butylpyrrole (**28**).[10] Dimethylcyclopentylmethylamine (**33**) was synthesized from 2,2-dimethylcyclopentanone (**32**) with tosylmethylisocyanide[11] followed by reduction of the nitrile. The 2,2-dimethylcyclopentylamine (**35**) was synthesized from 2,2-dimethylcyclopentanone (**34**) by reaction with ammonium formate followed by hydrolysis.[12]

The individual isomers of compound **19** were initially separated by chiral preparative HPLC (Chiracel OD, 15% 2-propanol, water). The absolute stereochemistry was later determined by synthesis of the individual isomers from intermediates of known stereochemistry. 2,2-Dimethylcycopentane carboxylic acid (**37**)[13] was coupled with (*S*)-α-methylbenzylamine to form a mixture of diastereomeric amides (Scheme 3). After separation of the amides, compound **38** was crystallized and the absolute configuration (**S**,**S**) was determined by X-ray analysis.[14] The individual amides were reduced to the amines which were coupled with the benzoic acid to give compounds **19(S)** and **19(R)**. The benzoic acid was synthesized from monomethylteraphthalate and *N*-hydroxypentamidine[15] followed by hydrolysis of the methyl ester.

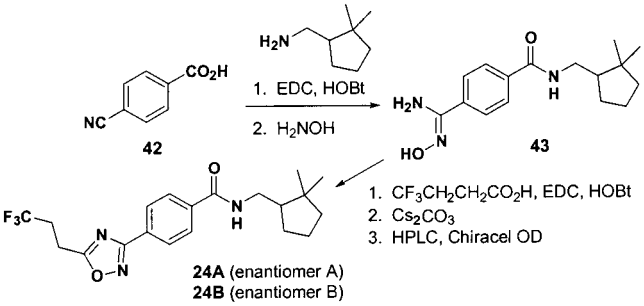
Scheme 3



Scheme 4



Scheme 5



The 1,2,4-oxadiazoles were synthesized by reaction of an acid or ester with *N*-hydroxyamidines[15] followed by dehydration (Scheme 4). Compound **24** was synthesized by reaction of cyanobenzoic acid (**42**) with the amine **33** followed by treatment with hydroxylamine. The *N*-hydroxyamidine **43** was coupled with trifluoropropanoic acid and then cyclized, and the isomers were separated by chiral preparative HPLC (Scheme 5).

Biology. Most compounds were first screened for I_{Ks} activity using *Xenopus* oocytes expressing the cloned I_{Ks} α-subunit, minK. Oocyte expression and two-microelectrode voltage clamp recordings were performed as described previously.[16] Compounds that showed sufficient block of the minK conducted current in oocytes were then tested for block of native I_{Ks} in isolated guinea pig ventricular myocytes. Myocytes were isolated as described previously.[17] Ionic solutions and voltage clamp procedures were designed to measure I_{Ks} in isolation from other currents.[18] Plasma levels of drugs were determined after oral dosing in rats. Compounds that showed plasma concentrations of >75 nM were then assayed for oral bioavailability individually. Oral bioavailability was determined by standard methods.

Supporting Information Available: SAR results of the compounds prepared using combinatorial methods, experimental details of the biological assays, synthetic procedures, analytical data, and X-ray crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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