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Discovery and synthesis of tetrahydroindolone-derived carbamates as Kv1.5 blockers

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Abstract—A novel class of tetrahydroindolone-derived carbamates has been discovered whose members are potent Kv1.5 blockers. The in vitro data show that compounds **6** and **29** are quite potent. They are also very selective over hERG (>450-fold) and L-type calcium channels (>450-fold).

Atrial fibrillation (AF) is the most common type of cardiac arrhythmia, affecting more than 2 million Americans.[1] AF can lead to thromboembolism, reduced left ventricular function, and stroke.[2–4] It is also independently associated with increased mortality. Current drug therapies for this type of arrhythmia are unsatisfactory.[5] Most currently marketed antiarrhythmic agents block the potassium channel, hERG, and the associated I_{Kr} repolarizing current which is present in both the atria and the ventricles. In contrast, the potassium channel Kv1.5, which underlies the ultra-rapid delayed rectifier K^+ current, IKur, is found selectively in the atria.[6–8] IKur is a major repolarizing current in the human atria and is not found in the human ventricles. Thus, Kv1.5 is an attractive molecular target for the treatment of atrial fibrillation or atrial flutter.[9,10] Theoretically, atrial selective antiarrhythmics that block only the Kv1.5 channel should avoid the induction of potentially fatal ventricular arrhythmias known as torsades de pointes, a serious side effect of many current antiarrhythmic agents that lack the proposed chamber selectivity.

Recently, we reported on tetrahydroindolone-derived semicarbazones as potent and selective Kv1.5 inhibitors.[11] In our efforts to broaden the diversity of our Kv1.5 inhibitors, we prepared isosteric tetrahydroindolone-derived carbamates, as a potential new class of Kv1.5 inhibitors. Herein, we report the synthesis and in vitro evaluations of these new analogs.

The compounds were prepared using the routes shown in Scheme 1.

The appropriate substituted dihydroindolones **1** were treated with hydroxylamine in the presence of KOH to generate dihydroindolone oximes **2**. The oximes **2** were then converted to the final desired carbamates **3**–**22** by treating with variety of arylisocyanates.

To generate the N-1 alkylated analogs, the indolones **1** were treated with MeI or other similar alkyating agents in the presence of NaH to afford *N*-alkyl indolones **23**. Again, the condensation of indolones **23** with hydroxylamine gave *N*-alkyl dihydroindolone oximes **24**. These were further converted to products **25**–**31** by reaction with arylisocyanates. Lastly, compounds with different alkyl linking groups between the aryl ring and the *N* of the carbamate were constructed by the treatment of **2** or **24** with carbonyldiimidazole (CDI) to yield **32** where R^3 is H or a small alkyl chain. This intermediate was then reacted in situ with different primary amines to afford final targets **33**–**37** (Scheme 2).

More than 120 carbamates were prepared and screened. Whole cell patch clamp electrophysiology was used to determine channel block in LTK^- cells expressing Kv1.5[12] and in HEK cells expressing hERG.[13] A FLIPR assay was used to determine channel block in HL-1 cells

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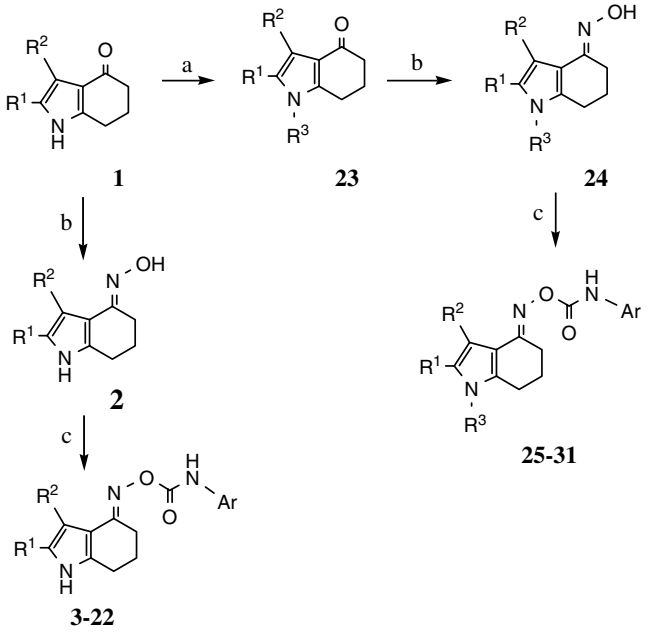


Scheme 1. Reagents and conditions: (a) MeI, NaH/dioxane, 70 °C, 1.5 h; (b) $NH_2OH{\cdot}HCl$ (excess)/KOH/EtOH H_2O, 70 °C, overnight; (c) aryl isocyanates/TEA/CH_2Cl_2, rt, 2 h.

expressing endogenous L-type calcium channels.[14] From the single point 1 μM percent inhibitions, a select group of compounds were further evaluated to obtain the IC_{50} values. As a reference, a known Kv1.5 benchmark by Icagen, ICA-32, had a measured IC_{50} value of 170 nM in our laboratory.

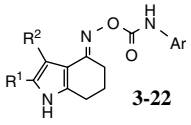
Compounds with desired inhibitory inhibition can be grouped into NH analogs (**3**–**22** and **33**–**37**), and *N*-1-alkyl analogs (**25**–**31**). Both classes of compounds provided very potent blockers.

In determining the SAR trends, the substituents on the dihydroindolone ring were first examined. The 2,3-dimethyl compounds (**3**–**5**) were less active than the corresponding 2-methyl, 3-ethyl compounds (**6**–**8**). In contrast, the 2,3-diethyl compounds (**18**, **19**) had approximately equal activity to their 2-methyl, 3-ethyl counterparts (**6**, **8**). This tolerance of larger substituents at C_2 or C_3 was also observed in the N-1 methyl series where R^1 = methyl or ethyl and R^2 = ethyl or isopropyl gave compounds with similar activity (**26**, **30**, and **31**). The R^1 and R^2 substituents were combined in the cyclohexyl fused compounds (**20**–**22**) which were active blockers in contrast to the previously reported fused cyclohexyl semicarbazones which showed little or no block of Kv1.5.[11]

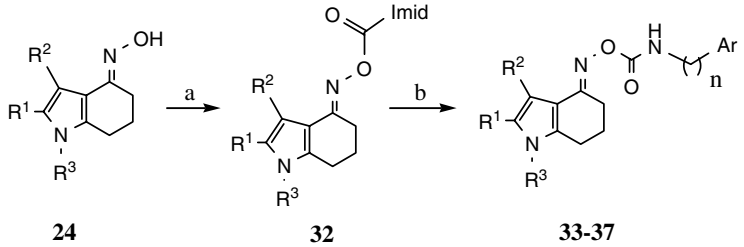
Another trend displayed in Table 2 concerns the size of the alkyl group on the N-1 position of the dihydroindolone ring. Compounds with the smaller methyl substituent were more active than the corresponding ethyl compounds (**25** (77%) and **26** (96%) vs **27** (35%) and **28** (71%)).

As shown in Table 1, the blockade of Kv1.5 was not sensitive to electronic effects of the substituents at the 4 position of the aromatic ring. The blocking activity for analogs with both electron-donating and electron withdrawing groups was very similar (**12**, **14**, and **15**, vs **7**, **8**, **9**, and **13**). In contrast, the size of alkyl substituents at the 4 position on the aryl ring has significant impact

Table 1.



Compound	R^1	R^3	Ar	% Inhibition of Kv1.5 at 1 μM	Kv1.5 IC_{50} (μM)
3	Me	Me	Phenyl	57	
4	Me	Me	4-Ac-Phenyl	60	
5	Me	Me	4-Cl-Phenyl	78	
6	Me	Et	Phenyl	91	0.067
7	Me	Et	4-Ac-Phenyl	91	
8	Me	Et	4-Cl-Phenyl	91	
9	Me	Et	4-F-Phenyl	90	0.275
10	Me	Et	4-*t*-Bu-Phenyl	6	
11	Me	Et	4-*i*-Pr-Phenyl	51	
12	Me	Et	4-Me-Phenyl	85	
13	Me	Et	4-CN-Phenyl	95	
14	Me	Et	4-OMe-Phenyl	90	
15	Me	Et	4-SMe-Phenyl	90	0.350
16	Me	Et	3-SMe-Phenyl	73	
17	Me	Et	2-SMe-Phenyl	41	
18	Et	Et	Phenyl	84	
19	Et	Et	4-Cl-Phenyl	87	
20	–$(CH_2)_3$–		Phenyl	79	
21	–$(CH_2)_3$–		4-Ac-Phenyl	79	
22	–$(CH_2)_3$–		4-Cl-Phenyl	58	



Scheme 2. Reagents: (a) CDI/dioxane; (b) $Ar(CH_2)_nNH_2$.

on inhibition. Smaller alkyl groups, such as the methyl analog (**12**), exhibit good activity (85%), while the larger isopropyl substituted analog (**11** (51%)) and the *tert*-butyl analog (**10** (6%)) showed reduced activity.

Substituents on the aryl ring in positions other than 4 appear to have considerable effects on inhibition. As shown in Table 1, analogs with substituents at the 2 position tend to have less activity (**17** (41%)) than those in the 3 position (**16** (73%)). Analogs with substituents in the 4 position (**15** (90%)) provided the most active analogs. This trend was similar for compounds substituted with chlorine or methyl substituents (data not shown).

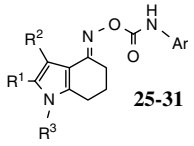
Next, the chain length between the aromatic ring and the nitrogen of the carbamate side was examined. As shown in Table 3, analogs without extended alkyl linkers (**6** (91%)) were more active than those with alkyl linkers $n = 1$ (**33** (70%)) and $n = 2$ (**34** (76%)). This trend was further substantiated with the 4-OMe phenyl isomers when the chain length was extended from $n = 0$ through $n = 2$ (data not shown).

In order to increase the solubility of the carbamate class, we prepared and evaluated several compounds containing the pyridinylethyl carbamate side chain. The position of nitrogen on the pyridine ring had a large impact on blocking inhibition. For example, the 2-pyridinyl isomer showed very good activity (**35** (91%)), while analogs with the 3- or 4-pyridinyl substitutions gave low activity (**36** (25%), and **37** (20%)).

In the carbamate series, our most potent analogs were **6** and **29** with IC_{50} values of 67 nM and 21 nM, respectively. Compound **6** was considerably more potent than its corresponding and previously published semicarbazone (IC_{50} = 125 nM).[11] While a patch value of 91% on the surface does not appear to be one of the most potent compounds, the rate at which that block occurred compared to the others resulted made us confident we could observe a low IC_{50} value. Additionally, carbamates **6** and **29** showed selectivity of more than $\geqslant$450-fold against the L-type-Ca channel (**6** ($IC_{50} > 30$ μM), and **29** ($IC_{50} = 20$ μM)), and $\geqslant$450-fold selectivity versus hERG (**6** ($IC_{50} > 30$ μM), and **29** ($IC_{50} > 30$ μM)). Low activity for Calcium and hERG was consistent throughout the class.

In conclusion, we have discovered a novel class of tetrahydroindolone-derived carbamates that are very potent blockers of the Kv1.5 channel. Some members also showed excellent selectivity for Kv1.5 inhibition over the calcium and hERG ion channels.

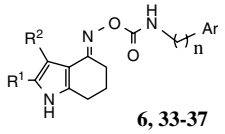
Table 2.



25-31

Compound	R^1	R^2	R^3	Ar	% inhibition of Kv1.5 at 1 μM	Kv1.5 IC_{50} (μM)
25	Me	Et	Me	Phenyl	77	
26	Me	Et	Me	4-Ac-Phenyl	96	0.368
27	Me	Et	Et	Phenyl	35	
28	Me	Et	Et	4-Ac-Phenyl	71	
29	Et	Et	Me	Phenyl	99	0.021
30	Et	Et	Me	4-Ac-Phenyl	92	
31	Me	*i*-pr	Me	4-Ac-Phenyl	96	

Table 3.



6, 33-37

Compound	R^1	R^2	N	Ar	% inhibition of Kv1.5 at 1 μM	Kv1.5 IC_{50} (μM)
6	Me	Et	0	Phenyl	91	0.067
33	Me	Et	1	Phenyl	70	
34	Me	Et	2	Phenyl	76	
35	Me	Et	2	2-Pyr	91	0.547
36	Me	Et	2	3-Pyr	25	
37	Me	Et	2	4-Pyr	20	

Acknowledgments

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12. Kv1.5 currents are recorded by the whole cell mode of patch clamp electrophysiology. Kv1.5 is stably overexpressed in either HEK or LTK-cells. Microelectrodes are pulled from borosilicate glass (TW150) and heat polished (tip resistance, 1.5–3 MΩ). The external solution is standard Tyrode's solution. The internal (microelectrode) solution contained: 110 mM KCl, 5 mM K_2APT, 5 mM K_4BAPTA, 1 mM $MgCl_2$, and 10 mM Hepes, adjusted to pH 7.2 with KOH. Command potentials are applied for 1 s to +60 mV from a holding potential of −70 mV using Axon software (pClamp 8.1) and hardware (Axopatch 1D, 200B). Compounds are prepared as 10–20 mM DMSO stocks and diluted to appropriate test concentrations. After stable currents are achieved, compounds are perfused onto the cells and the cells are pulsed every 5 s until no further changes in current are evident at a given compound concentration. Inhibition was measured at the end of the 1 s pulses and expressed relative to controls. Concentration–response curves are generated for appropriate compounds utilizing at least four concentrations and an $n = 3$. Curve fitting and IC_{50} estimation were done using Graphpad software (Ver. 4).
13. HERG currents are recorded by the whole cell mode of patch clamp electrophysiology as described by Hamill et al.[15] HERG is stably overexpressed in HEK cells. Microelectrodes are pulled from borosilicate glass (TW150) and heat polished (tip resistance, 1.5–3 MΩ). The external solution is standard Tyrode's solution. The internal (microelectrode) solution contained: 110 mM KCl, 5 mM K_2APT, 5 mM K_4BAPT, 1 mM $MgCl_2$, and 10 mM Hepes, adjusted to pH 7.2 with KOH. Command potentials are applied for 2 s to +20 mV from a holding potential of −80 mV using Axon software (pClamp 8.1) and hardware (Axopatch 1D, 200B). Tail currents are generated by returning to −40 mV for 2 s. Compounds are prepared as 10–20 mM DMSO stocks and diluted to appropriate test concentrations. After stable currents are achieved, compounds are perfused onto the cells and the cells are pulsed every 20 s until no further changes in current are evident at a given compound concentration. Inhibition of HERG is measured at the peak of the tail currents and expressed relative to controls. Initial HERG activity is estimated by single point determinations run at 10 μM. Concentration–response curves are generated for appropriate compounds utilizing at least four concentrations and an $n = 3$. Curve fitting and IC_{50} estimation were done using Graphpad software (Ver. 4).
14. HL-1 cells expressing endogenous L-type calcium channels are removed from culture flasks using trypsin, plated on fibronectin/gelatin-coated, clear-bottomed, black-walled 96-well microplates in Claycomb media (JRH Biosciences #51800) containing 10% fetal bovine serum, 4 mM L-glutamine, and 10 μM norepinephrine, and grown to confluency overnight. The next day, growth medium is aspirated from confluent cell monolayers and replaced with 100 μL per well Tyrode's solution (in mM: 130 NaCl, 4 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 20 Hepes, and 10 glucose, pH 7.35) and 50 μL per well FLIPR Calcium Assay kit, component A (#R-8033, Molecular Devices Corporation) and incubated for 60 min. in a 5% CO_2 37 °C incubator. Fifty microliters per well test compounds is added to the plates and further incubated for 15 min. in a 5% CO_2 37 °C incubator. All final solutions contain the anion exchange inhibitor, probenecid (2.5 mM). The 96-well plates are then placed in the center position of the FLIPR 1(Fluorometric Imaging Plate Reader, Molecular Devices Corporation). Cell monolayers in each well are simultaneously illuminated at 488 nm with an Argon ion laser, and fluorescence emission is monitored using a 510–570 nm bandpass filter and a cooled CCD camera. To depolarize the plasma membrane and activate L-type calcium channels, 50 μL per well of 20 mM KCl (final concentration) is dispensed simultaneously to all 96 wells using the FLIPR's automatic 96-well pipettor. Fluorescence measurements are captured for 5 min. following KCl addition. Calcium influx, expressed as % control, is calculated for each concentration of test compound and concentration–response curves and IC_{50} values are generated using GraphPad Prism 4.0.
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