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Discovery and synthesis of tetrahydroindolone derived semicarbazones as selective Kv1.5 blockers

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Abstract—A novel class of tetrahydroindolone-derived semicarbazones has been discovered as potent Kv1.5 blockers. In in vitro studies, several compounds exhibited very good potency for blockade of Kv1.5. Compound **8i** showed good selectivity for blockade of Kv1.5 vs hERG and L-type calcium channels. In an anesthetized pig model, compounds **8i** and **10c** increased atrial ERP about 28%, 18%, respectively, in the right atrium without affecting ventricular ERP.

Atrial fibrillation (AF) is the most common cardiac arrhythmia, affecting more than 2 million Americans.[1] AF can lead to thromboembolism, reduced left ventricular function or stroke, and is independently associated with increased mortality. Effective pharmacological treatment of atrial fibrillation remains an unmet medical need and current drug therapies for this type of arrhythmia are unsatisfactory.[2] Most current drug therapies target the hERG potassium ion channel which is present in both the atria and ventricles. Inhibition of hERG leads to QT prolongation and increases the incidence of the serious ventricular arrhythmia, torsade de pointe.

One strategy for safe, effective atrial antiarrhythmic drugs involves blockade of repolarizing ion channels that are found predominantly or only in the atria. The Kv1.5 potassium channel is an atrial-selective ion channel which underlies the ultra-rapid delayed rectifier K^+ current, IKur. This current is a major repolarizing current in human atria and is not found in human ventricles.[3] Thus, Kv1.5 is an attractive molecular target for treatment of atrial fibrillation or atrial flutter[4] and significant efforts have been made to identify novel blockers of Kv1.5. So far, several classes of compounds such as pyridazinones,[5] phosphine oxides,[6,7] indane sulfonamides,[8–10] and thiazolidinones[11] have been reported as potent Kv1.5 inhibitors by several pharmaceutical companies. Recently, Aventis published data on a new disubstituted bisaryl compound, (AVE0118),[12–15] and on anthranillic amides[16–18] as novel blockers of the Kv1.5 channel. AVE0118 blocks both IKur and Ito channels in vitro and shows atrial selectivity with no effect on the QT interval at the ventricular level in the chronically instrumented goat model. We describe herein the discovery of novel tetrahydroindolone-derived semicarbazones as potent blockers of the Kv1.5 channel.

Previous work from our laboratories primarily focused on the design and development of potassium channel blockers as Class III antiarrhythmic agents. Thus, our antiarrhythmic compound collection served as a starting point in the search for Kv1.5 blockers. We identified a phenylfuran-derived semicarbazone, PG-118668, as a potent inhibitor of the potassium channel. It demonstrated good efficacy in terminating atrial fibrillation in dog Y-shaped incision and vagatonic models[19] but was a potent hERG blocker. Further studies indicated that most analogs from this class blocked hERG with IC_{50} values ranging from sub-μM to 10 μM. This finding prompted us to search for ketone-derived semicarbazones with less hERG activity. Whole cell patch clamp electrophysiology was used to determine channel block in LTK^- cells expressing Kv1.5[20] and in HEK cells expressing hERG.[21] A FLIPR assay was used to

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determine channel block in HL-1 cells expressing endogenous L-type calcium channels.[22] When screening the ketone-derived semicarbazones, we discovered that the tetrahydro indolone derivative **8i** was a potent Kv1.5 inhibitor ($IC_{50} \sim 125$ nM). This compound also was selective for Kv1.5 vs the following channels: hERG (21 μM) and L-type Ca^{2+} (>30 μM).

The tetrahydroindolones were prepared using the routes shown in Scheme 1. The appropriate substituted diketones **1** were treated with hydroxylamine in the presence of KOH to generate their respective oximes **2**. The oximes **2** were then reacted with 1,3-cyclohexanedione to provide the corresponding tetrahydroindolones **3**. These tetrahydroindolones **3** were treated with MeI in the presence of NaH to afford *N*-methyl tetrahydroindolones **4**. Condensation of the tetrahydroindolones **3** and **4** with hydrazine or methyl hydrazine gave hydrazones or methyl hydrazones **5**, **6**, and **7** which were converted to the final desired semicarbazones **8**, **9**, **10**, and **11** by reaction with variety of arylisocyanates or arylisothiocyanates.

Shown in Figure 1, other semicarbazones like **12**, which was derived from tetrahydroindazolone, were prepared. Others such as **13**, **14**, and **15** were prepared from tetrahydrobenzofuranone, tetrahydro-benzothiophenone, and tetrahydroquinazolinone, using similar methods.

The scope of the tetrahydroindolone lead was further explored by: change of the R^1 and R^2 substituents on the tetrahydroindolone ring; replacement of the tetrahydroindolone ring with other heteroaromatic rings; and modifications of the isocyanate derived R^3 substituents.

As shown in Table 1, blockade of Kv1.5 was sensitive to the substitutions at the 2 and 3 positions of the tetrahydroindolone ring. For example, unsubstituted compounds (**8a,b**), analogs possessing more hindered alkyl groups such as 2-methyl-3-isopropyl (**8c,d**), or the 2,3-fused cyclohexane moiety (**8e**) showed little or no block of Kv1.5 at 1 μM concentration. In contrast, the 2,3-dimethyl, 2-methyl-3-ethyl, and 2,3-diethyl substituted derivatives (**8g–r**) exhibited significant block of Kv1.5. These results indicated that small structural changes in this area have significant impact on activity. Changes to the tetrahydroindolone core also significantly influenced activity. Replacement of the tetrahydroindolone core with other heteroaromatic cores (Fig. 1), such as 1-methyl tetrahydroindazolone, tetrahydrobenzofuranone, tetrahydrobenzothiophenone, and 2-methyl tetrahydroquinazolinone provided, inactive compounds **12**, **13**, **14**, and **15**.

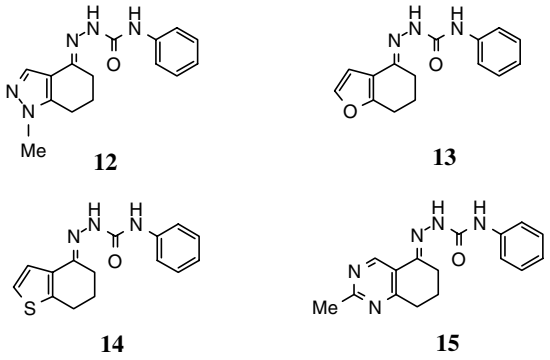
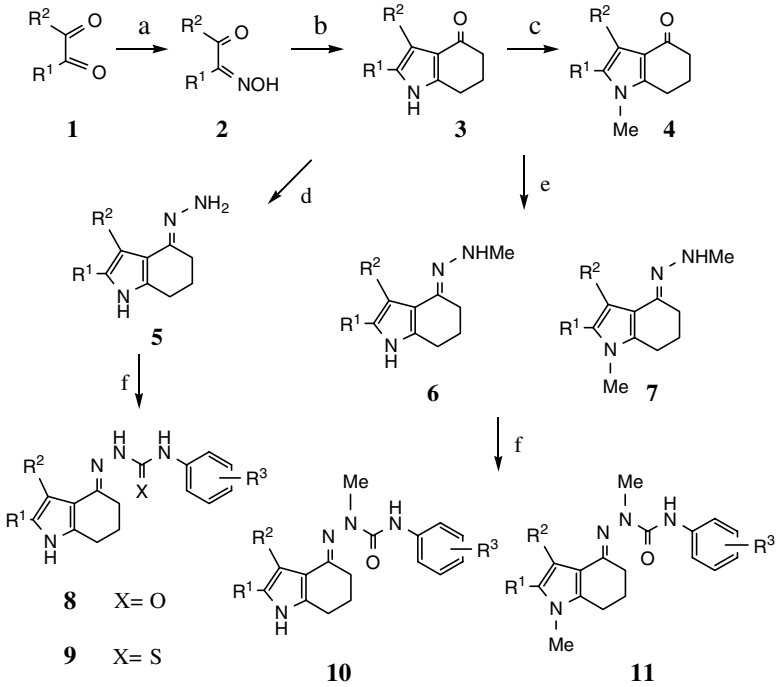
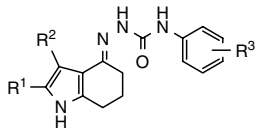


Figure 1. Semicarbazones derived from alternative scaffolds.



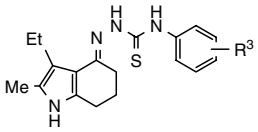
Scheme 1. Reagents and conditions: (a) NH_2OH/KOH, overnight; (b) 1,3-cyclohexanedione, Zn/HOAc, reflux, 3 h; (c) MeI, NaH/dioxane, 70 °C, 1.5 h; (d) NH_2NH_2 (excess)/dioxane, 60 °C, 7 h; (e) NH_2NHMe (excess)/dioxane, 60 °C; (f) arylisocyanates or arylisothiocyanates/MeOH, rt, 3 h.

Table 1. Kv1.5 Block by tetrahydroindolone-derived semicarbazones



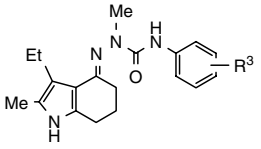
Compound	R^1	R^2	R^3	% Block of Kv1.5 at 1 μM	Kv1.5 IC_{50} (μM)
8a	H	H	H	Inactive	
8b	H	H	4-Cl	28	5.6
8c	Me	*i*-Pr	H	Inactive	
8d	Me	*i*-Pr	4-Cl	Inactive	
8e	$-CH_2(CH_2)_2CH_2-$		H	20	
8f	Me	Me	H	37	
8g	Me	Me	4-Cl	67	0.37
8h	Me	Me	4-CF_3	63	
8i	Me	Et	H	85	0.13
8j	Me	Et	4-Cl	65	0.44
8k	Me	Et	4-Me	50	
8l	Me	Et	4-SMe	80	0.28
8m	Me	Et	2-(*N*-Pyrrolidinyl)	60	0.75
8n	Et	Et	H	56	
8o	Et	Et	4-Cl	60	
8p	Et	Et	4-OMe	85	
8q	Et	Et	2-SMe	67	
8r	Et	Et	4-SMe	56	

Table 2. Kv1.5 Blockade by tetrahydroindolone-derived thiosemicarbazones



Compound	R^3	% Block of Kv1.5 at 1 μM	Kv1.5 IC_{50} (μM)
9a	3-Cl	78	
9b	4-Cl	71	
9c	3-Me	64	
9d	3-CF_3	91	0.20
9e	4-CF_3	52	

Table 3. Kv1.5 Blockade by *N*-methyl tetrahydroindolone-derived semicarbazones



Compound	R^3	% Block of Kv1.5 at 1 μM	Kv1.5 IC_{50} (μM)
10a	H	65	
10b	3-Cl	89	
10c	4-Cl	93	0.13
10d	4-SMe	78	
10e	4-Ac	86	

The blocking effects of the thiosemicarbazones (**9a–e**) against Kv1.5 were also examined. The data, summarized in Table 2, show these compounds displayed activity similar to the semicarbazones. A comparison of **9b** and **8j** suggests that replacing the oxygen with sulfur had little impact on activity.

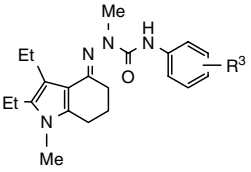
Replacement of the NH group of the semicarbazone side chain with an N–Me group, as shown in Table 3, provided analogs (**10a–e**) that showed similar or increased activity compared to the corresponding NH compounds. Most notably, the N–Me derivative **10c** was more potent than its direct NH comparator **8j** (IC_{50} = 0.13 μM vs. 0.44 μM) in blocking Kv1.5.

In the 2,3-diethyl substituted tetrahydroindolone analogs, the introduction of methyl groups on both N-1 of the tetrahydroindolone ring and the NH of the semicarbazone side chain provided compounds **11a–d** (Table 4). These analogs have similar or greater inhibitory activity for Kv1.5 compared to their non-methylated counterpart **8n–p**.

The compounds in Tables 3 and 4 also show that both electron-donating and electron-withdrawing R_3 substituents lead to active compounds with somewhat higher activity for compounds with electron-withdrawing substituents (**10b**, **c**, **e**; **11b**, **d**).

In the semicarbazone class, compounds **8i** and **10c** were profiled further in vivo. The tests were carried out in an anesthetized mini-pig model, similar to the model that Aventis recently reported.[17] The model was validated using the marketed class III agent dofetilide which

Table 4. Kv1.5 Blockade by N_1-methyl, *N*-methyl tetrahydroindolone-derived semicarbazones



Compound	R^3	% Block of Kv1.5 at 1 μM	Kv1.5 IC_{50} (μM)
11a	H	84	
11b	4-Cl	90	
11c	4-OMe	75	
11d	4-Ac	90	0.37

increased both atrial effective refractory period (ERP) and ventricular effective refractory period (ERP). Preliminary results showed that both compounds provided a dose-dependent increase in atrial ERP with no increase in ventricular ERP after a 15 min iv infusion. Figure 2 shows that right atrial ERP increased by 28% for **8i** at a dose of 10 mg/kg and 18% for **10c** at a dose of 30 mg/kg. At this same dose, there is no increasing on ventricular ERP.

In conclusion, we have discovered a novel class of tetrahydroindolone-derived semicarbazones that are potent blockers of the Kv1.5 channel. Some of these compounds show very good selectivity for Kv1.5 over L-type calcium and hERG channels. The in vivo pig results with compounds **8i** and **10c** show selective atrial ERP prolongations with no affect on ventricular ERP. The atrial-selective ERP increases in pigs are consistent with the atrial-selective presence of IKur in man. Thus, Kv1.5 blockers may offer an approach for the development of atrial-selective antiarrhythmic drugs for the treatment of AF (Fig. 3).

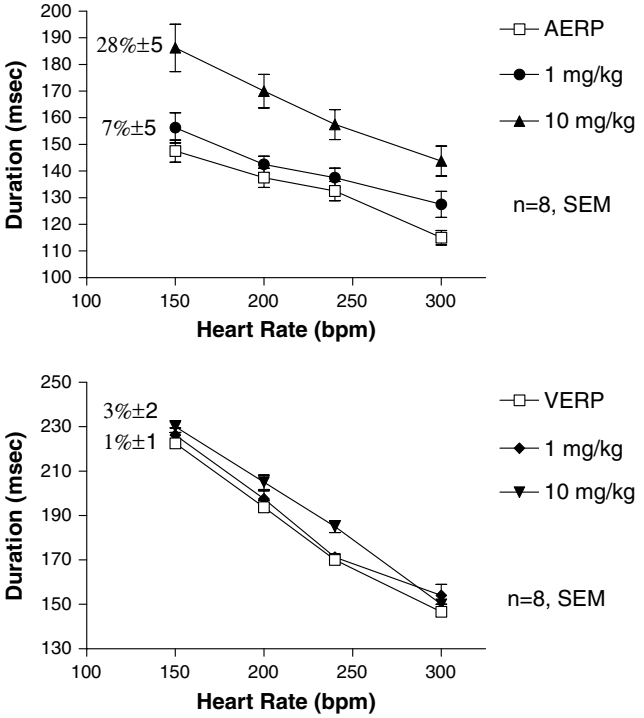


Figure 2. Anesthetized pig AERP and VERP changes for **8i** at 1 and 10 mg/kg doses.

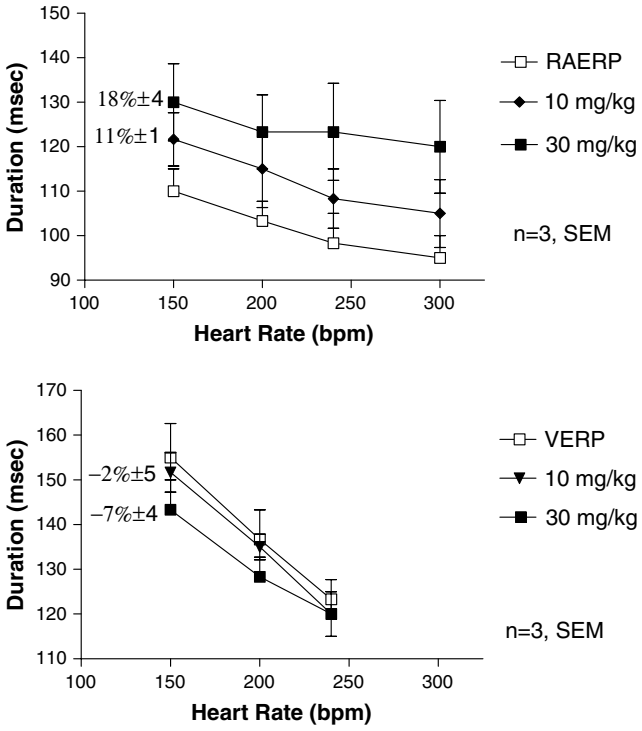


Figure 3. Anesthetized pig AERP and VERP changes for **10c** at 10 and 30 mg/kg doses.

Acknowledgments

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20. Kv1.5 currents are recorded by the whole cell mode of patch clamp electrophysiology. Kv1.5 is stably over expressed 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} estimating were done using Graphpad software (Ver. 4).
21. HERG currents are recorded by the whole cell mode of patch clamp electrophysiology as described by Hamill et al.[23] 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} estimating were done using Graphpad software (Ver. 4).
22. 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. 50 μL 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, 50μL 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 IC50 values are generated using GraphPad Prism 4.0.
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