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Discovery and in vitro/in vivo studies of tetrazole derivatives as Kv1.5 blockers

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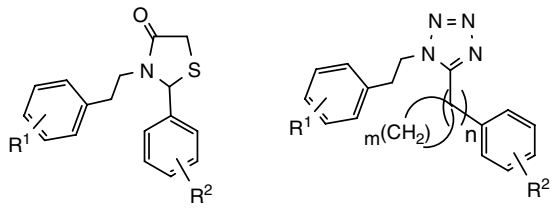
Abstract—A novel class of tetrazole-derived Kv1.5 blockers is disclosed. In in vitro studies, several compounds had IC_{50}s ranging from 180 to 550 nM. In vivo studies indicated that compounds **2f** and **2j** increased right atrial ERP about 40% without affecting ventricular ERP.

Atrial fibrillation (AF) is the most common chronic arrhythmia[1] and increases the risk of stroke and overall mortality. Treatment of this ailment remains problematic.[2] Most current drug therapies target the hERG potassium ion channel which is present in both the atria and the ventricles. Blockade of hERG leads to QT prolongation and increases the incidence of the serious ventricular arrhythmia, torsade de pointes.

One strategy for the development of 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] Significant efforts have been made to identify novel blockers of Kv1.5.[5–19] Icagen described the thiazolidinone derivatives **1** as potent and selective Kv1.5 blockers.[11] In a preceding publication, we reported that the ring sulfur was rapidly metabolized by oxidation. To overcome this metabolic liability, we describe herein replacement of the thiazolidinone scaffold with a tetrazole scaffold **2** (Fig. 1).

The synthesis of these compounds begins with conversion of appropriate acid derivatives **3** to the corresponding acid chlorides **4**. These acid chlorides **4** are then treated in situ with aryl ethyl amines to generate the amides **5**. The amides **5** are then reacted with PCl_5 and $TMSN_3$ to provide desired products **2(a–n)** in good yield (Scheme 1).

A variety of analogs were prepared (Table 1). Three analogs, **2a–2c**, with $n = 0$ were prepared and evaluated in vitro. While both **2a** and **2b** showed modest block of Kv1.5 at 1 μM, **2c** had greater activity and an IC_{50} value of 549 nM.[20] Since the planar tetrazole ring restricts the orientation of the 'lower' aromatic ring, we prepared tetrazoles **2d** and **2e** ($n = 1$, $m = 0$) with a one-carbon spacer to allow additional conformational



1 2

Figure 1. Comparison of thiazolidinone and tetrazole derivatives.

Keywords: Tetrazoles; Kv1.5 blocker; Atrial antiarrhythmic; In vivo efficacy.
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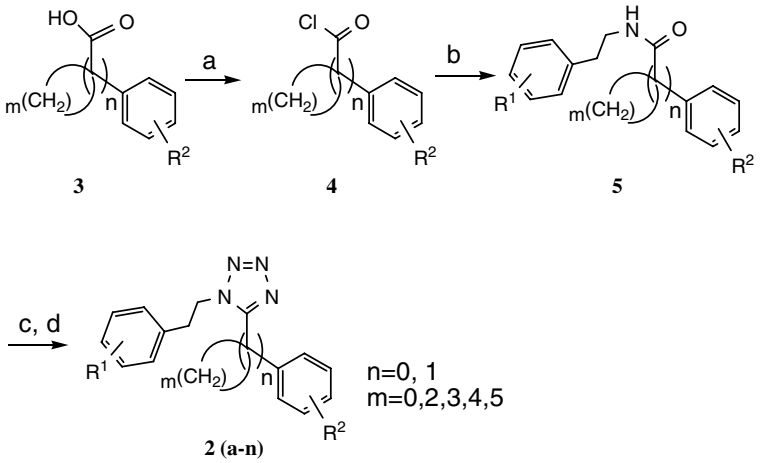


Scheme 1. Reagent and conditions: (a) $SOCl_2$, rt, 1 h; (b) aryl ethyl amines, THF, rt, 4 h; (c) PCl_5, CH_2Cl_2, −5 °C, 2 h; (d) $TMSN_3$, CH_2Cl_2, rt, overnight.

Table 1. In vitro inhibitory activity of tetrazoles against the Kv1.5 channel

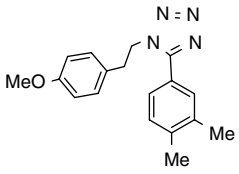
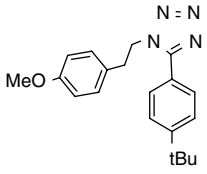
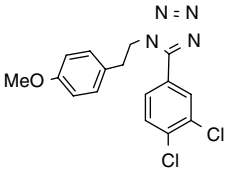
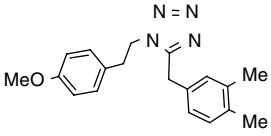
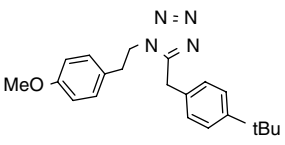
Compound	Structure	% Block of Kv1.5 at 1μM	Kv1.5 IC_{50} (μM)
2a		36	
2b		37	
2c		72	0.55
2d		75	0.33
2e		60	

Table 1 (*continued*)

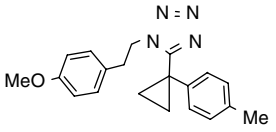
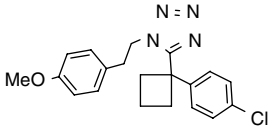
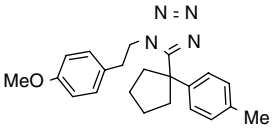
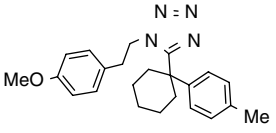
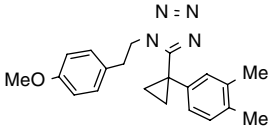
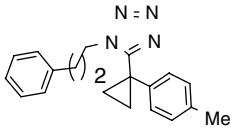
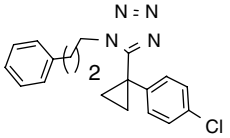
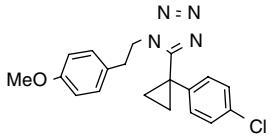
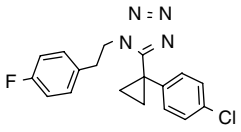
Compound	Structure	% Block of Kv1.5 at 1μM	Kv1.5 IC_{50} (μM)
2f		90	0.33
2g		20	
2h		89	0.40
2i		96	
2j		94	0.18
2k		72	0.48
21		81	
2m		74	
2n		47	

Table 2. In vivo anesthetized pig results for compounds **2c**, **2d**, **2f**, and **2j**

Compound	AERP prolongation		VERP prolongation		dp/dt_{max} change		BP change	
	10 mg/kg	30 mg/kg	10 mg/kg	30 mg/kg	10 mg/kg	30 mg/kg	10 mg/kg	30 mg/kg
2c	6 ± 3%	16 ± 7%	6 ± 7%	6 ± 7%	−24 ± 1%	−49 ± 4%	−6 ± 5%	−42 ± 4%
2d	3 ± 7%	23 ± 10%	0 ± 3%	0 ± 5%	−26 ± 5%	−32 ± 18%	−11 ± 13%	−21 ± 10%
2f	17 ± 2%	44 ± 5%	5 ± 4%	3 ± 3%	−1 ± 7%	−14 ± 14%	−5 ± 3%	−4 ± 1%
2j	16 ± 5%	37 ± 7%	9 ± 4%	4 ± 4%	−10 ± 1%	−12 ± 15%	7 ± 4%	19 ± 4%

freedom in the 'lower' aromatic ring. Both compounds are better Kv1.5 blockers compared to **2a** and **2b**, and compound **2d** exhibits a good potency with an IC_{50} value of 331 nM.

Following this finding, we then installed a cyclopropyl ring on the linker to the 'lower' aromatic ring to provide compounds **2f**, **2j**, and **2m** bearing the 4-methoxyphenethyl group in common. The in vitro results demonstrated that installation of this cyclopropyl ring increased the % block of Kv1.5. Compound **2d** has an IC_{50} value of 331 nM while the cyclopropyl ring-derived analog **2j** is about twice as potent with an IC_{50} value of 177 nM. Additionally, the substituents on the 'lower' phenyl ring have an impact on the activity of block Kv1.5 in these cyclopropyl ring-derived analogs. The 3,4-di-Me analog **2j** is more active than the 4-Me analog **2f** and the 4-Cl analog **2m**.

Further ring size variations indicated that analogs **2f**, **2h** and **2i** with three-, five-, and six-membered ring were active and potent Kv1.5 blockers. In contrast, analog **2g** with a four-membered ring showed significantly reduced blockade of Kv1.5 versus **2m**. These results demonstrate that activity is sensitive to the size of the cyclic rings. Furthermore, it is clear that compound **2n** with the electron-withdrawing group on the 'upper' phenyl ring (R_1 = F) is significantly less active against Kv1.5 (47%) compared to compound **2m** (74%) with electron-donating group (R_1 = OMe). Finally, compounds **2k** and **2l** show that the linker to the phenethyl group can be extended and still retain good Kv1.5 blockade.

Compounds **2c**, **2d**, **2f**, and **2j** were selected for in vivo evaluation in the anesthetized mini-pig (n = 3) which was used to investigate the effects on the atrial and ventricular effective refractory period (AERP and VERP).[21] Compounds were administered via 15 min iv infusions of 10 and 30 mg/kg doses. As shown in Table 2, compounds **2c** and **2d** provided small increases in AERP at a 10 mg/kg dose but larger increases at a 30 mg/kg dose. No changes in VERP were noted at either dose. However, reductions in contractility (dp/dt_{max}) and/or blood pressure were noted at both doses. Fortunately, improvements in both AERP prolongation and hemodynamic side effects were obtained with the addition of the cyclopropyl ring on the linker to the 'lower' aromatic ring. Compounds **2f** and **2j** showed dose-dependent increases of AERP and no effect on VERP. Compound **2f** had the best overall profile showing minimal effects on blood pressure or contractility. Figure 2 shows a typical dose–response curve for AERP prolongation. Additionally, compound **2f** also showed good selectivity for

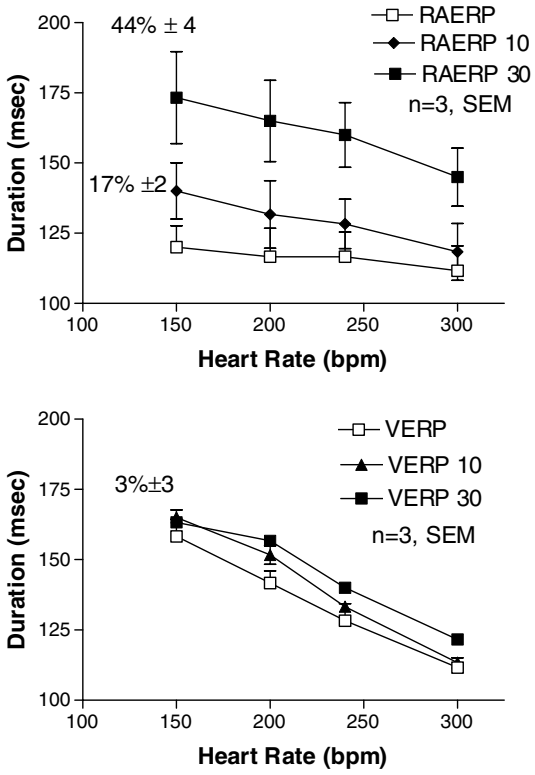


Figure 2. Anesthetized pig AERP and VERP changes of **2f** at different heart rates.

Kv1.5 block over hERG[22] (48-fold) and L-type calcium[23] (69-fold) channels consistent with atrial selectivity observed in vivo.

In conclusion, we have discovered a novel class of tetrazole derivatives that are potent blockers of the Kv1.5 channel. Analogs **2f** and **2j**, which contained the cyclopropyl ring on the linker to the 'lower' aromatic ring, displayed very good in vitro blockade of Kv1.5 as well as atrial-selective prolongation of ERP in vivo. The compound **2f** also lacked hemodynamic side effects. The atrial-selective ERP prolongation in pigs is consistent with the atrial-selective presence of IKur in human.[24] These findings support the potential for Kv1.5 channel blockers to provide atrial-selective antiarrhythmic drugs to treat AF.

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20. 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 megaohms). 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. In vivo assay: Vehicle: Compounds are dissolved to a final concentration of 20–50 mg/ml, first in dimethyl acetamide (DMAC) then adding the balance of propylene glycol 200 (PEG200) for a ratio of 20% DMAC/80% PEG200. *Mini Swine*: Minipigs of the Hanford or Sinclair strain weighing 15–30 kg are anesthetized with an IM injection of ketamine/xylazine followed, if needed, by 1–1.5% isoflurane to allow introduction of a venous catheter into the vena cava in the neck. Following incubation, IV pentobarbital is given and anesthesia is maintained via further IV boluses given during the study. Two electrode-tipped catheters are introduced via the jugular, one into the right atrium and the other into the right ventricle, and are used to pace the heart when needed. The carotid artery is cannulated and a pressure transducer-tipped catheter advanced into the left ventricle to track pressure development in the LV. An incision in the groin is used to access the femoral artery and vein. The artery is cannulated to monitor arterial pressure at the lower aorta and the vein is cannulated with an electrode-tipped catheter advanced into the right atrium to detect signal propagation. The arterial pressure, ECG, LV pressure, atrial electrogram, body temperature, and exhaled Pco2 are monitored continuously. When the surgical preparation is stable, baseline effective refractory periods (ERPs) are determined. The ERP is found by repeatedly pacing the heart chamber with current 2.5× the capture threshold at a given rate for 13 beats followed by a 3 s pause. With each pacing train the time interval between the 12th and 13th stimulus is shortened until the 13th does not cause signal propagation in the tissue. The interval that does not result in a propagated signal in 4 out of 5 repetitions is the ERP. Hearts are paced at rates of 150, 200, 240, and 300 beats per minute from the right atrium and the right ventricle. Compound is infused IV over 15 min, ERP determinations are made starting at the 12th minute of the infusion, and the animal is allowed to stabilize for about 15 min before another dose is given. After the final dose the ERPs are determined every 15 min until the values are back at baseline.
22. HERG currents are recorded by the whole cell mode of patch clamp electrophysiology as described by Hamill et al.[25] HERG is stably overexpressed in HEK cells. Microelectrodes are pulled from borosilicate glass (TW150) and heat polished (tip resistance, 1.5–3 megaohms). 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 deter-

minations 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).

23. HL-1 cells expressing endogenous L-type calcium channels are removed from culture flasks using trypsin, plated on fibronectin/gelatin-coated, clear-bottommed, 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 IC50 values are generated using GraphPad Prism 4.0.
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