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# Discovery of triazolopyridinone GS-462808, a late sodium current inhibitor (Late $I_{Na}i$) of the cardiac $Na_v1.5$ channel with improved efficacy and potency relative to ranolazine

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ABSTRACT

Previously we disclosed the discovery of potent Late $I_{Na}$ current inhibitor **2** (GS-458967, $IC_{50}$ of 333 nM) that has a good separation of late versus peak $Na_v1.5$ current, but did not have a favorable CNS safety window due to high brain penetration (3-fold higher partitioning into brain vs plasma) coupled with potent inhibition of brain sodium channel isoforms ($Na_v1.1$, 1.2, 1.3). We increased the polar surface area from 50 to 84 Å$^2$ by adding a carbonyl to the core and an oxadiazole ring resulting in **3** GS-462808 that had lower brain penetration and serendipitously lower activity at the brain isoforms. Compound **3** has an improved CNS window (>20 rat and dog) relative to **2**, and improved anti-ischemic potency relative to ranolazine. The development of **3** was not pursued due to liver lesions in 7 day rat toxicology studies.


Atherosclerotic narrowing of coronary vessels leads to ischemic heart disease (IHD) that has a high prevalence (7%) within the United States (US).[1] The initial stage of IHD is silent ischemia that often progresses to more severe ischemia that causes chest pain (angina), a condition that afflicts 9 million people in the US.[2] Ranolazine **1** was approved for the treatment of chronic angina in the US in 2006.[3] At therapeutic concentrations, ranolazine does inhibit cardiac late sodium current, although the mechanism of ranolazine's antianginal effect has not been determined. In an ischemic state, reactive oxygen species (ROS) can modify the cardiac sodium channel resulting in incomplete inactivation with repetitive opening of the inactivation gate resulting in a persistent sodium current that occurs late in the action potential, thus termed Late $I_{Na}$ current (Fig. 1).[4] Late $I_{Na}$ current leads to sodium overload in the cytoplasm and subsequent $Ca^{+2}$ overload through activity of the reverse mode Na–$Ca^{+2}$ exchanger, and ranolazine inhibits this process thereby lowering the ischemic burden (calcium imbalance and sequelae).[5] At its therapeutic concentration (2–8 μM in plasma), ranolazine inhibits a number of cardiac ion currents (e.g., $I_{Kr}$).[6] Ranolazine provides its antianginal benefit without causing bradycardia (slowing of heart rate) and/or lowering systemic blood pressure.[6] Ranolazine has been shown to inhibit S-T segment elevation in preclinical models of ischemia that correlates well with it's ability to inhibit S-T segment elevation in humans with angina undergoing exercise treadmill testing (MARISA study).[7] We describe our efforts to identify a 2nd generation Late $I_{Na}$ inhibitor with improved properties relative to ranolazine with less hERG and β-blocking activity.

Evidence provided through site directed mutagenesis studies suggest that ranolazine binds to the lidocaine binding site in the mouth of the cardiac sodium channel pore.[8] Late $I_{Na}$ current can be generated in vitro by the addition of toxins ATX-II or tefluthrin that bind to an external binding site that does not overlap with the lidocaine binding site (Fig. 1).[9] Ranolazine (**1**, Fig. 2) has an $IC_{50}$ 6.9 μM for ATX-II induced Late $I_{Na}$ inhibition (single cell manual patch).[6]

Peak $I_{Na}$ current is responsible for the propagation of the action potential from the pacemaker sinoatrial (SA) node through the atria and ventricles, and it is critical to have a good separation of Late $I_{Na}$ inhibition from peak $I_{Na}$ current. Ranolazine does not inhibit peak $I_{Na}$ current at therapeutic levels with a separation of >50 fold.[6]

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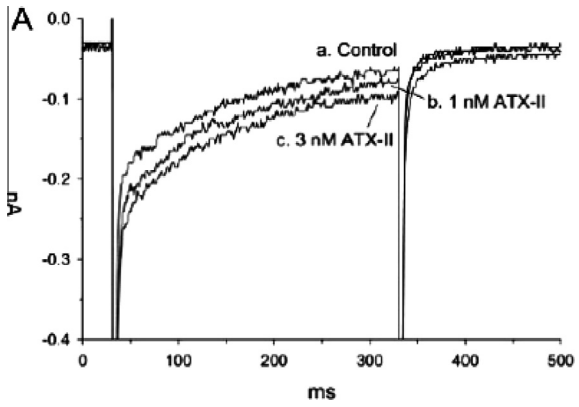


**Figure 1.** Late sodium channel current (Late $I_{Na}$) in a control experiment (a), or enhanced with aid of 1 nM (b) or 3 nM (c) ATX-II.

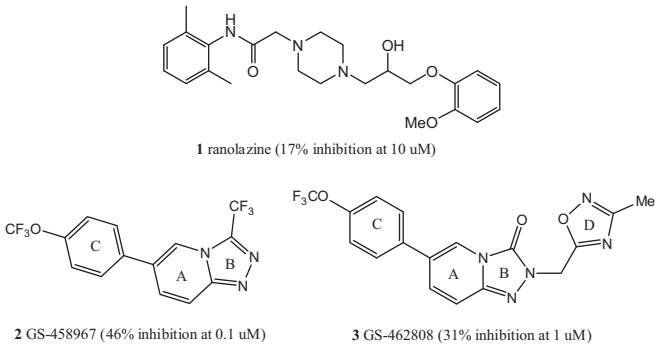


**Figure 2.** Ranolazine (**1**), potent Late $I_{Na}$ inhibitor (GS-458967, **2**), and selective Late $I_{Na}$ inhibitor (GS-462808, **3**).

Herein, we describe our efforts to discover a potent Late $I_{Na}$ current blocker selective against peak current, and demonstrate the anti-arrhythmic effects of our lead molecule **3** in isolated heart and in vivo ventricular arrhythmia models, respectively. Previously, we screened a number of in-house heterocyclic compounds without a basic group, to decrease the likelihood of hERG and β-blockade, for their Late $I_{Na}$ inhibitory activity using automatic patch clamp system ($hNa_v1.5$ α-subunit HEK-293) that led to the discovery of triazolopyridine **2** (Fig. 2).[10] The potent Late $I_{Na}$ current inhibitor **2** ($IC_{50}$ of 333 nM) has a good separation of Late $I_{Na}$ current inhibition from peak sodium current, but does not have a favorable CNS window due to high brain penetration (brain to plasma 3:1 partitioning) and high activity at brain sodium isoforms ($Na_v1.1$, 1.2, 1.3). We designed compound **3** with an increased polar surface area from 50 to 84 $Å^2$ by adding a carbonyl to the core and an oxadiazole ring with the hope of lowering brain penetration. We will describe the SAR leading to the discovery of [1,2,4]triazolo[4,3-*a*]pyridin-3(2*H*)-one **3** and it's improved properties relative to **2**.[11]

In general, the compounds were prepared via Suzuki coupling using a palladium catalyst, for example [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride, a bromo core molecule, and an appropriately substituted boronic acid derivative of formula R-Ar-$B(OH)_2$ in the presence of a base (potassium carbonate) using an inert solvent (degassed 2:1:1 toluene/isopropanol/water) at a temperature of 95 °C, typically for 3 days (**3**, Fig. 3).[11] Subsequently we alkylated the triazolone core using base (potassium carbonate) and alkyl halides in an inert solvent (*N*,*N*-dimethylacetamide) by heating to 110 °C for 2 h. 6-Bromo-[1,2,4]triazolo[4,3-*a*]pyridin-3(2*H*)-one core was prepared from the corresponding 2-hydrazinopyridine by reacting with carbonyldiimidazole (1.1 equiv) in acetonitrile at reflux temperatures for 2 h.

In optimization of our compounds to widen the CNS margin, we added the brain $Na_v$ 1.1 to our testing paradigm with stimulation at 10 Hz frequency. Lead compounds were tested at $Na_v$ 1.1, 1.2, and 1.3, but we found that activity for $Na_v$ 1.1 inhibition correlated well with activity at $Na_v$ 1.2 and 1.3, so we screened against $Na_v$ 1.1. Our testing paradigm starts with screening of our synthetic analogs for Late $I_{Na}$ inhibition at 10 μM concentration. With a few exceptions, compounds exhibiting 70% or greater inhibition at 10 μM were subsequently tested to determine an $IC_{50}$. Selected compounds were then counter-screened against Peak $I_{Na}$ inhibition at concentration close to their 50% block of Late $I_{Na}$—typically 1 μM. The screening for Peak $I_{Na}$ inhibition was performed at a pacing frequency 3 Hz, corresponding to 180 beats per minute.

Initially, we fixed the 4-trifluoromethoxyphenyl C ring and varied the D ring in our SAR studies on Late $I_{Na}$ current inhibition versus peak sodium current and $Na_v$ 1.1. The results of our study are shown in Table 1. Early on in our SAR studies, we prepared the 2-((3-methyl-1,2,4-oxadiazol-5-yl)methyl) D-ring **3** that afforded acceptable Late $I_{Na}$ inhibition ($IC_{50}$ = 1.9 μM), a good separation from Peak $I_{Na}$ inhibition, favorable metabolic stability, and most importantly lower activity at the brain isoform $Na_v$ 1.1. The unsubstituted triazolone core **7** retained the Late $I_{Na}$ inhibition and metabolic stability, but picked up more Peak $I_{Na}$ inhibition. The 2-((5-methyl-1,2,4-oxadiazol-3-yl)methyl) analog **8** was slightly less active for Late $I_{Na}$ inhibition, and exhibited more Peak $I_{Na}$ inhibition than **3**. Removing a nitrogen from the D ring of **3** as in oxazole **9** and isoxazole **10**, resulted in less Late $I_{Na}$ inhibition. Also, replacing the 5-methyl of **3** with the larger 5-ethyl **11** and 2,6-dichlorophenyl **12**, led to less Late $I_{Na}$ inhibition. The 2-((1-methyl-1*H*-1,2,4-triazol-3-yl)methyl) **13** shows the importance of D-ring electronics as it was completely inactive, in spite of having a similar steric shape as **3**. We tried to improve on oxazole **9** by either moving the methyl to the 5-position as in **14** or replacing with a cyclopropyl group **15**, but both compounds were inferior to **3** (Peak $I_{Na}$ inhibition and metabolic stability, respectively).

Next, we decided to explore the SAR of replacing the trifluoromethoxy group with either a 4-fluorophenoxy or 4-chlorophenoxy group using some of the favorable D-rings as shown in Table 2. The direct analogs of **3**, 4-fluorophenoxy **16** and 4-chlorophenoxy **21**, had improved Late $I_{Na}$ inhibition by 10 fold, but both compounds had too much Peak $I_{Na}$ inhibition and were less metabolically stable. The enhanced lipophilicity (measured Log *D* from 3.0 to 3.4) in this region really had a pronounced effect

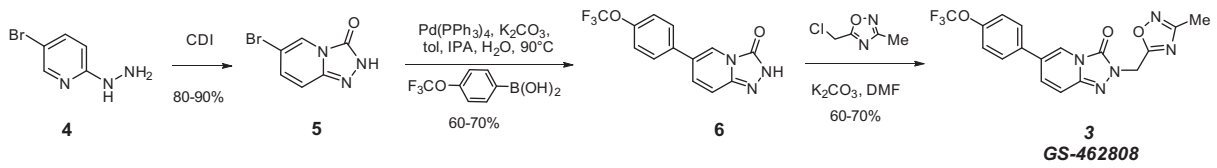


**Figure 3.** Preparation of **3**.