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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 vs. 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 (Figure 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 pre-clinical 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 (Figure 1).[9] Ranolazine (**1**, Figure 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]

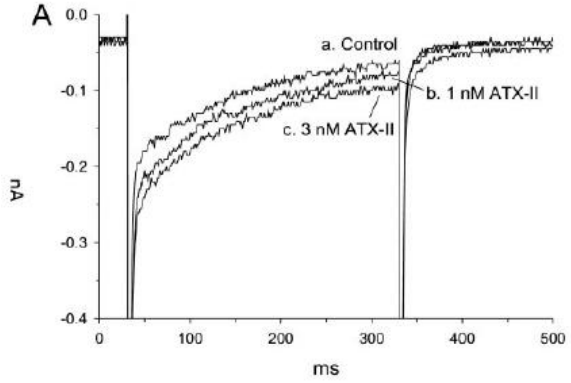


Figure 1. Late sodium channel current (Late I_{Na}) in a control experiment (a), or enhanced with aid of 1 nM (b) or 3 nM (c) ATX-II.

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** (Figure 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(2H)-one **3** and it's improved properties relative to **2**.[11]

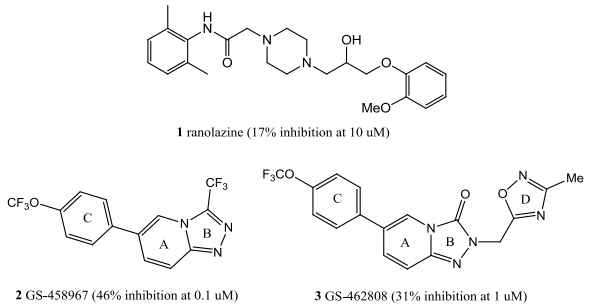
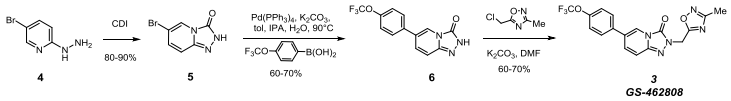


Figure 2. Ranolazine (**1**), potent Late I_{Na} inhibitor (GS-458967 , **2**), and selective Late I_{Na} inhibitor (GS-462808, **3**).

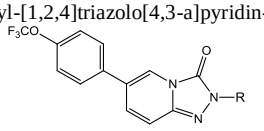
In general, the compounds were prepared via Suzuki coupling using a palladium catalyst, for example [1,1'-bis(diphenylphosphino)ferrocene]palladium(II) dichloride, a bromo core molecule, and an appropriately substituted boronic acid derivative of formula R-Ar-$B(OH)_2$ in the presence of a base (potassium carbonate) using an inert solvent (degassed 2:1:1 toluene:isopropanol:water) at a temperature of 95 °C, typically for 3 days (**3**, Figure 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(2H)-one core was prepared from the corresponding 2-hydrazinopyridine by reacting with carbonyldiimidazole (1.1 equivalents) in acetonitrile at reflux temperatures for 2 hours.

Figure 3. Preparation of **3.**



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.

Table 1. Sodium channel blocking properties of 4-trifluoromethoxyphenyl-[1,2,4]triazolo[4,3-a]pyridin-3(2H)-one



	R	Late Na IC_{50} μM	Mic. $T_{1/2}$ h:r:d	Peak $Na_v1.5$ block, % (μM) 3 Hz $Na_v1.5$	10 Hz $Na_v1.1$
3		1.9	308:198:236	10(10)	8(10)
7	H	1.9	390:89:190	24 (10)	
8		3.3	395:363:NT	10(10)	27(10)
9		2.5	144:41:160		28(10)
10		>10			
11		4.0			
12		3.5			
13		>10			
14		2.5	288:24:228		44(10)
15		3.0	39:39:108		

Initially, we fixed the 4-trifluoromethoxy phenyl 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 **7**, resulted in less Late I_{Na} inhibition. Also, replacing the 5-methyl of **3** with the larger 5-ethyl **11** and 2,6-dichlorophenyl **9**, led to less Late I_{Na} inhibition. The 2-((1-methyl-1H-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 on Late I_{Na} inhibition, but **16** and **21** are not coming off the ion channel fast enough resulting in Peak I_{Na} inhibition. Compound **18** and **21** are direct analogs of **8**, and they were much more potent for Late I_{Na} inhibition, but both have an unacceptable increase in Peak I_{Na} inhibition. Adding a polar hydroxyl group as in **19** (measured Log D 2.8) lessened the Peak I_{Na} inhibition substantially, but it was not metabolically stable. Changing to 2-((5-cyclopropyl-1,3,4-oxadiazol-2-yl)methyl) D-ring **17** enhanced metabolic stability, but it had too much Peak I_{Na} inhibition.

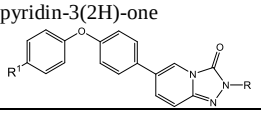
Compound **3** had the best overall properties with respect to low Peak I_{Na} and Na_v 1.1, so we continued to profile it. In manual patch assays, **3** had 10 fold lower activity than **2** at the brain isoforms when tested at 10 Hz (Table 3). [12,13] Subsequently, this translated to a larger CNS margin in both rat and dog (>20x). In addition, the increase in PSA for **3** relative to **2** may have accounted for diminished brain levels (brain vs. plasma 1.5:1 vs 3:1 for **2**, rat). The hERG IC_{50} was very similar for the two compounds.

We evaluated PK across the preclinical species, and we found a good *in vitro-in vivo* correlation for clearance (Table 4). The half-life was moderate in the monkey and rabbit, short in the rat, and relatively long in the dog that displayed a larger volume of distribution. Allometric scaling projected an 8 hour half-life in humans for compound **3**. The oral bioavailability for **3** was good in rat and dog. The free fraction for **3** was similar across the species in the 4-5% range.

Next, we looked at the anti-arrhythmic effects of our lead molecule **3** compared to ranolazine in isolated heart and *in vivo* Torsades des pointes model. In isolated rabbit hearts, we add the toxin ATX-II to induce Late I_{Na} that prolongs the mean action potential duration ($MAPD_{90}$) by approximately 100 msec. The addition of Late I_{Na} inhibitors ranolazine or **3** reverse the ATX-II effects in the isolated heart, albeit to a different degree and potency. Compound **3** reverses the ATX-II effect by 90% with an IC_{50} of 1.2 μM, but ranolazine provides only a partial reversal by 60% with an IC_{50} of 16 μM. Late I_{Na} prolongs the $MAPD_{90}$ in the isolated heart that *in vivo* would translate into a longer QT interval. Long QT-3 patients have mutations in their sodium channel that leads to improper inactivation of the channel where it repetitively opens and closes affording a persistent current late in the action potential duration.

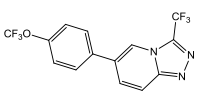
Ranolazine shortens the long QT interval in LQT3 patients that is consistent with Late I_{Na} inhibition. The ATX-II isolated heart model is an *ex vivo* model of LQT3 patients. Then, we determined the anti-arrhythmic effect of **3** in an *in vivo* model of chemically induced ventricular arrhythmia (Figure 5). In anesthitized rabbits we slowed the heart rate through the addition of an α-blocker (methoxyamine) followed by the addition of a hERG blocker (clofilium) that induced the control animals to have *torsade des pointes* (TdP).

Table 2. Sodium channel blocking properties of 4-phenoxyphenyl-[1,2,4]triazolo[4,3-a]pyridin-3(2H)-one

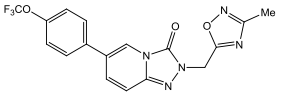


	R	Late Na IC_{50} nM or % Inhib nM	Mic. $T_{1/2}$ h:r:d	Peak $Na_v1.5$ block, % (μM) 3 Hz $Na_v1.5$	10 Hz $Na_v1.1$
16 **R^1=F**		215	127:85:217	33(0.3)	NT
17 **R^1=F**	H	1903	395:43:273	28 (1)	40(10)
18 **R^1=F**		541	207:145:167	46(1)	65(10)
19 **R^1=F**		981	81:32:181	21(10)	56(10)
20 **R^1=F**		740	395:395:287	69(1)	56(10)
21 **R^1=Cl**		134	45:34:94	47(1)	42(10)
22 **R^1=Cl**		60% @300 nM	395:390:354	40(1)	79(10)

Table 3. Sodium channel blocking properties of triazolopyridine **2** versus triazolonepyridine **3** (Manual patch clamp).



2 GS-458967



3 GS-462808

Assay	2	3
Late $Na_v1.5$ IC_{50} μM	0.25	1.33
Peak Nav 1.5 IC_{50} μM @ 1 Hz / 3 Hz	>10 / >3	>10 / >10
Nav 1.1 IC_{50} μM @ 1 Hz / 10 Hz	>10 / 0.4	>10 / 4.8
Nav 1.2 IC_{50} μM @ 1 Hz / 10 Hz	>10 / 0.4	>10 / 4.2
hERG IC_{50} μM	7.9	8.1

Compound **3** completely protected against Tdp at 3 μM. Ranolazine was only partially protective at much higher concentrations (14 uM) with 40% of the animals having Tdp.

Table 4. *In vitro* ADME and *in vivo* pharmacokinetic properties of compound **3**..

Clearance (L/h/kg)	*In vitro* (mic.)	*In vivo*
Rat:	0.52	0.719
Dog:	0.26	0.479
C. Monkey:	0.40	0.478
Human:	0.21	
Rabbit:		0.422
Terminal Half Life (h)		
Rat:		2.10
Dog:		17.3
Monkey:		4.78
Rabbit:		4.81
Vol. of dist. (L/kg)		
Rat:		1.95
Dog:		4.23
C. Monkey:		2.29
Rabbit:		2.24
Bioavailability (F)		
Rat:		44.3% [a]
Dog:		72.8% [b]
Protein binding (%)		
Rat:	95.4	
Dog:	94.3	
Human:	95.0	
Female Rabbit:	95.7	

[a] (15/10/75: NMP/Solutol HS 15/water)
[b] (5%EtOH, 40%PEG, 55% Water)

Figure 4. Reversal of ATX-II increase in $MAPD_{90}$ by compound **3** (1.2 uM IC_{50}, orange) and ranolazine (16 uM IC_{50}, red) in rabbit isolated heart.

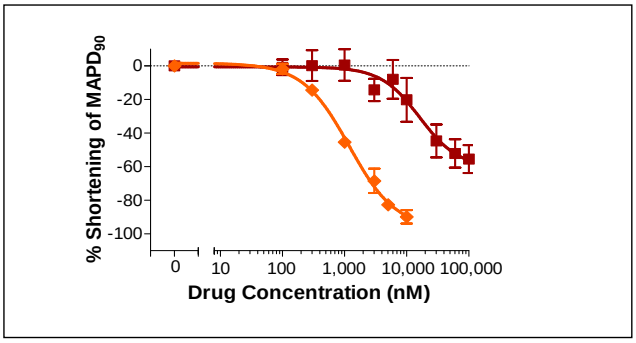
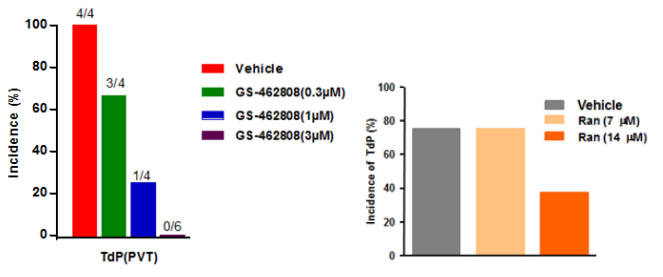


Figure 5. Protection from hERG induced ventricular arrhythmias by **3** (GS-462808) in anesthetized rabbits.



We went on to study **3** in 7 day rat and dog toxicology studies. We were gratified to find a much wider CNS margin (>20x) based on projected human C_{max} for **3** in both rat and dog, a dramatic improvement from the CNS margin for the initial compound **2** (5x rat, 3x dog). These results were consistent with both lower brain penetration and lower activity at the brain isoforms. Unfortunately, **3** led to liver lesions in the rat that were initially thought to be due to cleavage of the oxadiazole ring. Subsequently, we replaced the oxadiazole ring with an oxazole, and we still observed the liver lesion in the 7 day rat toxicology studies. We found that glutathione added to the [1,2,4]triazolo[4,3-a]pyridin-3(2H)-one core of both **3** and it's oxazole analog; therefore, ending further exploration of this series.

We designed **3** with an increased polar surface area (from 50 to 84) by adding a carbonyl to the core and an oxadiazole ring with the hope of lowering brain penetration. We found that **3** had decreased brain penetration than the previous lead **2**, and serendipitously had lower activity at the brain isoforms. Although we had established a wider CNS margin with **3**, the [1,2,4]triazolo[4,3-a]pyridin-3(2H)-one core had liabilities in rat toxicology studies. We will report our efforts to replace the core in due course.

Acknowledgments

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References and Notes

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[13] ^{1}H NMR of **3** (δ, d_6-DMSO, 400 MHz): 7.95 (dd, 1H) 7.54 (d, 2H), 7.40 (dd, 1H), 7.32 (d, 2H), 7.20 (dd, 1H) 5.43 (s, 2H), 2.40 (s, 3H). For experimental details see supplement.

Graphical Abstract

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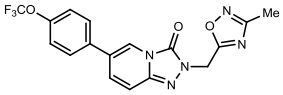

3 GS-462808

Luiz Belardinelli,[b] Jeff A. Zablocki.*[a]