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Discovery and optimization of aminopyrimidinones as potent and state-dependent Nav1.7 antagonists

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ABSTRACT

Clinical genetic data have shown that the product of the SCN9A gene, voltage-gated sodium ion channel Nav1.7, is a key control point for pain perception and a possible target for a next generation of analgesics. Sodium channels, however, historically have been difficult drug targets, and many of the existing structure-activity relationships (SAR) have been defined on pharmacologically modified channels with indirect reporter assays. Herein we describe the discovery, optimization, and SAR of potent aminopyrimidinone Nav1.7 antagonists using electrophysiology-based assays that measure the ligand-receptor interaction directly. Within this series, rapid functionalization at the polysubstituted aminopyrimidinone head group enabled exploration of SAR and of pharmacokinetic properties. Lead optimized *N*-Me-aminopyrimidinone **9** exhibited improved Nav1.7 potency, minimal off-target hERG liability, and improved rat PK properties.

Voltage-gated sodium (Nav) channels govern directly the excitability of pain-sensing neurons and are key to pain perception.¹ In addition, sodium channel antagonists including carbamazepine, oxcarbazepine, lidocaine, and mexiletine in some circumstances are effective analgesics.² These inhibitors, however, have little selectivity within the family of nine sodium channel isoforms and have limited clinical utility due to dose-limiting side effects on the cardiac and central nervous systems. Accordingly, inhibition of the sodium channel isoform(s) that govern pain without inhibition of the sodium channels governing cardiac, CNS, or motor function is a major goal of the field.

Gain-of-function and loss-of-function mutations in human point to Nav1.7 as the most promising drug target among the nine sodium channel isoforms (Nav1.1–Nav1.9).³ Fueling interest in Nav1.7, recent data show that small molecules can be made selective for Nav1.7, as previously shown for Nav1.3 and Nav1.8.^{4,5} In

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general, however, sodium channels have proven difficult to screen, and no subtype-selective sodium channel inhibitors have been made into drugs. The channels are voltage-activated and normally open and inactivate within milliseconds, making standard multiwell fluorescence-based live cell assays difficult. Few high-affinity ligands are available for binding assays; and sodium channels exist in many voltage-sensitive states with different pharmacology.⁶

In an effort to develop potent and selective Nav1.7 antagonists for the treatment of chronic pain, we carried out an electrophysiologybased screening and hit-to-lead campaign using the IonWorks[®] Quattro (IWQ) and PatchXpress[®] (PX) automated electrophysiology platforms, respectively.⁷ Using automated electrophysiology assays allowed us to quantitate directly receptor–ligand interactions with a readout of functional inhibition. Moreover, the precise voltage control of electrophysiology-based screening and driver assays enabled compound potency to be assessed against known gating states of the channel. Sodium channels inactivate after opening, and the nonselective sodium channel inhibitors used clinically likely gain their small therapeutic window via preferential inhibition or stabilization of inactivated states. Accordingly, compound potency was assessed

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Figure 1. Data for aminopyrimidinone hit 1. ^aElectrophysiology IC_{50} of partially inactivated channels is shown. Where applicable, bracket indicates IC_{50} against fully noninactivated channels determined with Man electrophysiology. Inhibitory activity represents an average of at least two determinations. ^bData obtained from MDR1-LLC-PK1 cells, pig kidney cells expressing human MDR1, at 5 μ M in the presence of 0.1% BSA and permeability estimated by averaging apparent permeability values in the apical to basolateral and basolateral to apical directions. ^cMale Sprague–Dawley rats were dosed intravenously at 0.5 mg/kg in 0.5 mL/kg of 100% DMSO. (See above-mentioned reference for further information.)

on hNav1.7 with an average 20% contribution from inactivated channels using the PX system. For key compounds, potency was verified with manual (Man) electrophysiology, both on average 20% inactivated channels and on fully noninactivated channels to verify that compounds indeed were preferential inhibitors/stabilizers of inactivated states.

Aminopyrimidinone **1** was identified as a potent and state-dependent inhibitor of hNav1.7 from a screening campaign (Fig. 1).^{8,9} This hit was unique in structure compared to reported Nav blockers.^{5,10} Although **1** lacked selectivity over the cardiac hNav1.5 (also measured with the PX system on channels with average 20% inactivation) and exhibited high clearance in rats, its favorable selectivity over hERG channels compared to other screening hits was promising. In addition, we anticipated that selectivity against hNav1.5 would be achieved if hNav1.7 potency could be further improved.

Flexible chemistry was developed in order to access analogs of **1** bearing different side-chains and head groups. Side-chain analogs **8** and **14–24** were prepared by selective S-alkylation of aminothiopyrimidinone **2** with alkyl bromides (Scheme 1, Eq. 1). Similarly, compounds **9**¹²–**13** with different head groups could be prepared by selective S-benzylation of the corresponding aminothiopyrimidinones (**3**¹³–**7**) and 1-(bromomethyl)-4-(*tert*-butyl)benzene (Eq. 2).

Further derivatization of compound **8** was readily achieved to explore modifications of the aminopyrimidinone head group (Scheme 2). For example, the amino-group of **8** was transformed to amides (**25–26**), carbamate (**27**), and sulfonamide (**28**) via acylation, peptide coupling, or reaction with either (Boc)₂O or mesyl chloride. Taking advantage of the enaminone reactivity of **8**, regioselective chlorination afforded **29**. Finally, **9** and **30** were prepared via a Mitsunobu reaction of **8** and MeOH.

The synthesis of triazinone **34** was achieved with three consecutive S_NAr reactions of trichloro triazine **31**, with 4-*tert*-butylbenzyl



Scheme 1. Preparation of 8-24 via chemoselective S-alkylation of 2-7 with alkyl bromides. Reagents and conditions: (a) NaOH in EtOH/H₂O, 50 °C.



Scheme 2. Synthesis of analogs by derivatization of lead 8. Reagents and conditions: (a) AcCl or cyclopropanecarbonyl chloride, Et₃N, DCM, rt (25, 22%; 26, 71%); (b) (Boc)₂, DMAP, Et₃N, DCM, rt (47%); (c) MsCl, Et₃N, DCM, rt (69%); (d) NCS, CHCl₃, rt (60%); (e) MeOH, DEAD, PPh₃, THF, rt (9, 30%; 30, 21%).



Scheme 3. Preparation of triazinone 34. Reagents and conditions: (a) 4-tert-butylbenzyl mercaptan, (i-Pr)₂NEt, THF, 0 °C (96%); (b) NH₃ in MeOH, rt (50%); (c) TFA, water, rt (100%).

Table 1

Alkyl thio modifications

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[<u> </u>	NH P
H ₂ N	N	S

Compound	R	hNav1.7 PX IC ₅₀ (µM)	HLM/RLM CL _{int} (µL/min/mg)
1	$\bigvee \longrightarrow$	0.94	257/224
14	$\sim\sim\sim$	1.3	49/124
15	\searrow	>30	ND/ND
16	$\mathbf{\mathbf{\mathbf{\mathbf{\mathbf{\mathbf{\mathbf{\mathbf{\mathbf{\mathbf{\mathbf{\mathbf{\mathbf{\mathbf{\mathbf{\mathbf{\mathbf{\mathbf{$	20	<14/<14

Table 2

Structure-activity relationship of benzyl analogs

Compound	\mathbb{R}^1	hNav1.7 PX IC_{50} (μ M)	HLM/RLM CL _{int} (µL/min/mg)
16	Н	20	<14/<14
17	4-F	>30	ND/ND
18	4-Cl	4.7	22/<14
19	4-Me	5.4	ND/ND
20	$4-CF_3$	3.2	<14/<14
21	4-0CF ₃	2.2	<14/25
8	4- <i>t</i> -Bu	0.68	<14/28
22	4-Ph	>30	ND/ND
23	4-SO ₂ Me	>30	ND/ND
24	4-CN	>30	ND/ND

mercaptan, followed by NH₃, then water in the presence of TFA (Scheme 3).

We initiated medicinal chemistry efforts around aminopyrimidinone hit **1** with the goals of improving potency, selectivity, and PK properties while maintaining state-dependence and hERG selectivity. All potent hNav1.7 blockers within this series were tested against the cardiac channel hNav1.5 assay. At the onset, our strategy was to improve PK properties by removing the potential sites of oxidative metabolism. Thus, truncating the methyl chain by one carbon (**14**) reduced the intrinsic clearance (CL_{int}) in liver microsomes (Table 1). However, further truncation eventually abolished potency (**15**). The potency could be rescued somewhat by capping the aminopyrimidinone head group with a benzyl (**16**) that imparted significantly improved microsomal clearance because most of the metabolic soft spots from the linear carbon chain of hit **1** had been removed.

From a structural overlay of **1** and **16**, we hypothesized that the potency of microsomally stable compound **16** could be substantially improved by strategic incorporation of hydrophobic groups



Figure 2. Data for aminopyrimidinone lead **8**. ^aElectrophysiology IC_{50} of partially inactivated channels is shown. Where applicable, bracket indicates IC_{50} against fully noninactivated channels determined with Man electrophysiology. Inhibitory activity represents an average of at least two determinations. ^bData obtained from MDR1-LLC-PK1 cells, pig kidney cells expressing human MDR1, at 5 μ M in the presence of 0.1% BSA and permeability estimated by averaging apparent permeability values in the apical to basolateral and basolateral to apical directions. ^cMale Sprague–Dawley rats were dosed intravenously at 0.5 mg/kg in 0.5 mL/kg of 100% DMSO.

at the *para*-position of the phenyl ring (Table 2). The IC_{50} was improved by fourfold with a chlorine substituent (**18**). As the 4-substituent increased in size, the potency further improved (**17–21**, **8**). However, the *para*-phenyl variant **22** was not active at concentrations up to 30 μ M. Incorporation of polar groups such as SO₂Me (**23**), and CN (**24**) was detrimental to inhibitory activity. These modifications revealed that 4-*t*-butyl analog **8** was comparably potent to hit **1** and highlighted the overall potency trend that lipophilic substitution at the *para*-position was preferred. Notably, these benzyl analogs were in general more stable in liver microsomes compared to their alkyl counterparts (Table 2 vs Table 1).

Manual patch-clamp on 20% inactivated channels confirmed the potency of lead **8** determined by the PX studies (Fig. 2).⁹ Potency was approximately sixfold weaker on fully noninactivated channels, confirming the property of state dependence. Although **8** still lacked selectivity over hNav1.5, the maintenance of hERG selectivity and the improvement in microsomal clearance over hit **1** were promising. Unfortunately, the low CL_{int} did not translate in vivo, as rat clearance of **8** exceeded hepatic blood flow.

Although the CL_{int} in liver microsomes was low, preliminary data showed that phase II metabolism involving O-glucuronidation was the predominant clearance mechanism.¹⁴ Since the glucuronidation was likely to occur on the aminopyrimidinone head group of **8**, we decided to probe the site of glucuronidation and to investigate the SAR around this aminopyrimidinone ring (Table 3). First, the des-amino **35**¹⁵ and hydroxyl **11** analogs were inactive up to 30 μ M, suggesting that the 6-amino group was critical. The acetyl **25** or the cyclopropylcarbonyl **26** analog was tolerated but these compounds were unstable in human and rat liver microsomes.¹⁶ Carbamate **27** was moderately active and sulfonamide **28** was

Table 3

Amino group at R⁶ position is important for potency



inactive. Second, modulating the steric and electronic properties of the aminopyrimidinone ring at the five position with a chlorine **29** reduced potency. 5-Amino compound **12** was inactive. Finally, bicyclic analog **13** and triazinone **34** (Scheme 3) were also inactive up to 30μ M.

Table 4

Determination of the preferred binding tautomer

Next, we sought to cap the pyrimidinone with a small methyl group at either the oxygen or the ring nitrogens. This exercise could also allow us to determine the active tautomer of the aminopyrimidinone **8** and provide insightful information on how aminopyrimidinone antagonists interact with the sodium channel to guide further SAR exploration (Table 4). Based on ground state energies,¹⁷ we suspected that the pyrimidin-4(3*H*)-one tautomer (**8**) rather than either the pyrimidin-4-ol (**36**) or pyrimidin-4(1*H*)-one tautomer (**37**) was predominant. In the proton NMR of **8**, we observed a broad singlet at 11.5 ppm corresponding to an *NH*-amide; however, we were not able to conclusively determine the predominant tautomer.¹⁸ In order to probe the active species, we selectively installed a methyl group at each reactive position (**8** \rightarrow **9**, **36** \rightarrow **30**, and **37** \rightarrow **10**).¹⁹ As expected, pyrimidin-4(3*H*)-one containing compound **9** was the active methylated compound.

Methylation served to increase the potency of our lead 8 by fourfold as measured by manual electrophysiology (Fig. 3).^{9,20} Lead 9 showed state dependence when tested with manual electrophysiology (IC₅₀ = $0.50 \,\mu$ M on fully noninactivated channels). In addition to exceptional potency and state dependence, hERG activity was also minimal. Importantly, installation of this methyl group led to a substantially improved pharmacokinetic profile as clearance was reduced significantly (CL = 1.1 L/h/kg), possibly due to suppressed glucuronidation. Unfortunately, compound **9** showed no selectivity over hNav1.5, a concern for cardiac toxicity. Due to its excellent PK properties, compound 9 was studied in vivo via intraperitoneal injection ([unbound plasma] = 0.024 µM; [brain]/ [plasma] = 3.3). Unfortunately, in open field studies, compound 9 caused severe reductions in total basic movement (81%) and an almost complete reduction in rearing behavior (98%). This obviated drawing conclusions from other in vivo models, as the rats were no longer moving. Therefore, due to poor tolerability, apparent off-target behavioral effects and a risk for cardiotoxicity, this series was not further pursued.

In conclusion, we have described the discovery and the hit-tolead optimization of aminopyrimidinones as a new class of potent and state-dependent hNav1.7 antagonists. Even though the potency of lead **9** was improved by fivefold compared to hit **1**, the selectivity ratio against the cardiac hNav1.5 channel was very similar. Our strategy to remove the oxidative metabolic sites resulted in low CL_{int} in liver microsomes. However further examination revealed phase II mediated clearance. Gratifyingly, a chemoselective N-methylation of the lead **8** improved the inhibitory activity and



^a All of the calculations were done at the HF/6-31+G(d) level of theory by using Gaussian03. Solvation corrections were computed by using CPCM method with UAKS cavity model.

A. Manual electrophysiology recording



B. Data summary

hNav1.7 PX IC ₅₀ (μM)	0.42 ^a
hNav1.7 Man IC ₅₀ (μM)	$0.080 \ [0.50]^{a}$
hNav1.5 PX IC ₅₀ (μM)	0.20 ^a
hERG dofetilide binding, derived Ki (μM)	>15
HLM / RLM CL _{int} (µL/min/mg)	164 / 57
Solubility ¹¹ - SIF, PBS, 0.01 N HCl (µg/mL)	14, 10, 1
Permeability (10 ⁻⁶ cm/s) / Efflux ratio	16 / 1.0 ^b
CL (L/h/kg)	1.1 ^c
Vss (L/kg)	2.6
t _{1/2} (h)	3.9

Figure 3. Potent block of Nav1.7 by *N*-methyl aminopyrimidinone lead **9**. **A**. Percent Nav1.7 current remaining as a function of concentration of compound **9**, at holding potentials where Nav1.7 was fully noninactivated and where Nav1.7 was 20% inactivated (average of *n* = 2 cells for each condition). Curves were fitted to the equation $I/10 = 1/{1+(concentration/IC_{50})n}$. **B**. ^aElectrophysiology IC₅₀ of partially inactivated channels is shown. Where applicable, bracket indicates IC₅₀ of fully noninactivated channels. Inhibitory activity represents an average of at least two determinations. ^bData obtained from MDR1-LLC-PK1 cells, pig kidney cells expressing human MDR1, at 5 µM in the presence of 0.1% BSA and permeability estimated by averaging apparent permeability values in the apical to basolateral and basolateral to apical directions. ^cMale Sprague–Dawley rats were dosed intravenously at 0.5 mg/kg in 0.5 mL/kg of 100% DMSO.

reduced rat in vivo clearance possibly by suppressing glucuronidation, while maintaining state-dependence and excellent selectivity over hERG.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.111.

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- PX protocol: hNav1.7 or hNav1.5-expressing HEK 293 cells were voltage clamped at a membrane potential that produced average 20% fractional inactivation. Dose-response curves were built from an ensemble of singleconcentration tests on many cells. For details of electrophysiology and in vivo assays, see Bregman, H. et al.
- 9. Manual electrophysiology was used to determine compound potency on noninactivated channels (holding voltage set to -140 mV) and on channels with holding voltage set and adjusted as necessary during the experiment to produce steady 20% inactivation. Values are average of *n* = 2 cells. Compounds 1 and 8 blocked and reversed with fast kinetics. Compound 9 had a slower offrate but still blocked with rapid onset.
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- 12. An alternative synthesis of compound **9** via a Mitsunobu reaction is shown in Scheme 2.
- This compound was purchased from HDH Pharma Inc. LC–MS (ESI) Calcd for C₅H₇N₃OS: [M]⁺ = 157. Found [M+H]⁺ = 158. ¹H NMR (Bruker, 400 MHz, DMSOd₆) d ppm 11.73 (br s, 1H), 6.38 (br s, 2H), 4.86 (s, 1H), 3.43 (s 3H).
- 14. Metabolite identification in fresh rat hepatocytes confirmed that lead **8** underwent glucuronidation. Full scan positive ion mode of the incubated material showed parent **8** at 20.1 min, m/z = 290 and glucuronide adduct at 18.7 min, m/z = 466.
- 15. This analog was purchased from Otava.
- 16. The parent compound was not detected on the LC-MS after 15 min of incubation with rat liver microsomes.
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- 18. The carbon shift data supported the structure of pyrimidin-4(1*H*)-one **37**. However, line-broadening in proton and carbon spectra suggested the double-bond was likely moving between the two ring-nitrogens suggesting that pyrimidin-4(1*H*)-one **37** and aminopyrimidin-4(3*H*)-one **8** were in equilibrium in DMSO-d₆.
- Each structure was confirmed by 1D and 2D NMR analyses. ¹H NMR chemical shifts in DMSO-d₆, LCMS and HPLC retention times distinguished and confirmed each methylated isomer. Chemical shifts of methyl group were 3.21, 3.33, and 3.78 ppm for **9**, **10**, and **30** respectively. The ¹H NMR chemical shift of the O-methylated analog (**30**) was shifted down field as expected. ¹H NMR (Bruker, 400 MHz, DMSO-d₆) d ppm for **9**: 7.31–7.41 (dd, *J* = 26, 8.5 Hz, 4H), 6.51 (br s, 1H), 4.93 (s, 1H), 4.37 (s, 2H), 3.21 (s, 3H), 1.26 (s, 9H); for **10**: 7.34 (m, 4H), 6.55 (s, 2H), 4.94 (s, 1H), 4.30 (s, 2H), 3.33 (s, 3H), 1.26 (s, 9H); for

30: 7.33 (m, 4H), 6.72 (s, 2H), 5.45 (s, 1H), 4.28 (s, 2H), 3.78 (s, 3H), 1.25 (s, 9H). LC–MS retention times were 2.33, 2.65, and 1.62 min for **9**, **30**, and **10**, respectively. HPLC retention times were 7.51, 7.25, and 6.37 min for **9**, **30**, and **10**, respectively.

20. Eighty nanomolar is approaching the most potent sodium channel inhibitors reported. Most nonselective sodium channel inhibitors have potency well over a micromolar. In our hands, mexiletine IC₅₀ on 20% inactivated channels was 56 mM (Bregman, H. et al.).