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Discovery of Selective, Orally Bioavailable, N-linked Arylsulfonamide $Na_v1.7$ Inhibitors with Pain Efficacy in Mice

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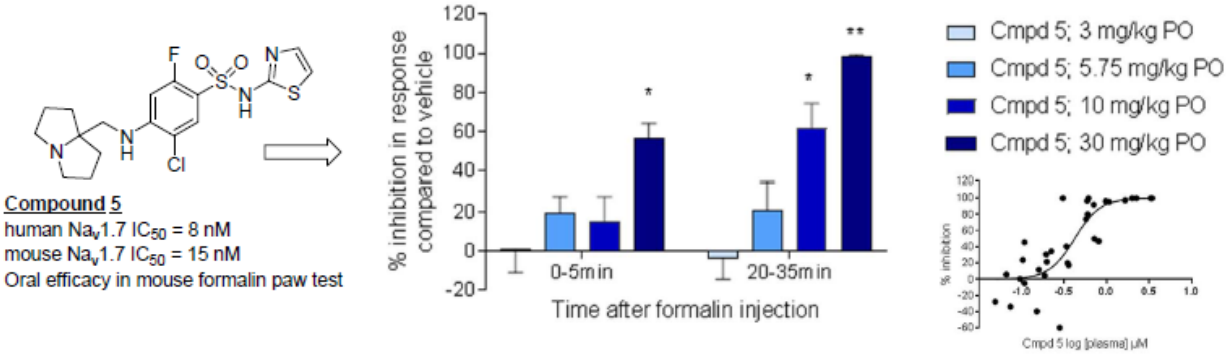
Abstract

The voltage-gated sodium channel $Na_v1.7$ is a genetically validated target for the treatment of pain with gain-of-function mutations in man eliciting a variety of painful disorders and loss-of-function mutations affording insensitivity to pain. Unfortunately, drugs thought to garner efficacy via Na_v1 inhibition have undesirable side effect profiles due to their lack of selectivity over channel isoforms. Herein we report the discovery of a novel series of orally bioavailable arylsulfonamide $Na_v1.7$ inhibitors with high levels of selectivity over $Na_v1.5$, the Na_v isoform responsible for cardiovascular side effects, through judicious use of parallel medicinal chemistry and physicochemical property optimization. This effort produced inhibitors such as compound **5** with excellent potency, selectivity, behavioral efficacy in a rodent pain model, and efficacy in a mouse itch model suggestive of target modulation.



Keywords: $Na_v1.7$, ion channel, pain, arylsulfonamide, selectivity

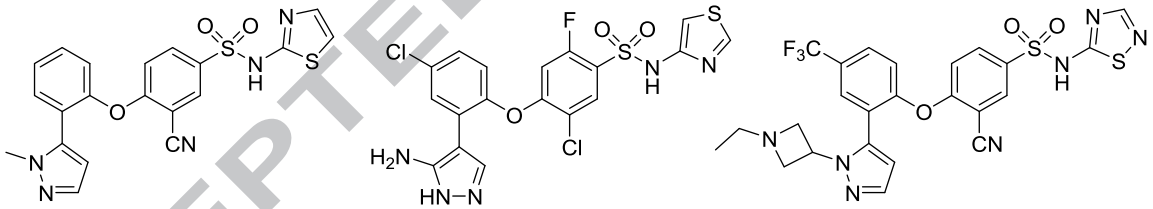
It is estimated that 15% of the US population currently suffers from chronic pain conditions. In addition to the debilitating nature of these ailments, this spectrum of diseases confers a significant economic burden of approximately $600 billion per year in the US due to increased medical costs and lost productivity in the workforce.[1] A range of pharmaceutical agents are utilized to treat chronic pain. First line treatments include anticonvulsants (pregabalin and gabapentin),[2] antidepressants (i.e. duloxetine and desipramine),[3] and non-steroidal anti-inflammatory drugs (NSAIDs, i.e. ibuprofen and naproxen).[4] All current first-line treatment options have significant limitations including incomplete efficacy and undesirable side effect profiles. Opioids (i.e. morphine, oxycodone, and hydrocodone) are effective agents for chronic pain management; however, these are not considered first line options due to the potential for tolerance, potential for addiction and abuse, and narrow therapeutic indices with adverse effects consisting of constipation, decreased respiration, sedation, and nausea.[5,6] For these reasons, novel, safe, and effective chronic pain treatments remain an area of significant unmet medical need and a focus of pharmaceutical research.[7]

A more recent option for chronic pain management is the topical application of lidocaine in the form of a patch (Lidoderm®) approved for the treatment of postherpetic neuralgia, a painful skin condition resulting from shingles.[8] The mechanism of action of lidocaine is thought to arise from non-selective blockade of Na_v1 voltage-gated sodium channels (VGSCs); key regulators of electrical signaling in a variety of tissues through the generation and propagation of action potentials via sodium ion flux in response to changes in membrane voltage potential.[9] There are nine VGSCs currently known, $Na_v1.1$ – 1.9, that possess differential tissue distribution patterns and associated pharmacology.[10]

$Na_v1.1$, 1.2, and 1.3 are expressed in the central nervous system (CNS) while $Na_v1.4$ and 1.5 are expressed in skeletal tissue and cardiac muscle, respectively. $Na_v1.6$ is expressed widely throughout the CNS and peripheral nervous system (PNS), and $Na_v1.7$, 1.8, and 1.9 are restricted mainly to the PNS. Recent human genetic studies have revealed that loss-of-function (LOF) mutations in $Na_v1.7$ afford channelopathy-associated insensitivity to pain suggesting that blockade of this VGSC might be a contributing factor to efficacy for agents such as lidocaine.[11] Additional investigations revealed that gain-of-function (GOF) mutations in $Na_v1.7$ elicit painful conditions such as erythromelalgia and paroxysmal extreme pain disorder further linking $Na_v1.7$ and pain.[12] Unfortunately, systemic exposure to local anesthetics such as lidocaine can engender severe cardiovascular and CNS side effects through blockade of $Na_v1.5$ in the heart and Na_v channels in the CNS ($Na_v1.1$ / $Na_v1.2$), respectively, thus limiting their therapeutic utility.[13] The combination of human genetic validation for $Na_v1.7$ and marketed therapeutics which target Na_v1 channels has drawn extremely high levels of interest and investment from the scientific community with the goal of developing a safe and effective therapeutic for patients suffering from chronic pain.[14]

Historically, identifying selective inhibitors of $Na_v1.7$ has been extremely challenging due to high levels of homology among the Na_v1 channels. Indeed, the binding site for lidocaine resides in the pore region of the channels which is almost completely conserved; consistent with the non-selective nature of the drug.[15] Pioneering work by Pfizer and Icagen described the discovery of arylsulfonamides such as PF-04856264 (**1a**, Figure 1) that potently inhibited $Na_v1.7$ through a binding site distinct from the pore region (DIV = domain four: S2-S3 = between segments 2 and 3) with

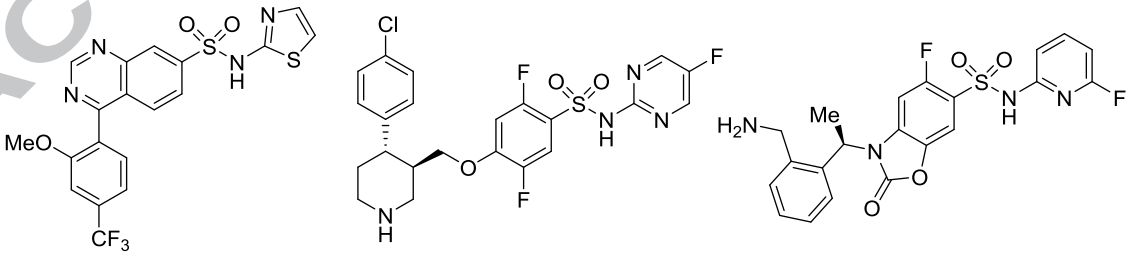
unprecedented selectivity over $Na_v1.5$, [16] potentially ameliorating the risk of cardiotoxicity.[17] This important finding has inspired further chemistry efforts from Pfizer affording the clinical candidate PF-05089771 (**1b**, Figure 1),[18] an X-ray crystal structure of GX-936 (**1c**, Figure 1) bound to DIV of human $Na_v1.7$ from scientists at Genentech and Xenon,[19] and several arylsulfonamide subseries efforts reported by Amgen (**1d**, Figure 1),[20] Genentech / Xenon (**1e**, Figure1),[21] and MSD (**1f**, Figure 1).[22] One hallmark feature of the arylsulfonamide series referenced above is the use of aromatic motifs in Western SAR. This observation suggested that Western aromatic motifs might be necessary to achieve adequate target potency. Herein we describe initial parallel chemistry efforts to identify novel Western SAR devoid of aromatic motifs and further medicinal chemistry efforts to optimize potency, selectivity, physicochemical properties, oral bioavailability, and behavioral pain efficacy in mice.



PF-04856264 (1a)
$Na_v1.7$ IC_{50} = 28 nM
$Na_v1.5$ IC_{50} > 10 μM

PF-05089771 (1b)
$Na_v1.7$ IC_{50} = 11 nM
$Na_v1.5$ IC_{50} = 25 μM

GX-936 (1c)
Potent $Na_v1.7$ inhibitor
Utilized in channel X-ray structure



Amgen (1d)
$Na_v1.7$ IC_{50} = 160 nM
$Na_v1.5$ IC_{50} > 30 μM

Xenon (1e)
$Na_v1.7$ IC_{50} = 11 nM
$Na_v1.5$ IC_{50} > 10 μM

MSD (1f)
$Na_v1.7$ IC_{50} = 39 nM
$Na_v1.5$ IC_{50} = 33 μM

Figure 1. Known arylsulfonamide (**1a** – **1f**) $Na_v1.7$ inhibitors from Pfizer / Icagen, Genentech, Amgen, Xenon, and MSD.

The generic aryl sulfonamide scaffold (**2**) shown in Figure 2 was utilized as a template for design of nucleophilic aromatic substitution (S_NAr) libraries taking advantage of the MSD collection of alcohol- and amine-containing building blocks lacking aromatic motifs. Initial library exploration afforded several interesting leads including compound **3** containing a novel (tetrahydro-1*H*-pyrrolizin-7a(5*H*)-yl)methanamine (3.3.0 amine hereafter) Western substituent.[23] This compound possessed moderate potency against $Na_v1.7$ (inactivated state potency)[24] of 112 nM, no activity against $Na_v1.5$ (> 30 μM), and good binding affinity to the same binding site as PF-04856264.[25] Encouraged by this finding, compound **3** was profiled further and was found to be very polar (LogD = 0.0) with low permeability (P_{app} = 2; Table 1). Arylsulfonamide **3** had limited oral bioavailability (%F = 4), consistent with its permeability and lipophilicity profile, and low intrinsic clearance (CL_{int}) in rat. Hence, additional optimization focused on increasing lipophilicity without compromising pharmacokinetics.

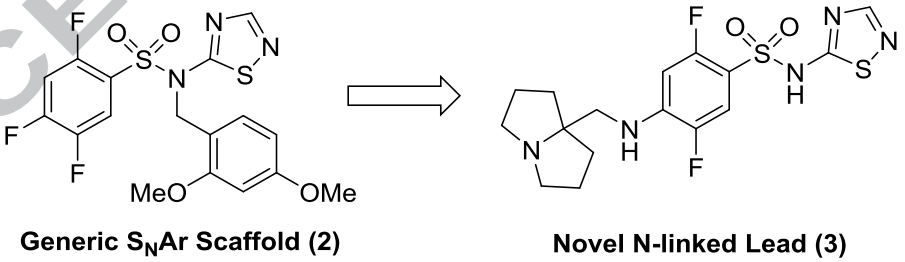
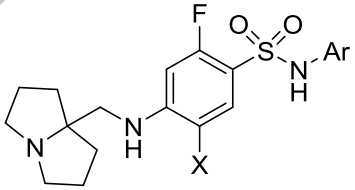


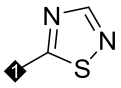
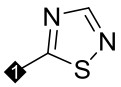
Figure 2. Generic arylsulfonamide scaffold (**2**) utlized in library design via S_NAr chemistry yielding novel $Na_v1.7$ inhibitor leads such as **3**.

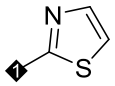
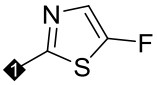
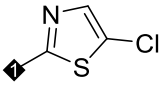
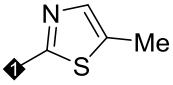
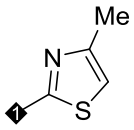
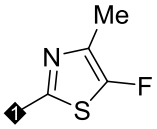
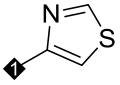
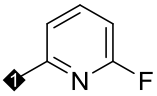
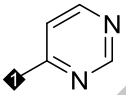
Modification of the 5-position fluorine to chlorine on the central aromatic ring (**4**, Table 1) increased lipophilicity (LogD = 0.4), improved $Na_v1.7$ potency by an order of

magnitude, and maintained selectivity over $Na_v1.5$. Unfortunately, rat CL_{int} increased significantly and permeability was not improved. Exploration of substituted thiazole sulfonamides based upon compound **4** afforded compounds **5** – **11** which all possessed moderate to excellent $Na_v1.7$ potency, high levels of selectivity over $Na_v1.5$, and increased LogD (0.7 – 1.3; Table 1). Surprisingly, compounds **5** -**7** and **9** still displayed low permeability (P_{app} = 2 - 6) but were orally bioavailable in rat, indicating that high levels of permeability were not a prerequisite for oral bioavailability.[26] A selection of 6-membered ring sulfonamides was also synthesized to probe SAR, and representative examples **12** and **13** showed decreased $Na_v1.7$ potency compared to **5** suggestive of tight sulfonamide SAR. Although **12** significantly increased permeability, the increase in rat CL_{int} deterred further analog design using this core template. Arylsulfonamide **5** possessed the best balance of potency, selectivity, rat CL_{int}, and oral bioavailability and was utilized for further compound design.

Table 1. Arylsulfonamide core SAR.



Compound	Ar	X	$Na_v1.7$ IC_{50} (nM)[a]	$Na_v1.5$ IC_{50} (nM)[a]	LogD[b] / PSA (Å)	P_{app} (10^{-6} cm/s)[c]	Rat CL_{int}[d] / %F
3		F	112	>30000	0.0 / 96	2	62 / 4
4		Cl	10	7200	0.4 / 95	2	660 / ND

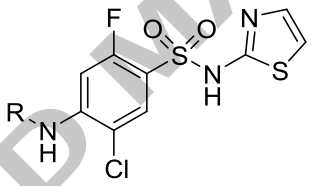
5		Cl	8	>30000	0.7 / 77	2	210 / 40
6		Cl	5	1900	1.0 / 77	2	230 / 55
7		Cl	12	17600	1.3 / 77	3	780 / 45
8		Cl	142	24200	1.1 / 78	9	560 / ND
9		Cl	44	22500	1.1 / 78	6	71 / 36
10		Cl	30	>30000	1.3 / 78	5	630 / ND
11		Cl	91	>30000	0.7 / 78	20[e]	ND / ND
12		Cl	45	19000	1.0 / 77	23	610 / 26
13		Cl	215	>30000	-0.2 / 92	2	61 / ND

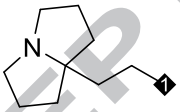
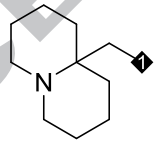
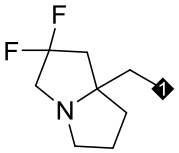
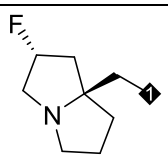
(a) Estimated inactivated state potency as measured by PatchXpress® in HEK293 cells stably expressing human Nav1.7 or Nav1.5; IC_{50} values are estimated from $\geq$ 3 cellular measurements at varying compound concentrations; (b) MSD HPLC LogD assay; (c) Monolayer assay in MDCK cells; (d) Rat CL_{int} = (84*rat CL) / [rat f_u * (84-rat CL)]; units mL/min/kg; IV: 0.05 mpk cassette dosing or 2 mpk single dosing in DMSO/PEG400/water (20/60/20); PO: 10 mpk in PEG400/Tween90/water (40/10/50); (e) permeability determined in LLCPK cell line.

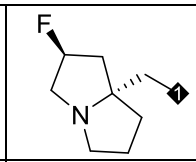
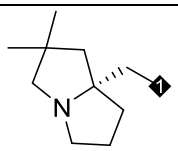
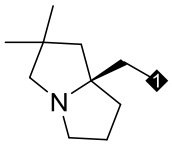
Western amine SAR utilizing the core template of arylsulfonamide **5** is displayed in Table 2. Addition of one carbon atom to the linker of **5** afforded **14** which decreased $Na_v1.7$ potency by over an order of magnitude, increased rat CL_{int} substantially, and had no impact on permeability. Somewhat surprisingly, the (octahydro-9a*H*-quinolizin-9a-yl)methanamine (4.4.0) substituent in **15**[27] dramatically increased permeability (P_{app} =

32) compared to **5**, albeit at the expense of potency and pharmacokinetics. Modifying the basicity of the 3.3.0 amine via the *beta*-fluorine effect improved permeability for compounds **16** and **18**,[28] however, potency was diminished on $Na_v1.7$. A substitution pattern which was tolerated with regard to $Na_v1.7$ potency was dimethyl-3.3.0 ring system in **19** and **20**. This pair of enantiomers had excellent selectivity over $Na_v1.5$, moderate permeability (9 – 11), lipophilicity (LogD = 1.7), rat CL_{int} and oral bioavailability. Compound **19** possessed a very comparable profile to **5** (Table 1) with slight differentiation in permeability (P_{app} = 9), hence both compounds were moved forward for additional characterization.

Table 2. Western Amine SAR.[a]

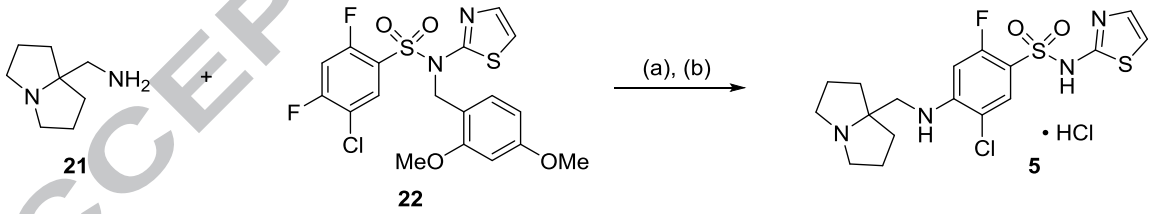


Compound	R	$Na_v1.7$ IC_{50} (nM)	$Na_v1.5$ IC_{50} (nM)	LogD / PSA (Å)	P_{app} (10^{-6} cm/s)	Rat CL_{int} / %F
14		129	24200	0.6 / 80	2	1600 / 30
15		136	>30000	2.2 / 76	32	3000 / ND
16 (racemic)		375	>30000	1.7 / 77	23	NA / NA
17 (enant. A)		160	>30000	1.5 / 77	ND	ND / ND

18 (enant. B)		210	5700	1.5 / 77	11	810 / 57
19 (*R*)		12	>30000	1.7 / 78	9	460 / 61
20 (*S*)		27	>30000	1.7 / 78	13	850 / 45

(a) See Table 1 legend for definition of terms.

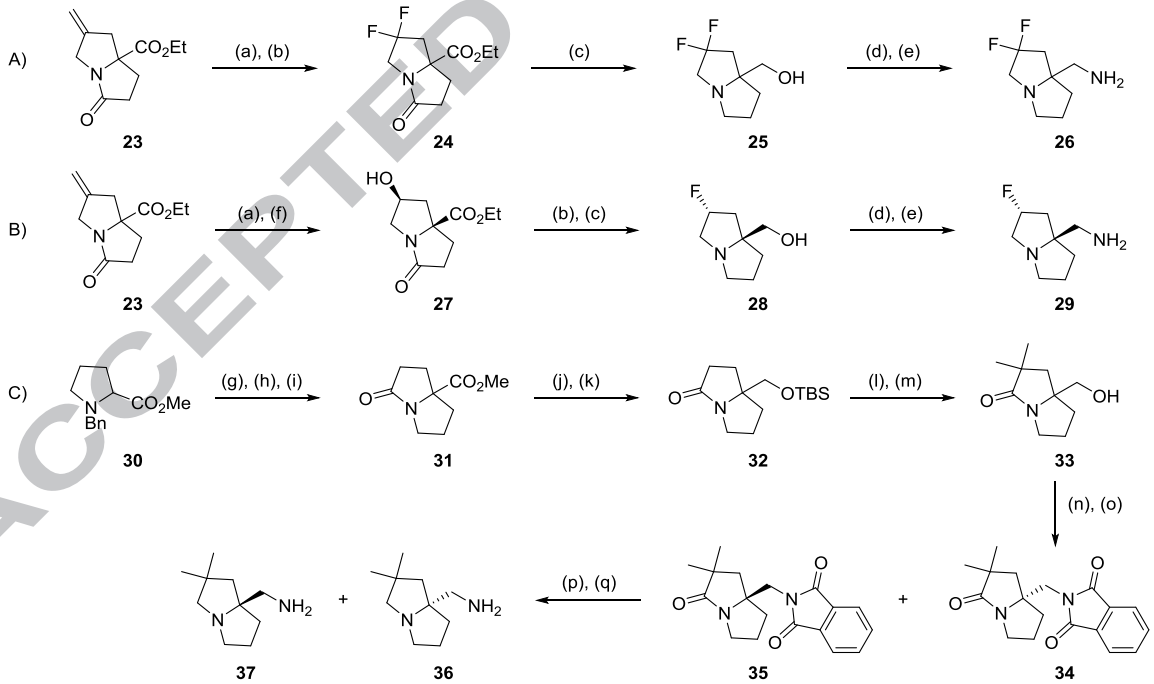
Representative synthetic routes for the compounds in Tables 1 and 2 are exemplified in Schemes 1 and 2. Compound **5** was synthesized from commercially available (tetrahydro-1*H*-pyrrolizin-7a(5*H*)-yl)methanamine **21** [29] and known 2,4-dimethyoxybenzyl-protected sulfonamide scaffold **22**[30] under nucleophilic substitution reaction conditions, deprotected under acidic conditions, and transformed to the HCl salt to afford **5** in moderate overall yield. This synthetic approach was utilized for all compounds in the preceding tables.



Scheme 1. Synthesis of compound **5**. Reagents and reaction conditions: (a) Et_3N, DMF, 40 °C, 18 h, 62%; (b) TFA, DCM, 25 °C, 1 h, then 2.0 M HCl / MeOH, 80%: DMF = *N*,*N*-dimethylformamide, DCM = dichloromethane.

Substituted or homologated 3.3.0 amines were assembled using several methods (Scheme 2). Known intermediate **23**[31] was ozonized and subsequently difluorinated to afford **24** which was globally reduced to afford **25** in moderate yield (Scheme 2A).

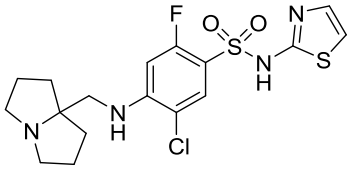
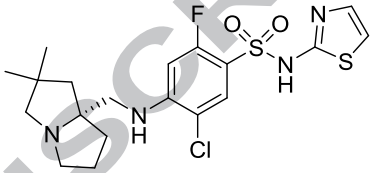
Alcohol **25** was converted to primary amine **26** via standard mesylate formation, azide displacement, and hydrogenation sequence in modest yield. Alternatively, the ozonolysis product of intermediate **23** could be stereoselectively reduced with $NaBH_4$ to afford alcohol **27** which can be inverted through a DAST fluorination to afford **28** (Scheme 2B). This intermediate underwent the same alcohol to primary amine interconversion sequence as before to afford **29**. Finally, methyl benzylprolinate **30** was alkylated with ethyl-3-bromopropionate, de-benzylated and cyclized to afford **31** which was selectively reduced ($NaBH_4$) and TBS protected (**32**). Dimethylation of the lactam was achieved under LDA / MeI conditions to afford **33** in modest yield after TBS deprotection. The resulting alcohol was taken through a phthalimide Mitsunobu / hydrazine deprotection sequence to afford amines **36** and **37**, respectively, with a chiral resolution occurring at the phthalimide stage (Scheme 2C).[32]



Scheme 2. Representative synthesis of amine monomers. Reagents and reaction conditions: A) (a) ozone, DCM, 25 °C, then Me_2S, 67%; (b) DAST, THF, 0 °C, 18 h, 57%; (c) $LiAlH_4$, THF, 0 °C to reflux, 4 h, 80%; (d) MsCl Et_3N, DCM, 0 °C, 1 h, aqueous work up, then NaN_3, DMF, 80 °C, 18 h, 83%; (e) 10% Pd/C, MeOH, H_2 (1 atm), 0 °C, 2 h, ~ 100%; B) (a) see A-a; 67%; (f) $NaBH_4$, MeOH, 0 °C, 5 min, 86%; (b) DAST, DCM, 0 °C to 25 °C, 18 h; 74%; (c); see A-c; 99%; (d) see A-d; 71%; (e); see A-e; ~ 100%; C) (g)

LDA, HMPA, 3-bromopropionate, THF, -78 °C to 25 °C, 16 h, 22%; (h) 10% Pd/C, cat. formic acid, MeOH, H_2 (50 psi), 25 °C, 10 h, 92%; (i) toluene, reflux, 16 h, 81%; (j) $NaBH_4$, MeOH, 0 °C, 30 min, 75%; (k) TBSCl, imidazole, DCM, 0 °C to 25 °C, 5 h, 100%; (l) LDA, MeI, THF, -78 °C, (reaction performed twice for bis-addition), 3 h, 36% overall; (m) 2.0 M HCl, MeOH, 1 h, 76%; (n) phthalimide, PPh_3, DEAD, THF, 0 °C to 25 °C, 18 h, 71%; (o) SFC separation; IC column, 5 to 40% EtOH in CO_2; (p) hydrazine hydrate, EtOH, reflux, 4 h, 54%; (q) BH_3•DMS, THF, 25 °C ~ quant. DAST = diethylaminosulfurtrifluoride; MsCl = methanesulfonyl chloride; LDA = lithium diisopropylamide; HMPA = hexamethylphosphoramide; TBSCl = *tert*-butyldimethylsilyl chloride; DEAD = diethylazodicarboxylate; LiHMDS = lithium bis(trimethylsilyl)amide.

Table 3. Additional profiling of compounds **5** and **19**.

Compound	 Compound **5**	 Compound **19**
Molecular Weight	431 g/mol	459 g/mol
pK_a (sulfonamide / amine)	6.7, 9.8	7.0, 9.3
Solubility (μM, pH 2 and 7)[a]	159, 43	143, 28
HPLC LogD[b] / P_{app}[c]	0.7 / 2	1.7 / 9
Mouse $Na_v1.7$ IC_{50} (nM)[d]	15 nM	13 nM
Mouse PPB	59%	82%
CYP IC_{50} (3A4/2C9/2D6, μM)	> 50, > 50, 32	> 50, > 50, 3.1
PXR EC_{50} (μM)	16	5.6

(a) MSD HPLC kinetic solubility assay; (b) MSD HPLC logD assay; (c) Monolayer assay in MDCK cells; (d) Estimated inactivated state inhibition potency as measured by PatchXpress® in HEK293 cells stably expressing mouse Nav1.7; IC_{50} values are estimated from $\geq$ 3 cellular measurements at varying compound concentrations.

Compounds **5** and **19** possessed moderate molecular weight, zwitterionic character, and moderate kinetic solubility at neutral and acidic pH (Table 3). As stated above, the two compounds represented a 1 log unit range of lipophilicity (0.7 versus 1.7) and apparent differentiation in permeability (2 versus 9 x 10^{-6} cm/s), and the impact of these properties on *in vivo* performance was of high interest to our team. $Na_v1.7$ potency shifts in preclinical species, especially rodents, were recently identified in the literature.[33] Hence, we were delighted to observe that compounds **5** and **19** possessed a mouse $Na_v1.7$ IC_{50} of 15 and 13 nM, respectively; sufficient to support *in vivo* efficacy evaluation. Both compounds had adequate unbound fraction in mouse plasma to interpret our *in vivo*

experiments, and compound **5** displayed excellent functional selectivity in a general off-target screening panel with no hits of less than 400-fold selectivity.[34] Both compounds were not potent CYP inhibitors with the exception of moderate activity at CYP2D6,[35] and did elicit moderate PXR activation. Compound **5** demonstrated minimal CNS penetration when dosed orally to mice with less than 1% and 6% of the plasma exposure existing in the CSF and brain compartments (30 mpk oral dose), respectively. While this same experiment was not performed on **19**, this compound was a Pgp substrate in the rat (LLCPK cell line) with a BA/AB ratio of 17.[36] These two experiments partially alleviated concerns around CNS penetration of these molecules which could elicit known toxicity from blockade of Na_v1 isoforms ($Na_v1.1$ and 1.2) expressed in the CNS (*vide supra*). On the basis of the data above, both compounds **5** and **19** were advanced into behavioral pain models in mice.

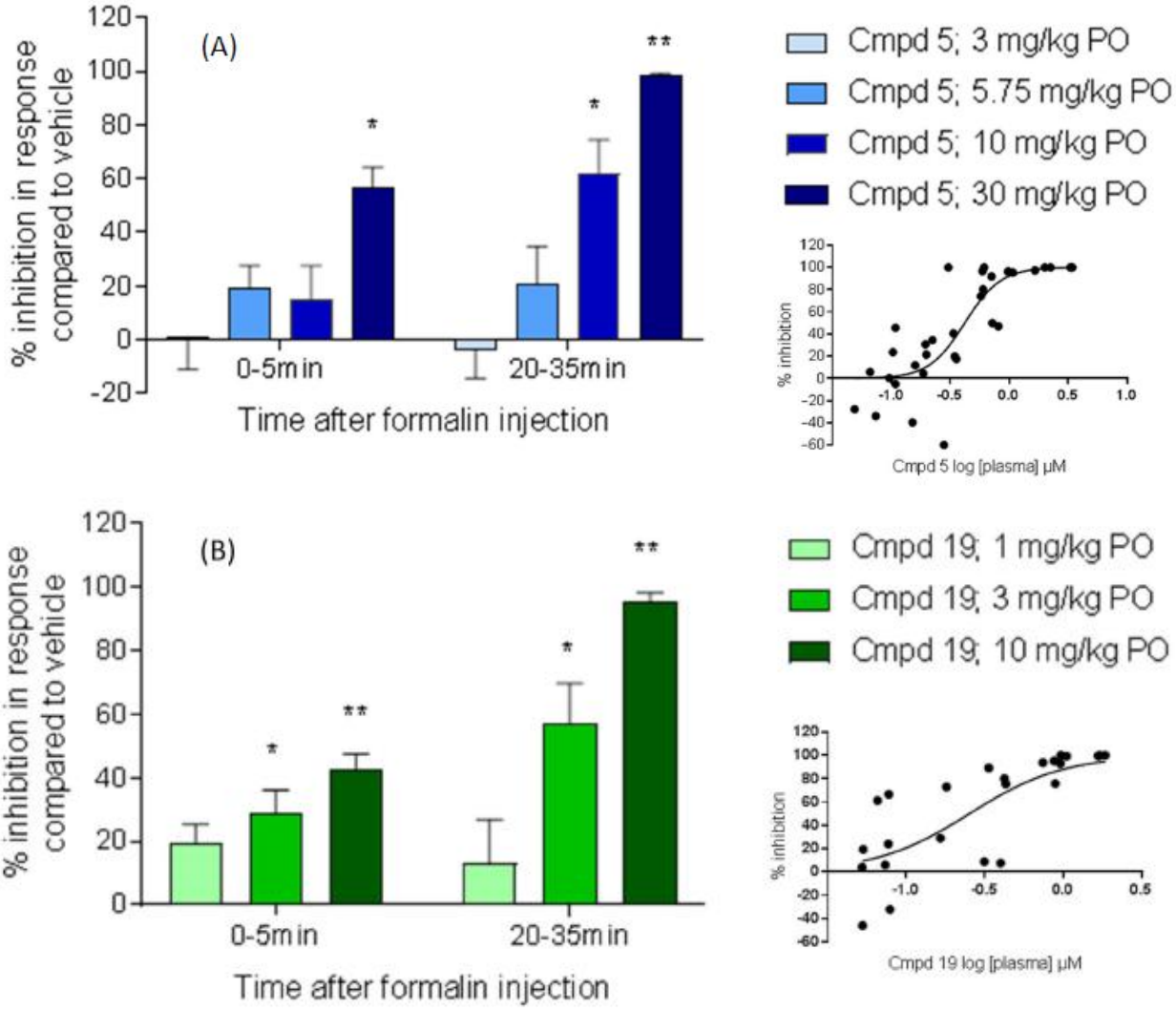
A common assay to evaluate antinociceptive effects of potential pain therapeutic agents is the mouse formalin paw test assay.[37] When a dilute solution of formalin is injected into the hind paw of a mouse, characteristic biting and licking of the affected area is elicited. The efficacy assessment is composed of two phases; an acute phase (or Phase I) generally reflective of direct activation of nociceptors and a tonic phase (or Phase II) related to inflammatory responses. Compounds **5** and **19** were administered orally two hours prior to formalin challenge, and both compounds demonstrated statistically significant, dose-dependent reversal of these effects in the acute phase of the experiment (0 – 5 minute period post formalin injection) and the tonic phase of the experiment (20 – 35 minute period post formalin injection) with full reversal of formalin effects in the tonic phase (Figure 3). Plasma concentrations were evaluated at the end of

the experiment with an unbound plasma concentration IC_{50} of 170 nM (C_p = 420 nM) and 45 nM (C_p = 250 nM) for **5** and **19**, respectfully, during the tonic phase. These exposures were 11-fold (**5**) and 3.5-fold (**19**) over the mouse $Na_v1.7$ IC_{50} value, respectively.

Recently, GOF mutations in $Na_v1.7$ have been linked to paroxysmal itch in humans.[38] Hence, a model of histamine-induced itch was developed to compare the pain efficacy observed for the compounds characterized above to effects that our team viewed as suggestive of $Na_v1.7$ target modulation. Briefly, intradermal treatment of histamine to mice engendered a significant increase in the number of scratching events over the course of 15 minutes (Figure 4). Oral administration of compound **5** two hours prior to histamine challenge demonstrated dose-dependent blockade of these scratching events with statistically significant, complete blockade occurring at the same oral dose as that used in the mouse formalin paw test (30 mpk). This suggested that the efficacy observed in the mouse formalin paw test was mediated through the inhibition of $Na_v1.7$.

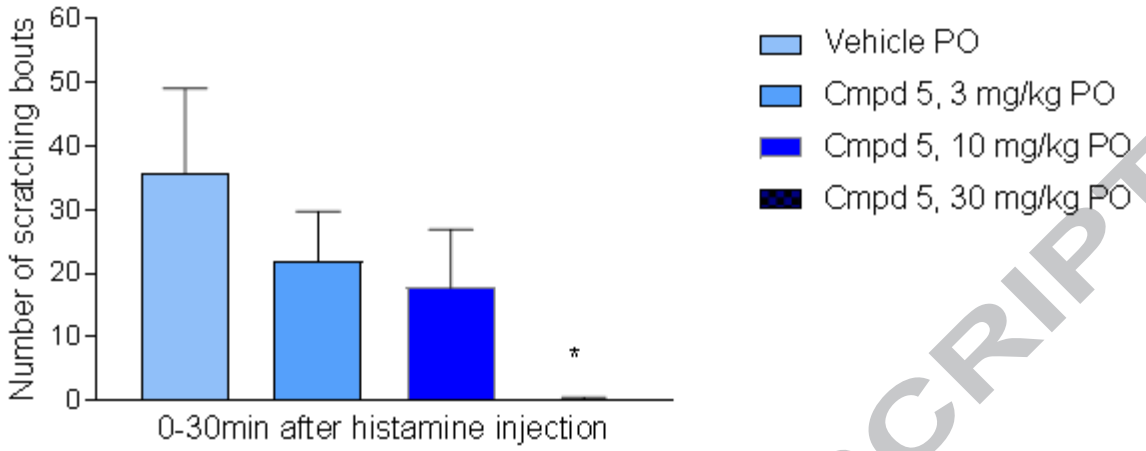
In conclusion, parallel synthetic efforts utilizing an arylsulfonamide core template led to the discovery of the novel Western 3.3.0-amine class of arylsulfonamide $Na_v1.7$ inhibitors represented by compound **3**. Medicinal chemistry targeting the balance of potency, selectivity over $Na_v1.5$, PSA, and lipophilicity afforded advanced compounds **5** and **19**: potent, selective, and orally bioavailable inhibitors of $Na_v1.7$ with limited CNS penetration. Arylsulfonamides **5** and **19** were effective at reversal of formalin-induced nociceptive events in mice, and the efficacy of **5** was consistent with $Na_v1.7$ target modulation as evidenced by efficacy in the mouse itch assay. Additional research in the $Na_v1.7$ inhibitor arena will be published in due course.

Figure 3. Oral efficacy of compounds **5** and **19** in mouse formalin paw test.[a]



(a) Measurement of formalin-induced nociceptive behaviors in mice (C57BL/6 mice) following administration of vehicle (40% PEG400 / 10% Tween 80 / 50% water) or rising doses of compounds **5** (Panel A) and **19** (Panel B) PO. Compounds were administered 2 hours prior to formalin injection to coincide with T_{max} from separate pharmacokinetic studies (3 hours). Pharmacokinetics were evaluated 3 hours post administration. Data were analyzed using within-subject ANOVA to determine main effects and one sample t-test to compare to vehicle (N = 8 / group); * P > 0.01, ** > 0.001.

Figure 4. Oral efficacy of compound **5** in mouse itch assay.[a]



(a) Measurement of histamine-induced nociceptive behaviors in mice (C57BL/6 mice) following administration of vehicle (40% PEG400 / 10% Tween 80 / 50% water), 3, 10, and 30 mg/kg of compound **5** PO. Compound **5** was administered 2 hours prior to histamine injection to coincide with T_{max} from separate pharmacokinetic studies (3 hours). Data were analyzed using within-subject ANOVA to determine main effects and one sample t-test to compare to vehicle (N = 8 or 9 / group); * P > 0.01.

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[23] All oxygen-linked variants of the 3.3.0 amine template were greater than 20-fold less potent than the analogous nitrogen-linked compounds.

[24] Please see the Supporting Information section for experimental procedures regarding $Na_v1.7$ and $Na_v1.5$ potency determinations via PatchXpress electrophysiology assays.

[25] The "arylsulfonamide" binding site potency for **3** was measured by a radioligand displacement assay with a K_i = 42 nM using a proprietary Merck radioligand residing in the structural class from reference 22. No binding was detected for compound **3** in a standard local anesthetic binding site displacement assay with K_i > 30 μM.

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[34] General off-target screening (Ricerca panel, 145 assays) of compound **5** revealed several initial binding hits including adrenergic activity (α1D, α2A, α2B), serotonin ($5HT_{2B}$), and muscarinic (M1) activites. Second messenger assays for adrenergic activities resulted in functional antagonist activity with an IC_{50} ~ 8 μM (all 3 isoforms listed), M1 IC_{50} and EC_{50} > 30 μM, and $5HT_{2B}$ IC_{50} = 3.8 μM. Compound **19** was only tested in selected off-target assays and demonstrated a similar profile to **5**.

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