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ARTICLE

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Identification of a Potent, State-Dependent Inhibitor of Nav1.7 with Oral Efficacy in the Formalin Model of Persistent Pain

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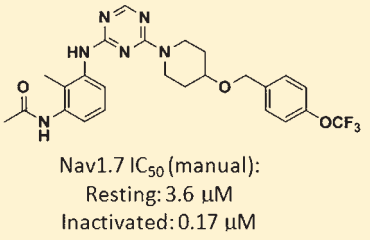
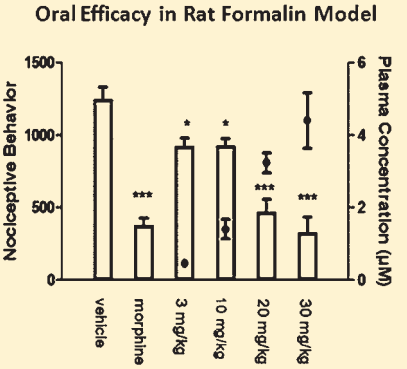
Supporting Information

ABSTRACT:





Clinical human genetic studies have recently identified the tetrodotoxin (TTX) sensitive neuronal voltage gated sodium channel Nav1.7 (SCN9A) as a critical mediator of pain sensitization. Herein, we report structure−activity relationships for a novel series of 2,4-diaminotriazines that inhibit hNav1.7. Optimization efforts culminated in compound **52**, which demonstrated pharmacokinetic properties appropriate for in vivo testing in rats. The binding site of compound **52** on Nav1.7 was determined to be distinct from that of local anesthetics. Compound **52** inhibited tetrodotoxin-sensitive sodium channels recorded from rat sensory neurons and exhibited modest selectivity against the hERG potassium channel and against cloned and native tetrodotoxin-resistant sodium channels. Upon oral administration to rats, compound **52** produced dose- and exposure-dependent efficacy in the formalin model of pain.

INTRODUCTION

Voltage-gated sodium ion channels have long been recognized as potentially attractive drug targets for the treatment of chronic pain, including disorders such as fibromyalgia, painful diabetic neuropathy, osteoarthritis, and cancer-related pain.[1] Existing antiepileptic or antiarrhythmic drugs that are sodium channel inhibitors have some efficacy against several forms of chronic pain,[2] and intramuscular administration of tetrodotoxin is effective for relief of pain associated with cancer or chemotherapy.[3] These treatments, however, are limited by side effects that are presumably due to the inhibition of the sodium channels that influence other brain, heart, or muscle function. Among the nine members of the voltage-gated sodium channel family (Nav1.1−Nav1.9),[4] the Nav1.7 (SCN9A) gene stands out as a critical control point for pain. Clinical genetic studies by several groups have found that loss of function truncation mutations of SCN9A result in the complete abrogation of the ability to perceive pain, without additional major physiological abnormalities.[5−8] Conversely, gain of function mutations in SCN9A that increase channel opening cause chronic pain in the absence of injury.[9−11] Accordingly, much effort has been focused on generating potent inhibitors of Nav1.7 that lack inhibitory effects on other members of the sodium channel family.[12−22] This functional selectivity may be achieved via a combination of subtype selectivity for Nav1.7 and state dependence, a property by which compounds

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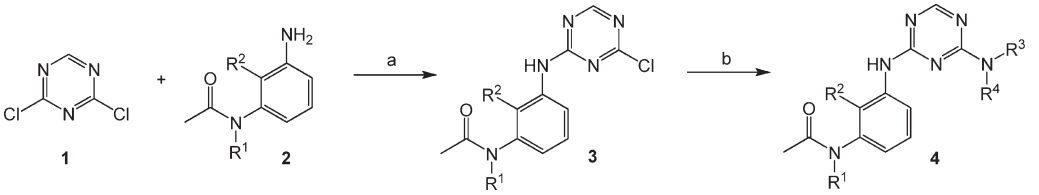
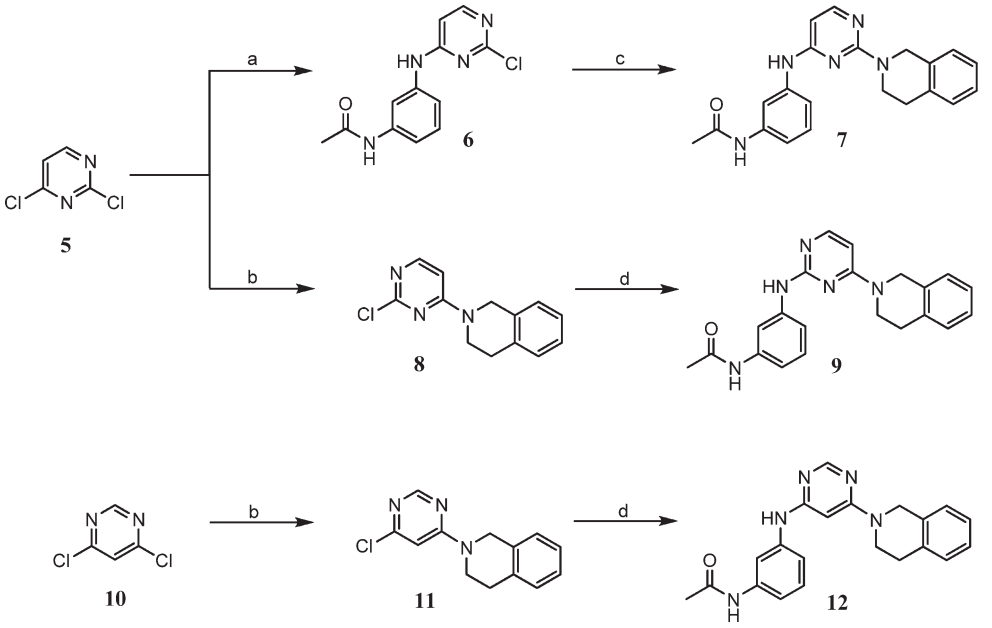
Scheme 1. Preparation of Diaminotriazines 4[a]



[a] Reagents: (a) DIEA, DMF, 0−20 °C; (b) DIEA, DMF, NHR^3R^4, 20 °C. R^1, R^2 = H, Me; R^3, R^4 = varied.

Scheme 2. Preparation of Diaminopyrimidines 7, 9, 12[a]



[a] Reagents: (a) *N*-(3-aminophenyl)acetamide, DIEA, *i*-PrOH, 0−20 °C, 31%; (b) 1,2,3,4-tetrahydroisoquinoline, DIEA, *i*-PrOH, 0−20 °C, 88% (**8**), 82% (**11**); (c) 1,2,3,4-tetrahydroisoquinoline, TFA, *i*-PrOH, 90 °C, 50%; (d) *N*-(3-aminophenyl)acetamide, TFA, *i*-PrOH, 90 °C, 80% (**9**), 34% (**12**).

preferentially inhibit the inactivated states of sodium channels thought to have greater prevalence in diseased tissue such as the hyperexcitable neurons driving chronic pain.[23,24] Here we report the characterization, structure−activity relationship (SAR), and optimization of a series of Nav1.7 inhibitors derived from a novel small molecule scaffold identified using a high-throughput electrophysiology screen.[25] This effort produced a tool compound with novel biophysical properties that was effective in the rat formalin model of pain and represents a step toward identifying new therapeutics for the control of pain via subtype-specific state-dependent inhibition of sodium ion channels.

CHEMISTRY

The preparation of diaminotriazine **4** proceeded via two sequential nucleophilic aromatic substitution reactions (S_NAr) (Scheme 1). Reaction of a 3-aminoacetanilide (**2**) with 2,4-dichlorotriazine (**1**) in the presence of *N*,*N*-diisopropylethylamine (DIEA) led to the clean formation of monochlorotriazine **3**,[26] which could be either isolated chromatographically prior to the subsequent nucleophilic aromatic substitution or treated in one pot directly with primary or secondary amines at ambient temperature leading to the formation of desired product (**4**).[27,28] This protocol was found to be general and reproducible for a wide range of anilines and amines with the order of addition being paramount. For example, reaction of primary or secondary amines directly with 2,4-dichlorotriazine led to a mixture of mono and bis-substitution adducts.

The diaminopyrimidine derivatives **7**, **9**, and **12** were prepared from the respective dichloropyrimidine precursors in analogous two-step processes (Scheme 2). Substitution at the more electrophilic 4-position of 2,4-dichloropyrimidine (**5**) by 3-aminoacetanilide proceeded smoothly under general basic conditions, providing intermediate 2-chloropyrimidine **6** which upon heating with 1,2,3,4-tetrahydroisoquinoline in the presence of trifluoroacetic acid (TFA) afforded pyrimidine **7**. By reversal of the sequence of nucleophilic additions, pyrimidine **9** was isolated via the intermediacy of chloropyrimidine **8**. Starting with 4,6-dichloropyrimidine (**10**), an analogous sequence of basic and subsequent acidic S_NAr reactions afforded 4,6-disubstituted derivative **12**.

The synthesis of corresponding N-methylated derivative **15** proceeded via the intermediacy of aryl bromide **14** which was prepared employing a one pot double S_NAr procedure (Scheme 3). Sequential exposure of 2,4-dichlorotriazine (**1**) with 3-bromo-*N*-methylaniline (**13**) and 1,2,3,4-tetrahydroisoquinoline, respectively, in *N*,*N*-dimethylformamide (DMF) furnished **14**, which

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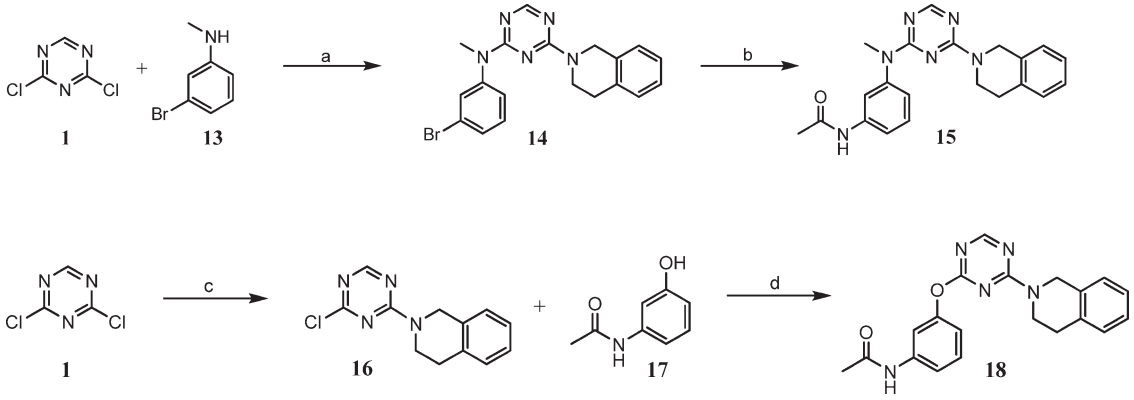
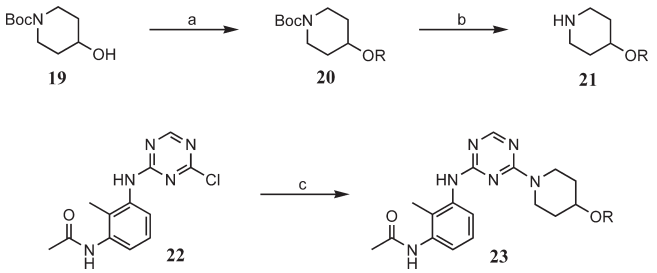
Scheme 3. Preparation of N-Modified or Substituted Triazines 15 and 18[a]



[a] Reagents: (a) (i) DIEA, 3-bromo-*N*-methylaniline, DMF, 0−20 °C; (ii) tetrahydroisoquinoline, 20 °C, 25%; (b) acetamide, X-Phos, Cs_2CO_3, $Pd_2(dba)_3$, 1,4-dioxane, 110 °C, 35%; (c) 1,2,3,4-tetrahydroisoquinoline, DIEA, DMF, 0−20 °C, 54%; (d) NaH, DMF, 20 °C, 75%.

Scheme 4. Preparation of Piperidine Ether Substituted Diaminotriazines 23[a]



[a] (a) (i) NaH, DMF, 0−20 °C; (ii) RX; (b) TFA, DCM, 20 °C; (c) **21**, DIEA, DMF, 20 °C.

was coupled with acetamide under Buchwald−Hartwig conditions to yield triazine **15**. Derivative **18**, in which the aniline nitrogen is replaced with an oxygen, was prepared by reaction of chlorotriazine **16** with the sodium alkoxide of *N*-(3-hydroxyphenyl)acetamide (**17**) in DMF at ambient temperature.

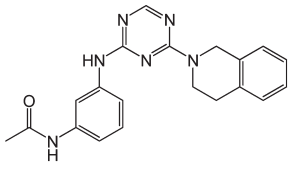
The preparation of modified benzyloxypiperidines **21** was amenable to a parallel synthetic approach (Scheme 4). Deprotonation of *N*-Boc-4-hydroxypiperidine (**19**) with sodium hydride in DMF followed by alkylation with benzyl halides afforded N-protected piperidines **20**. TFA mediated cleavage of the *tert*-butoxycarbonyl (Boc) group of **20** afforded secondary amines **21** which were purified by a catch and release protocol utilizing strong cation exchange (SCX-2) chromatography.[29] Piperidines **21** were treated with DIEA in DMF in the presence of chlorotriazine **22** at ambient temperature to provide desired compounds **23**.

RESULTS AND DISCUSSION

Nonselective sodium channel inhibitors such as mexiletine that are used off-label as third-line treatments for pain display preferential inhibition of inactivated channel states. This form of state-dependent potency is likely the feature that confers their modest therapeutic window.[23,30] We sought a state-dependent small molecule inhibitor of Nav1.7 with sufficient potency and pharmacokinetic properties to achieve target coverage with oral dosing in order to determine if it would be effective in a rat formalin model of persistent pain. Accordingly, compounds were screened by automated high throughput patch-clamp electrophysiology (PatchXpress) on live cells expressing Nav1.7. We utilized a voltage protocol that enabled assessment of compound state dependence with a holding voltage set to inactivate approximately 20% of the channels. Our screen identified lead compound **24** as a potent, state-dependent inhibitor having structural features unique among known sodium channel antagonists. Manual whole-cell patch-clamp experiments on Nav1.7 confirmed the initial PatchXpress results, with IC_{50} = 0.9 μM for Nav1.7 with partially inactivated channels (20% inactivated) and IC_{50} > 30 μM recorded on noninactivated channels.[31] Additionally, compound **24** exhibited modest selectivity over the cardiac sodium channel Nav1.5 (IC_{50} = 5.4 μM) and the hERG (human ether a-go-go-related gene) potassium channel (IC_{50} = 3.8 μM) (Figure 1). A major challenge we confronted was the poor pharmacokinetic (PK) profile of lead compound **24**, which had high clearance (4.0 (L/h)/kg). Although compound **24** had good permeability, it possessed poor solubility, an issue to be addressed en route to identifying an agent suitable for oral dosing (Figure 1). We embarked on a comprehensive SAR evaluation around **24** with the goals of improving potency and PK properties.

Initial studies were focused on modification of the core and aminophenylacetamide moieties. It was determined that most point mutations led to an erosion of potency (see Table 1). All three pyrimidine derivatives constituting single point nitrogen to carbon−hydrogen atom replacements were prepared (**7**, **9**, **12**). Of this set only 4,6-pyrimidine derivative **12** was equipotent with triazine **24**, while the 2,4 pyrimidine analogues (**7**, **9**) were weakly active. Methylation of the aniline or amide nitrogen (**15** and **25**, respectively) or replacement of the aniline NH with oxygen (**18**) resulted in a dramatic loss of potency. On the basis of these results, SAR evaluations of the tetrahydroisoquinoline portion of the molecule were performed with the triazine and 3-aminophenylacetamide groups locked as the core and left-hand-side fragments, respectively. The 4,6-pyrimidine core would be revisited once the rest of the molecule had been optimized and will be discussed later.

We suspected that the electron rich and conformationally rigid tetrahydroquinoline moiety was a liability in terms of oxidative metabolism and solubility and therefore sought to identify the



24

hNav1.7 PX IC_{50} (μM): 1.9

hNav1.7 Manual IC_{50} (μM): 0.90, [>30, noninactivated]

hNav1.5 PX IC_{50} (μM): 5.4

hERG PX IC_{50} (μM): 3.8

Solubility (μg/mL) (0.01N HCl / SIF (pH 6.8) / PBS (pH 7.4)): 42 / 31 / 1

Permeability (Papp average AB/BA) / Efflux Ratio: 29.3 / 1.2

HLM / RLM CL (μl/min/mg): 166 / 219

Rat IV PK: CL: 4.0 L/h/kg, AUC (0.5 mpk): 126 ng· h /mL

Figure 1. In vitro activity and pharmacokinetic properties of compound **24**.

minimal critical pharmacophore. Upon examination of the right-hand-side (RHS) SAR (see Table 2) it was determined that truncation of the tetrahydroisoquinoline was unfavorable. For example, compounds containing amino (**26**) and unsubstituted piperidine (**27**) groups had poor activity. The introduction of various small substituents at the 4-position did not provide a boost in potency. For example, methyl substitution (**28**) and methoxy (**29**) and hydrophilic groups such as a primary carboxamide (**30**) were not tolerated. However, we were delighted to learn that submicromolar potency could be realized with the appropriate larger hydrophobic modification at the 4-position of the piperidine. For example, 4-phenyl substituted compound (**31**) exhibited submicromolar potency. Introducing polar substitution in addition to hydrophobicity in this region of chemical space also led to a drop in potency (**33** and **34**). However, potency was retained when one- or two-atom homologations of phenylpiperidine (**31**) were made, as in phenoxypiperidine (**35**) and benzyloxypiperidine (**36**), respectively. Interestingly, replacement of the oxygen atom of **35** with a methylene (**32**) led to a significant reduction in potency.

The potencies and microsomal stabilities of **35** and **36** were maintained relative to **24**, and the solubility properties were markedly improved (see Table 3). The observed improvement in solubility is possibly due to the additional elements of rotational freedom present in the phenoxypiperidine and benzyloxypiperidine moieties or the addition of a heteroatom relative to the tetrahydroisoquinoline. Compounds exhibiting acceptable potency (<2 μM) and low to moderate in vitro intrinsic clearance (CL_{int}) in rat liver microsomes (RLM) (<200 (μL/min)/mg) were assessed in rat PK studies (see Table 3). Both phenoxypiperidine (**35**) and benzyloxypiperidine (**36**) derivatives had low clearance in rats (0.59 and 0.45 L/(h·kg), respectively), and moderate to good oral bioavailability (F) (F = 52% and 19% for compounds **35** and **36**, respectively). Because of their significantly improved PK properties relative to lead **24**, compounds **35** and **36** were chosen for further SAR examination with the goal of increasing target coverage by a combination of potency and PK improvements.

Because the 2-aminotriazine motif is a hinge-binder element present in many kinase inhibitors,[27] aminotriazine **36** was screened at 1 μM against a panel of 48 kinases and was found to inhibit six kinases by greater than 50% (Abl, 82%; Fyn, 85%; Lyn, 74%; Src, 81%; Mst2, 76%; BTK, 54%). It was envisioned that introduction of a substituent adjacent to the linker binder element may disrupt the kinase inhibitory activity via a combination of steric and/or conformational effects. Accordingly, compound **37** was

Table 1. SAR: Variations to the Core and Left-Hand-Side Polar Moieties

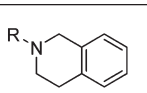
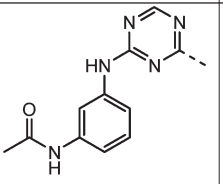
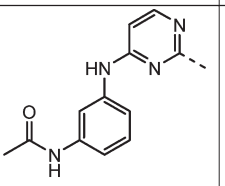
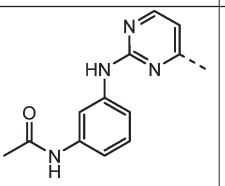
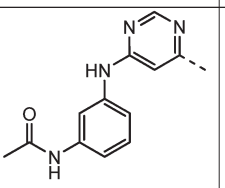
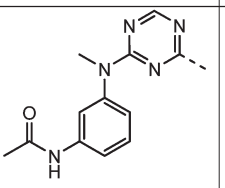
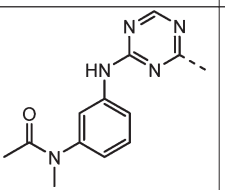
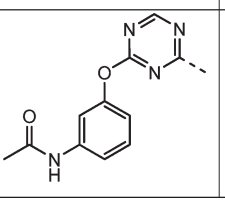
		
	R	*Nav1.7 PX IC_{50} (μM)*
24		1.9
7		>10
9		>10
12		2.5
15		> 10
25		> 10
18		>10

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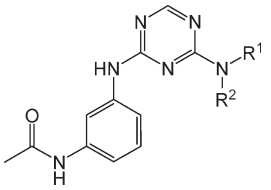
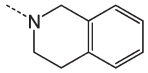
Table 2. SAR: Variations to the Right-Hand-Side Amine Moiety



		Nav1.7 PX IC_{50} *(μM)*
24		1.9
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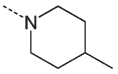
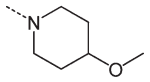
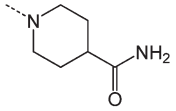
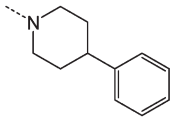
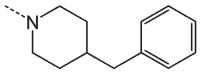
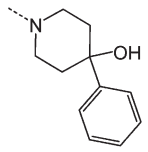
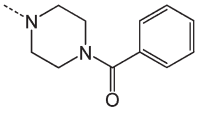
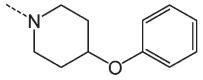
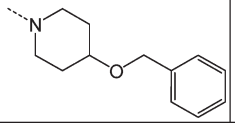
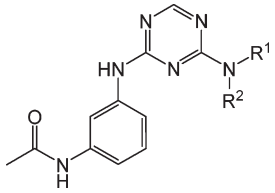
27		> 10
28		> 10
29		> 10
30		> 10
31		0.7
32		>10
33		9.9
34		> 10
35		1.4
36		1.4

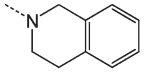
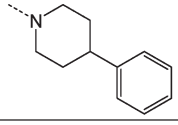
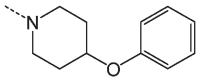
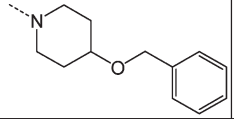
prepared, featuring a methyl group at the 2-position of the aminophenylacetamide moiety. This perturbation led to a reduction in kinase inhibitory activity, with **37** inhibiting no kinase by greater than 10% from the same panel of 48 kinases. We found that, in general, introduction of a 2-methyl substituent retained potency and microsomal stability (see Table 4 for a comparison of des-methyl (**36**, **31**, **35**) and methylated analogues (**37**−**39**)). Therefore, optimization of lead compounds **37** and **39** was initiated with *N*-(3-amino-2-methylphenyl)acetamide fixed, and further piperidine ether modifications were explored using a focused library synthetic approach.

The SAR of the aryloxypiperidine series derived from lead **39** closely paralleled that of the benzyloxypiperidine series derived from lead **37** in terms of substitution about the aryl ring of the piperidine ether moiety. Therefore, only the SAR for the benzyloxypiperidine series will be reported in detail with the most potent of the aryloxypiperidine series described (Table 5). Methyl substitution at the benzylic position (**40**) was not tolerated and was not further explored. Substitution of the aromatic ring revealed subtle differences for the small fluorine substituent (**41**−**43**) and more pronounced preference for the para position with the bulkier trifluoromethyl derivatives (**44**−**46**). Broader exploration of para-substitution indicated that hydrophobic substitution was preferred but with a limit to the amount of steric bulk that this region of chemical space would accommodate. For example, *p*-Me and *p*-*i*-Pr substitution afforded submicromolar compounds **47** and **48**, respectively, but there was a dramatic loss in potency with *t*-Bu substitution (**49**). Polar substituents with hydrogen bond donors, such as primary carboxamide **50**, also resulted in a substantial loss of potency. Electron rich substitutents, such as *p*-OMe (**51**), were not tolerated. Alternatively, substitution of the para position with a trifluoromethoxy group (**52**) afforded a potent analogue (0.5 μM). Although alkyl substituted derivatives **47** and **48** afforded submicromolar potency, they were not further pursued because of relatively poor selectivity over the hERG channel (IC_{50} of 1.93 and 0.44 μM, respectively) compared to **46** and **52**, (IC_{50} > 10 μM). The corresponding aryloxy derivatives containing *p*-trifluoromethyl (**53**) and trifluoromethoxy (**54**) groups also exhibited submicromolar potency (0.8 and 0.3 μM, respectively) with good microsomal stability in rat microsomes (HLM/RLM CL_{int} = 157/38 and 110/33 (μL/min)/mg, respectively). Combining the optimized piperidine aryl ether fragment with the previously identified pyrimidine core (**12**) yielded **55**, which had submicromolar potency (0.9 μM) but higher CL_{int} in RLM (HLM/RLM CL = 117/193 versus 91/46 ((μL/min)/mg) for **52**). Thus, compounds that imparted the greatest balance of potency and microsomal stability were further evaluated in terms of selectivity and pharmacokinetic properties.

In order to determine the best candidate for evaluation in pain models, a more detailed selectivity and PKDM analysis of **46** and **52**−**54** was conducted (Table 6). Comparison of aryloxy ether leads (**53**, **54**) with benzyloxy ether analogues (**46**, **52**) revealed that the benzyloxy ether subseries demonstrated superior selectivity over Nav1.5 and the hERG potassium channel and were therefore chosen for evaluation in rat iv and po PK studies. Gratifyingly, the low CL_{int} in RLM of **46** and **52** translated into low clearance (0.48 and 0.42 L/(h·kg), respectively). Furthermore, compounds **46** and **52** were found to possess moderate and good oral bioavailability (27% and 67%, respectively). Compound **52** was selected for further profiling and evaluation in the rat

Table 3. Potency, PK, and Solubility Properties of Selected Derivatives[a]



		*Nav1.7 PX IC_{50} (μM)*	*RLM CL_{int} (μl/min/mg)*	*Rat IV CL (L/(h-kg))*	*Rat PO F (%)*	*Permeability Papp (ave AB/BA) (μcm/s) / Efflux Ratio*	*Solubility (μg/mL) (0.01N HCl/ SIF (pH 6.8)/ PBS (pH 7.4))*
24		1.9	219	4.0	--	29.3 / 1.2	42/31/1
31		0.7	247	--	--	--	--
35		1.4	127	0.59	52%	26.1 / 1.2	>200/110/11
36		1.4	185	0.45	19%	22.2 / 1.1	157/86/15

[a] Rat PK studies were conducted with male Sprague−Dawley rats as follows: iv dosing, 0.5 mg/kg in 0.5 mL/kg of 100% DMSO; oral administration, 2 mg/kg in 10 mL/kg of vehicle (30% hydroxypropyl-β-cyclodextrin, pH 2.2 adjusted with methanesulfonic acid for **35** or 1% Tween 80, 2% hydroxypropyl methylcellulose, pH 2.2 adjusted with HCl for **36**). Data were obtained from MDR1-LLC-PK1 cells, pig kidney cells expressing human MDR1, at 5 μM in the presence of 0.1% BSA, and permeability was estimated by averaging apparent permeability values in the apical to basolateral and basolateral to apical directions.

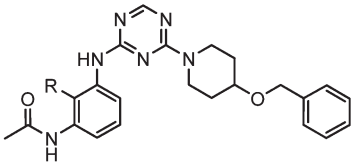
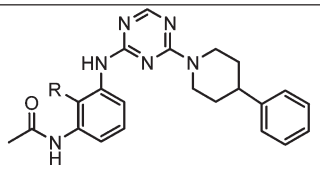
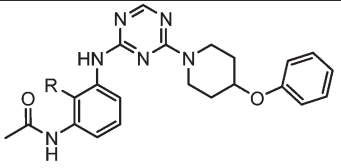
formalin model of persistent pain based on its balance of potency, selectivity, and oral exposure.

For a more accurate measure of activity, compound **52** was evaluated using manual whole-cell patch-clamp electrophysiology and was confirmed as a potent and state-dependent blocker of Nav1.7, with IC_{50} = 170 nM on channels that were 20% inactivated and IC_{50} = 3.6 μM on fully noninactivated channels (average, n = 2 trials). All of the more than 20 analogues tested with this protocol showed strong state dependence. Application of the modulated receptor model with single affinities for resting (K_R) and inactivated (K_I) channels[32−34] gives an extrapolated affinity of K_I = 35 nM for binding of **52** to fully inactivated channels and a ratio K_R/K_I = 103. That is, the compound is approximately 100-fold more potent on inactivated than on resting (noninactivated) channels. By comparison, mexiletine measured with the same protocol had IC_{50} = 56 μM on partially inactivated Nav1.7 and IC_{50} = 340 μM on noninactivated Nav1.7, for an extrapolated K_I = 13 μM and ratio K_R/K_I = 26, demonstrating weaker state dependence than **52**. To verify the suitability of **52** for testing in rat models of pain, **52** was tested on sodium channels recorded from cell bodies of individual sensory neurons dissociated from rat dorsal root ganglia (DRG) (see Supporting Information, Figure S1). Consistent with its strongly state-dependent inhibition of cloned channels and its potent inhibition of Nav1.7 and Nav1.3, **52** (at 1 μM) inhibited the fast-inactivating TTX-sensitive sodium channels of sensory neurons (90% inhibition) but only with the holding voltage set to produce partial channel inactivation. Compound **52** weakly inhibited (15% inhibition) the slow-inactivating TTX-resistant sodium channels of sensory neurons.

In addition to its strong state dependence, **52** also displayed some selectivity within the sodium ion channel family (Table 7). Selectivity for Nav1.7 over the tetrodotoxin resistant Nav1.5 and Nav1.8 subtypes was modest but clear, at 6.5-fold and 13-fold, respectively, whereas selectivity over Nav1.3 and Nav1.4 was not observed.[35−37] A greater than 50-fold selectivity margin over the hERG channel and a greater than 100-fold margin over the Kv1.5 channel was observed. Compound **52** exhibited weak or no activity against an extended panel of pain related targets (TRPM8, TRPV3, TRPV4, P_2X_7), a GPCR panel of 13 receptors, and a CEREP panel. In addition, when **52** was screened in an Ambit kinase panel (400 kinases) at 1 μM, no kinase was inhibited by greater than 75% and only 13 kinases were inhibited by more than 50% (Table 7).

The formalin model measures spontaneous rather than evoked pain and involves the sensitization of central neurons

Table 4. Comparison of Rat Liver Microsomal Clearance and Potency between des-Methyl and Methylated LHS Derivatives

Compound	R	Structure	RLM CL_{int} (μl/min/mg)	Nav 1.7 PX IC_{50} (μM)
36	H		187	1.4
37	CH_3		111	5.0
31	H		247	0.7
38	CH_3		134	0.6
35	H		127	1.4
39	CH_3		93	1.3

in response to a peripheral insult. This situation is perhaps somewhat analogous to the clinical conditions of erythromelalgia and paroxysmal extreme pain disorder, which are considered to be spontaneous pain disorders triggered by activation of Nav1.7, likely in peripheral neurons.[5] The formalin model is also appealing since its automated end point removes operator bias from the quantitation of animal pain.[38,39]

Compound **52** showed dose-dependent efficacy in the formalin model, with statistically significant analgesic effects at 3 mg/kg, matched by dose-dependent increases in plasma concentration (Figure 2A, Supporting Information Figure S2). The maximum effect (20−30 mg/kg) was equivalent to that produced by morphine. To verify that this apparent analgesic efficacy was not due to sedation or other effects causing a nonspecific reduction in movement, the same doses of **52** were also tested in a rat open field movement assay (Figure 2B, Supporting Information Figure S2). Total plasma concentrations were comparable between the open field and the formalin experiment. The 3 and 10 mg/kg doses of **52** were not significantly different from the vehicle control. While the 20 mg/kg dose elicited a statistically significant 29% reduction in movements, the reduction exhibited was not to the extent where conclusions drawn from the formalin test would be definitively confounded. Typically a large reduction in open field activity, approximately 50% or more, needs to be achieved before interpretation of a therapeutic effect in the formalin assay could be confounded.[40] Once this threshold is crossed, the rat has difficulty responding naturally to formalin stimulus. The 30 mg/kg dose also produced a significant reduction in open field movement. Because of the 52% reduction in movement produced at this dose, interpretation of the formalin result is difficult for reasons stated above. We conclude that compound **52** gives analgesic efficacy starting at plasma concentrations of approximately 0.5 μM (3 mg/kg dose), with full efficacy at approximately 3 μM (20 mg/kg dose). The potency of **52** incubated with 5% human serum albumin (HSA) on Nav1.7 (20% inactivated, PX) was 3.3 μM. The unbound plasma concentration (0.014 μM) at the minimum efficacious dose (3 mg/kg) was approximately 10- to 15-fold below the measured IC_{50} on partially (~20%) inactivated Nav1.7 channels (0.17 μM). Human and rat Nav1.7 amino acid sequences are 96% homologous and 93% identical; no pharmacological differences between human and rat are known for existing sodium channel blockers.

Biochemically, sodium channels have six known binding sites for neurotoxins and one defined binding site for small molecule inhibitors such as local anesthetics.[41,42] The binding epitope for local anesthetics, formed mostly by residues in the sixth transmembrane domain of the third and fourth pseudosubunits of the sodium channel peptide sequence,[43] has significant overlap with the binding site for batrachotoxin.[42,44−46] Although batrachotoxin binding is often used as a driver assay for the discovery and characterization of sodium channel inhibitors,[47] its binding epitope is highly conserved among sodium channel isoforms, suggesting that inhibitors acting at a different site might be better candidates for building an Nav1.7-selective molecule. To help understand the observed subtype selectivity of **52**, displacement assays were performed with tritiated batrachotoxin (^{3}H-BTX) and with a tritiated triazine lead compound (^{3}H-**36**) (Table 8, Supporting Information Figure S3). Both ^{3}H-BTX and ^{3}H-**36** showed saturable specific binding to membranes from Nav1.7-expressing cells. Interestingly, **52** did not displace ^{3}H-BTX (K_i > 10 μM) binding to Nav1.7 but did displace ^{3}H-**36** with K_i = 0.4 μM, close to its IC_{50} on partially inactivated Nav1.7. By contrast, as expected, the local anesthetic tetracaine inhibited ^{3}H-BTX binding with K_i = 0.5 μM, again very close to its IC_{50} = 0.3 μM on ~20% inactivated Nav1.7. Tetracaine had little effect on ^{3}H-**36**

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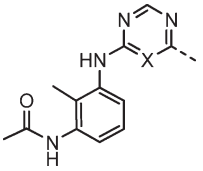
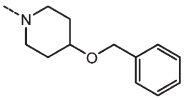
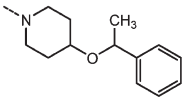
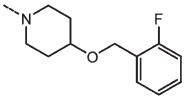
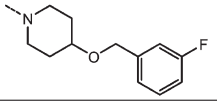
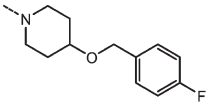
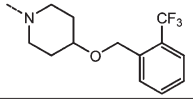
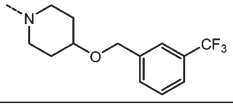
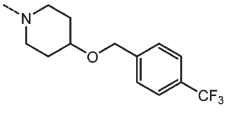
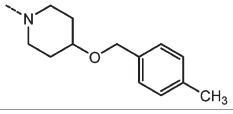
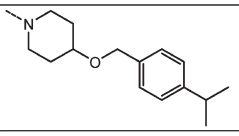
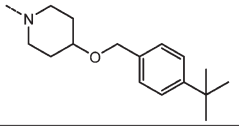
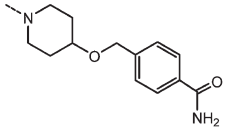
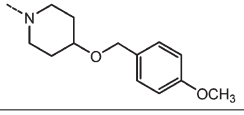
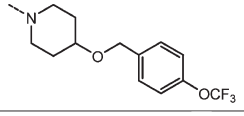
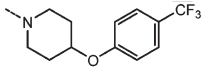
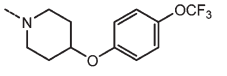
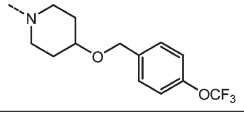
Table 5. SAR: Variations to the RHS Piperidine



	X =	RHS fragment	$Na_v1.7$ PX IC_{50} (μM)
37	N		5.0
40	N		>10
41	N		3.4
42	N		5.0
43	N		2.6
44	N		8.1
45	N		1.2
46	N		0.4
47	N		0.9

	X =	RHS fragment	$Na_v1.7$ PX IC_{50} (μM)
48	N		0.6
49	N		>10
50	N		>10
51	N		>10
52	N		0.5
53	N		0.8
54	N		0.3
55	CH		0.8

binding (K_i = 12 μM), again consistent with separate binding sites for triazines and for batrachotoxin/local anesthetics. Results show that **52** (and likely other compounds within the triazine chemical series) do not bind at the consensus local anesthetic/batrachotoxin site of Nav1.7 and suggest that inhibitors discussed herein recognize an undescribed epitope for small molecules. Since compound **52** has some selectivity for different sodium channels, this binding site may define an epitope to which different sodium channel isoforms contribute different amino acid residues.

In conclusion, a high-throughput screening campaign using a functional electrophysiology platform identified **24** as a modestly potent but highly state-dependent inhibitor of Nav1.7 with poor PK properties. A broad structure−activity relationship evaluation with emphasis on optimization of potency and PK produced compound **52**, a more potent and strongly state dependent inhibitor of Nav1.7 that demonstrated dose and exposure dependent efficacy in the formalin model of pain. Compound **52** had selectivity over many ion channels and GPCRs, including some selectivity within the sodium channel family, likely acting via a novel site. Accordingly, the triazine series described here, including compound **52**, may be valuable for the further investigation of the in vivo physiological roles of tetrodotoxin-sensitive sodium channels in pain pathways.

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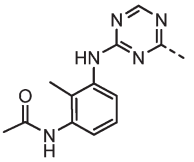
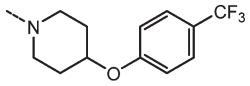
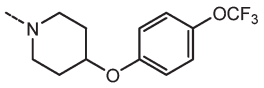
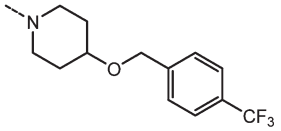
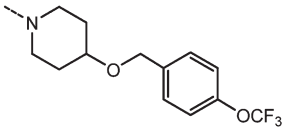
Table 6. Selectivity and Oral PK Comparisons of 53, 54, 46, and 52[a]

		hERG PX IC_{50} *(μM)*	*$Na_v1.5$ PX* IC_{50} *(μM)*	*$Na_v1.7$ PX* IC_{50} *(μM)*	*HLM/RLM* CL_{int} *(μl/min/mg)*	*Rat IV CL (L/(h-kg))*	*F (%)*
							
53		4.1	0.5	0.8	157 / 38	--	--
54		2.6	0.3	0.3	110 / 33	--	--
46		> 10	6.8	0.4	93 / 53	0.48	27%
52		> 10	5.2	0.5	91 / 46	0.42	67%

[a] Rat PK studies were conducted as follows: iv dosing, 0.5 mg/kg using 100% DMSO; with oral dosing, 2 mg/kg dosing using a formulation of 1% hydroxypropyl methylcellulose (HPMC), 5% Tween 80, 94% water.

EXPERIMENTAL SECTION

Electrophysiology. Sodium currents were recorded with the whole-cell configuration of the patch-clamp from Nav1.7 expressed in HEK293 cells using either conventional operator recording (manual electrophysiology as described)[48] or with the PatchXpress planar patch clamp automated electrophysiology system (Molecular Devices, Inc.). For manual recordings, cells were cultured under standard conditions, split, and plated into 35 mm dishes either 1 day before or on the same day of recording, and culture medium was exchanged for external solution containing (in mM) NaCl 140, KCl 2, $CaCl_2$ 2, $MgCl_2$ 1.1, HEPES 10, glucose 11, pH 7.4 (NaOH). Internal solution contained (in mM) CsCl 62.5, CsF 75, $MgCl_2$ 2.5, EGTA 5, HEPES 10, pH 7.2 (CsOH). Following establishment of the whole-cell patch-clamp configuration, cells were lifted off the bottom of the dish with the patch pipet and positioned directly in front of a microarray of glass tubes (each tube internal diameter, ~1 mm) with each tube containing continuously flowing control or test solution. A full current−voltage relation was recorded from each cell, and cells with poor space clamp as indicated by discontinuous $I-V$ relation or by prolonged or nonexponential current kinetics were discarded. For each sodium channel, IC_{50} values for test compounds were measured with a holding voltage set to produce approximately 20% steady-state fractional inactivation, and this voltage was reset as needed after application and washoff of each individual compound concentration to maintain fractional inactivation at 20%. For measurement of state-dependent pharmacology, the relevant parameter is not the absolute voltage to which the cell is clamped, but the fractional inactivation produced by a given voltage. Current was evoked with a 20 ms test pulse to −20 or −10 mV, and current amplitudes were corrected for leak by subtracting the current at the end of the test pulse (leak current) from the peak current during the test pulse (sodium channel current plus leak current). IC_{50} values for fully noninactivated (resting) channels were determined with the holding voltage set to either −140 or −120 mV (reported in brackets). For automated planar patch-clamp recordings, cells were used in suspension as per manufacturer's protocols. The voltage dependence of inactivation was measured for each cell individually, and the holding voltage was set to produce 20% fractional inactivation before application of each concentration of test compound. Time courses of current were corrected for rundown manually with DataXpress software. To record from individual rat dorsal root ganglion neurons, the dorsal root ganglia were dissected from rats aged 3−10 days and the ganglia enzymatically digested with collagenase and Dispase followed by papain as described.[49] Individual neurons were released via gentle trituration and plated into a 35 mm dish and used on the same day or the first day after dissection. Currents were evoked by 20 ms depolarizations to a voltage at or near the peak of the current−voltage curve: approximately −20 mV for TTX-sensitive sodium currents and +10 mV for TTX-resistant sodium currents. At least four different concentrations of test compound at half log units were applied individually, with washout, recovery of current, and resetting of holding voltage between each individual concentration. Percent inhibition as a function of compound concentration was pooled from at least n = 10 different cells, with two to three data points per concentration, to produce a single IC_{50} curve. A benchmark aminotriazine run as positive

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Table 7. In Vitro Selectivity Profile of 52

receptor	IC_{50} (μM)
hNav1.7[a]	0.17 (3.6)
hNav1.5[a]	1.1 (>10)
hNav1.3[a]	0.3 (>10)
hNav1.4[a]	0.4 (>10)
rNav1.8[a]	2.2
hERG[b]	>10
hTRPV3, hTRPV4[b]	>10
hTRPM8[b]	>20
Kv1.5[b]	>33.3
rP_2X_7[b]	>10
Cerep (at 10 μM)[c]	A3 (h), TP (TXA2/PGH2) (h), σ (nonselective), Na^+ (site 2) (85% inhibition)
GPCR panel (13)	>50
kinase (Ambit)[d]	0, 13, 387

[a] IC_{50} numbers for sodium channels displayed in this table were taken with manual electrophysiology on ~20% inactivated channels. The value in parentheses is the IC_{50} recorded on fully noninactivated channels determined with a very negative holding potential, usually −140 mV. [b] IC_{50} values for hERG and Kv1.5 were recorded with electrophysiology; values for TRP and P2X receptors were recorded with fluorescence-based live-cell functional assays. [c] Displayed >70% inhibition of control specific binding at indicated receptor. Site 2 Na^+ was measured from rat cortex, which contains a mix of sodium channel isoforms and likely minimal Nav1.7, possibly accounting for the lower affinity seen for binding to hNav1.7 directly (Figure 2). [d] Number of kinases exhibiting % inhibition in the presence of 1 μM **52**: >50%, 25−50%, and <25%, respectively.

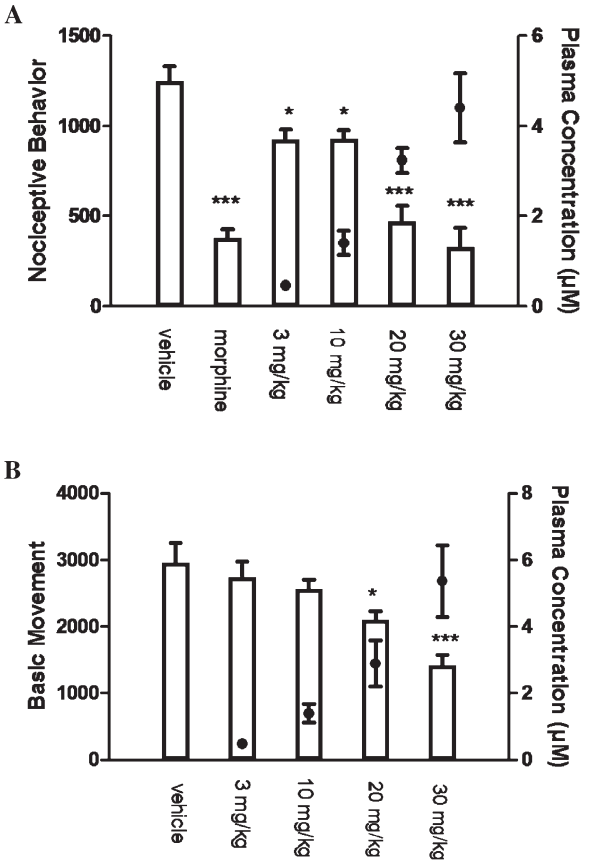


Figure 2. (A) Time course of formalin-induced flinches in animal cohorts administered vehicle, morphine sulfate (subcutaneous dosing, 2 mg/kg), or 3, 10, 20, or 30 mg/kg compound **52**: (left *y*-axis) total flinches in phase 2a as a function of dose; (right *y*-axis) mean plasma concentrations in the formalin experiment for 3, 10, 20, 30 mg/kg doses were 0.5, 1.4, 3.2, 4.4 μM. (B) (left *y*-axis) time course of movement following administration of either vehicle or compound; (right *y*-axis) mean plasma concentrations in the open-field experiment for 3, 10, 20, 30 mg/kg doses were 0.5, 1.4, 2.9, 5.4 μM. *, $p < 0.05$, ***, $p < 0.001$ versus vehicle group (one-way ANOVA followed by Dunnett's test).

control produced mean IC_{50} = 1.4 μM and standard deviation of 0.6 μM from n = 25 individually calculated IC_{50} curves.

hERG (human ether-a-go-go-related gene) currents were recorded with the whole-cell configuration of the patch clamp from hERG expressed in HEK293 cells (Millipore) using the PatchXpress planar patch-clamp automated electrophysiology system (Molecular Devices, Inc.) per the manufacturer's protocol. The external recording solution contained (in mM) NaCl (135), KCl (4), $MgCl_2 \cdot 6H_2O$ (1), $CaCl_2$ (1.8), HEPES (10), and glucose (10). pH was adjusted to 7.4 with NaOH, and the final osmolarity was set at 310 mOsm. The internal recording solution contained (in mM) KCl (70), KF (60), $MgCl_2 \cdot 6H_2O$ (2), HEPES (10), EGTA (10), and MgATP (5). pH was adjusted to 7.4 with KOH, and the final osmolarity was set at 280 mOsm.

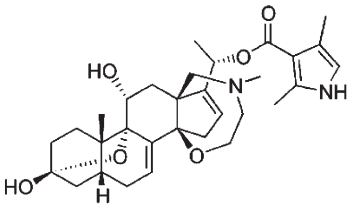
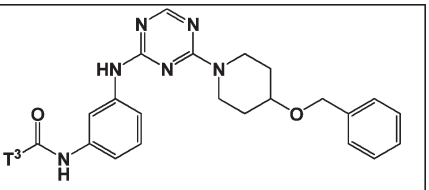
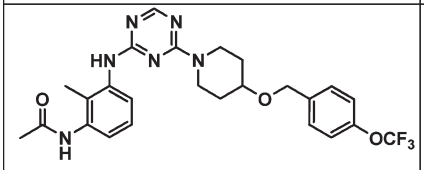
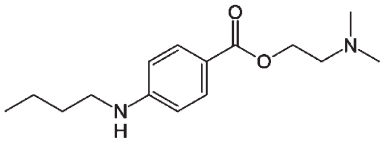
Cells were voltage clamped at −80 mV holding potential, and the hERG current was activated by a depolarizing step first to −50 mV for 500 ms to serve as a baseline for the outward tail current, then to +30 mV for 2 s to activate the channels, and finally back to −50 mV for 2 s to remove the inactivation and record the deactivating outward tail current.

All test articles in solid format were dissolved in 100% dimethylsulfoxide (DMSO, Sigma) to a final stock concentration of 10 mM. Serial dilutions were prepared in DMSO, and 3 μL aliquots were subsequently transferred to 900 mL of external recording solution in a 96 well plate containing glass vials to minimize compound lost because of nonspecific binding. The final DMSO concentration in the external recording solution was 0.3%. The compound plate was then sonicated for 15 min before each experiment to ensure adequate mixing of the test article in external recording solution.

Binding. Membranes were prepared from 293 cells stably expressing Nav1.7, and binding of tritiated batrachotoxin (Perkin-Elmer) and of radiolabeled triazine (compound ^{3}H-**36**) was tested with modifications of the methods of Brown.[50] ^{3}H-BTX binding was assayed in 96-well plates with 50 μg of membrane in each well, in binding buffer containing (in mM) choline chloride 130, HEPES 50, glucose 5.5, $MgSO_4$ 0.8, KCl 5.4, plus 0.1% BSA (Sigma) and 1 protease inhibitor cocktail tablet (Roche) per 50 mL of solution. Reactions were incubated for 1 h at 200 rpm shaking speed, and the product was filtered over 0.5% polyethylenimine-treated GF/C filter plates (Perkin-Elmer), washed six times, and dried. Counts were assayed with a MicroBeta imager (Perkin-Elmer), and binding was expressed as counts per minute (CPM). Nonspecific binding was determined by co-incubation with 100 μM veratridine (^{3}H-BTX binding) or of excess of cold compound **36**. B_{max} and K_d were calculated from the Langmuir equation. K_i values for individual compounds were determined from experimentally determined IC_{50} via $K_i = IC_{50}/(1 + (\text{radioligand concentration})/K_d)$. For competition binding experiments, radioligands were used at concentrations near K_d.

In Vivo Pharmacology. Formalin and open-field testing were carried out as previously described.[48] In brief, 50 μL of 2.5% formalin diluted in saline was injected intraplantar, and flinching behaviors were quantitated for the 40 min following injection using an automated nociception analyzer from the University of California, San Diego.[38] Formalin evoked the characteristic biphasic response in which phase 2a flinches (10−40 min after formalin injection) represent persistent pain

Table 8. Epitope Binding Affinities for 52 and Tetracaine at BTx and ^{3}H-36 Binding Sites

	 BTX site (K_i: μM)	 ^{3}H-36 site (K_i: μM)
 52	> 10	0.4
 Tetracaine	0.5	12

associated with central sensitization. Test compound was administered po 120 min prior to formalin injection to $n = 8$ animals per dose in vehicle of 30% hydroxypropyl-β-cyclodextrin, pH set to 1.5 with methane sulfonic acid. Positive control morphine sulfate was administered sc 30 min prior to formalin injection to $n = 8$ animals in a vehicle of saline. Movement of naive (non-formalin-treated) animals in an open-field box was assayed with a photobeam activity system (San Diego Instruments) and reported as number of beam breaks binned in 5 min intervals and as total beam breaks over the entire test session. Test compounds ($n = 8$ animals per dose) were administered such that movement recording began at the same time after compound administration as did the beginning of the formalin model. Statistical significance was as follows: *, $p < 0.05$, ***, $p < 0.001$ by one-way ANOVA and Dunnett's post hoc test.

Chemistry. General. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Dry organic solvents (CH_2Cl_2, CH_3CN, DMF, etc.) were purchased from Aldrich packaged under nitrogen in Sure/Seal bottles. Reactions were monitored using Agilent 1100 series LC−MS with UV detection at 254 nm and a low resonance electrospray mode (ESI). Medium pressure liquid chromatography (MPLC) was performed on a CombiFlash Companion (Teledyne Isco) with RediSep normal-phase silica gel (35−60 μm) columns or Biotage Isolera using SNAP columns normal phase silica and UV detection at 254 nm. Preparative reversed-phase HPLC was performed on a Gilson (215 liquid handler), YMC-Pack Pro C18, 150 mm × 30 mm i.d. column, eluting with a binary solvent system A and B using a gradient elution (A, H_2O with 0.1% TFA; B, CH_3CN with 0.1% TFA) with UV detection at 254 nm. The analytical SFC chromatograph was a SFC method development station sold by Thar (Pittsburgh, PA, U.S.) equipped with a Waters ZQ mass spectrometer (Milford, MA, U.S.). The preparative SFC chromatograph was a SFC Prep 80 from Thar (Pittsburgh, PA, U.S.). Purity for final compounds was greater than 95% unless otherwise noted and was measured using Agilent 1100 series high performance liquid chromatography (HPLC) systems with UV detection at 254 nm (system A, Agilent Zorbax Eclipse XDB-C8 4.6 mm × 150 mm, 5 μm, 5−100% CH_3CN in H_2O with 0.1% TFA for 15 min at 1.5 mL/min; system B, Waters Xterra 4.6 mm × 150 mm, 3.5 μm, 5−95% CH_3CN in H_2O with 0.1% TFA for 15 min at 1.0 mL/min). Exact mass confirmation was performed on an Agilent 1100 series high performance liquid chromatography (HPLC) system (Santa Clara, CA, U.S.) by flow injection analysis, eluting with a binary solvent system A and B (A, water with 0.1% FA; B, ACN with 0.1% FA) under isocratic conditions (50% A/50% B) at 0.2 mL/min with MS detection by an Agilent G1969A time of flight (TOF) mass spectrometer (Santa Clara, CA, U.S.). ^{1}H NMR spectra were recorded on a Bruker AV-400 (400 MHz) spectrometer at ambient temperature or on a Varian 400 MHz spectrometer. Chemical shifts are reported in ppm from the solvent resonance (DMSO-d_6 2.50 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants, and number of protons.

***N*-(3-(4-Chloro-1,3,5-triazin-2-ylamino)phenyl)acetamide (3).** To a brown solution of *N*-(3-aminophenyl)acetamide (8.19 g, 54.5 mmol) in 1,2-dimethoxyethane (81 mL) was added diisopropylethylamine (23.7 mL, 136 mmol), and the mixture was cooled in an ice−water bath. A suspension of 2,4-dichloro-1,3,5-triazine (9.0 g, 60.0 mmol) in 1,2-dimethoxyethane (195 mL) was cooled in an ice−water bath, then added to the cooled *N*-(3-aminophenyl)acetamide solution. The solution was allowed to stir and warm on its own to room temperature for 16 h. To the pale brown solution was added ethyl acetate and 1 M HCl (400 mL). The aqueous phase was extracted with ethyl acetate (300 mL, 2×). The combined organics were dried with Na_2SO_4, filtered, and dried under reduced pressure. Upon standing a solid formed in the aqueous

phase, which was collected by filtration. The combined solids were triturated with CH_2Cl_2 to remove any yellow impurities, providing the title compound (13.18 g, 91.7% yield) as a light yellow solid. LC−MS (ESI) m/z: 264.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.74 (s, 1H), 10.04 (s, 1H), 8.62 (s, 1H), 7.87 (br s, 1H), 7.39 (br. s, 1H), 7.34−7.22 (m, 2H), 2.04 (s, 3H).

***N*-(3-(4-Chloro-1,3,5-triazin-2-ylamino)-2-methylphenyl)-acetamide (22).** A solution of 2,4-dichloro-1,3,5-triazine (5.00 g, 33.3 mmol) in *N*,*N*-dimethylformamide (44.5 mL) and *N*,*N*-diisopropylethylamine (6.38 mL, 36.7 mmol) was cooled in an ice−water bath, and *N*-(3-amino-2-methylphenyl)acetamide (5.47 g, 33.3 mmol, 71.7% yield) was added. The mixture was allowed to slowly warm to room temperature and was stirred for a total of 18 h. To the resulting dark orange solution was added water (~200 mL) and the turbid mixture cooled in the refrigerator overnight leading to the formation of a yellow precipitate which was collected via vacuum filtration and the solid washed with minimal ethyl acetate and excess water. The solid was dried under reduced pressure to provide the title compound (6.64 g, 72%) as a yellow solid. LC−MS (ESI) m/z: 278.1 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.27 (s, 1H), 9.39 (br s, 1H), 8.38−8.60 (m, 1H), 7.31 (d, J = 6.65 Hz, 1H), 7.19 (t, J = 8.00 Hz, 1H), 7.12 (d, J = 8.00 Hz, 1H), 2.06 (s, 3H), 2.03 (s, 4H).

***N*-(3-(2-(3,4-Dihydroisoquinolin-2(1*H*)-yl)pyrimidin-4-ylamino)phenyl)acetamide (7).** To a flask charged with 2,4-dichloropyrimidine (1.00 g, 6.7 mmol) in 15 mL of 2-propanol at 0 °C was added diisopropylethylamine (2.33 mL, 13.4 mmol) followed by *N*-(3-aminophenyl)acetamide (1.01 g, 6.7 mmol). The resulting mixture was stirred at 0 °C for 15 min, then at ambient for 21 h. The resulting mixture was dried under reduced pressure and purified by silica gel chromatography (0−100% ethyl acetate in hexanes), providing (**6**) *N*-(3-(2-chloropyrimidin-4-ylamino)phenyl)acetamide (554 mg, 31%). LC−MS (ESI) m/z: 263.0 (M + 1).

To a flask charged with *N*-(3-(2-chloropyrimidin-4-ylamino)phenyl)-acetamide (150.0 mg, 571 μmol) and 1,2,3,4-tetrahydroisoquinoline (79.7 μL, 628 μmol) were added 2-propanol (3 mL) and trifluoroacetic acid (132 μL, 1.71 mmol). The resulting mixture was heated at 90 °C for 23 h. The resulting mixture was concentrated, dissolved in EtOAc, transferred to a separatory funnel, and washed with saturated aqueous $NaHCO_3$. The organic phase was washed with brine. The combined aqueous washes were extracted with ethyl acetate. The combined organic washes were dried with Na_2SO_4, filtered, and dried under reduced pressure. The crude residue obtained was purified by silica gel chromatography (0−100% CH_2Cl_2/CH_3OH (90:10) in CH_2Cl_2), providing the title compound (103 mg, 50%) as a white solid. LC−MS (ESI) m/z: 360.0 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.90 (s, 1H), 9.27 (s, 1H), 8.17 (br s, 1H), 7.95 (d, J = 5.67 Hz, 1H), 7.39 (d, J = 7.83 Hz, 1H), 7.28−7.11 (m, 5H), 7.00 (d, J = 7.92 Hz, 1H), 6.08 (d, J = 5.67 Hz, 1H), 4.86 (s, 2H), 3.97 (t, J = 5.87 Hz, 2H), 2.85 (t, J = 5.67 Hz, 2H), 2.07 (s, 3H). HRMS calcd for $C_{21}H_{21}N_5O$ $(M + H)^+$ 360.1819, found 360.182.

***N*-(3-(4-(3,4-Dihydroisoquinolin-2(1*H*)-yl)pyrimidin-2-ylamino)phenyl)acetamide (9).** To a flask charged with 2,4-dichloropyrimidine (1.00 g, 6.7 mmol) in 15 mL of 2-propanol at 0 °C was added diisopropylethylamine (2.33 mL, 13.4 mmol) followed by 1,2,3,4-tetrahydroisoquinoline (0.894 g, 6.7 mmol). The resulting mixture was stirred at 0 °C for 15 min, then at ambient for 24 h. The resulting mixture was dried under reduced pressure and purified by silica gel chromatography (0−60% ethyl acetate in hexanes), providing (**8**) 2-(2-chloropyrimidin-4-yl)-1,2,3,4-tetrahydroisoquinoline (1.45 g, 88%). LC−MS (ESI) m/z: 246.0 (M + 1).

To a flask charged with 2-(2-chloropyrimidin-4-yl)-1,2,3,4-tetrahydroisoquinoline (150.0 mg, 610 μmol) and *N*-(3-aminophenyl)-acetamide (101 mg, 672 μmol) were added 2-propanol (3 mL) and trifluoroacetic acid (141 μL, 1.83 mmol). The resulting mixture was heated at 90 °C for 23 h. The resulting mixture was concentrated, dissolved in EtOAc, transferred to a separatory funnel, and washed with saturated aqueous $NaHCO_3$. The organic phase was washed with brine. The combined organic washes were dried with Na_2SO_4, filtered, and dried under reduced pressure. The crude residue obtained was purified by silica gel chromatography (0−100% CH_2Cl_2/CH_3OH (90:10) in CH_2Cl_2), providing the title compound (176 mg, 80%) as a white solid. LC−MS (ESI) m/z: 360.0 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.79 (s, 1H), 9.01 (s, 1H), 8.11 (s, 1H), 7.98 (d, J = 6.06 Hz, 1H), 7.33 (d, J = 7.83 Hz, 1H), 7.28−7.17 (m, 4H), 7.14 (t, J = 8.00 Hz, 1H), 7.07 (d, J = 8.00 Hz, 1H), 6.30 (d, J = 6.06 Hz, 1H), 4.77 (s, 2H), 3.83 (t, J = 5.33 Hz, 2H), 2.90 (t, J = 5.87 Hz, 2H), 2.05 (s, 3H). HRMS calcd for $C_{21}H_{21}N_5O$ $(M + H)^+$ 360.1819, found 360.1823.

***N*-(3-(6-(3,4-Dihydroisoquinolin-2(1*H*)-yl)pyrimidin-4-ylamino)phenyl)acetamide (12).** To a flask charged with 4,6-dichloropyrimidine (250 mg, 1.68 mmol) were added 2-propanol (6.7 mL) and *N*,*N*-diisopropylethylamine (308 μL, 1.76 mmol). The solution was cooled in an ice−water bath prior to the addition of 1,2,3,4-tetrahydroisoquinoline (224 μL, 1.68 mmol). The mixture was stirred and allowed to slowly warm on its own to room temperature, leading to the formation of a white precipitate after 1 h. The solid was collected via vacuum filtration and the solid washed with excess 2-propanol, then dried under reduced pressure, providing a white solid 2-(6-chloropyrimidin-4-yl)-1,2,3,4-tetrahydroisoquinoline (**11**) (340 mg, 1.384 mmol, 82% yield). LC−MS (ESI) m/z: 246.0 (M + 1).

To a flask charged with 2-(6-chloropyrimidin-4-yl)-1,2,3,4-tetrahydroisoquinoline (150 mg, 0.610 mmol) was added 2-propanol (4.1 mL) followed by 3′-aminoacetanilide (110 mg, 0.733 mmol) and trifluoroacetic acid (118 μL, 1.53 mmol). The vessel was sealed and heated overnight at 95 °C, providing a light brown suspension. The mixture was cooled to room temperature, then in a −20 °C freezer for 1 h. The resulting solid was collected via vacuum filtration and washed with excess 2-propanol. The filtrate was concentrated under reduced pressure and the oil obtained purified by silica gel chromatography (0−100% CH_2Cl_2/CH_3OH/NH_4OH (90:10:1) in CH_2Cl_2), providing the title compound (75 mg, 34.2% yield) as a white solid. LC−MS (ESI) m/z: 360.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.87 (s, 1H), 9.07 (s, 1H), 8.22 (d, J = 0.59 Hz, 1H), 7.88−7.85 (m, 1H), 7.28 (td, J = 1.82, 7.52 Hz, 1H), 7.26−7.11 (m, 6H), 6.08 (d, J = 0.59 Hz, 1H), 4.67 (s, 2H), 3.76 (t, J = 5.92 Hz, 2H), 2.89 (t, J = 5.87 Hz, 2H), 2.04 (s, 3H). HRMS calcd for $C_{21}H_{21}N_5O$ $(M + H)^+$ 360.1819, found 360.1823.

***N*-(3-((4-(3,4-Dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-yl)(methyl)amino)phenyl)acetamide (15).** To a flask charged with 2,4-dichloro-1,3,5-triazine (0.500 g, 3.33 mmol) was added *N*,*N*-dimethylformamide (13.3 mL) and *N*,*N*-diisopropylethylamine (1.17 mL, 6.67 mmol), respectively. The resulting yellow solution was cooled in an ice−water bath prior to the addition of 3-bromo-*N*-methylaniline (0.425 mL, 3.33 mmol). The solution was allowed to stir and warm on its own to room temperature over 3.5 h, leading to clean conversion to desired triazine monochloride species. To the mixture was added 1,2,3,4-tetrahydroisoquinoline (0.444 mL, 3.33 mmol), and stirring at room temperature continued overnight. The orange solution was dried under reduced pressure and the crude material purified by silica gel chromatography (0−100% CH_2Cl_2/CH_3OH/NH_4OH (90:10:1) in CH_2Cl_2), providing a mixture of product and a major aliphatic impurity. The orange oil was dissolved in DCM and diethyl ether was added leading to the formation of an orange precipitate which was collected via vacuum filtration and washed with diethyl ether. The filtrate was dried under reduced pressure, providing product as an orange oil, *N*-(3-bromophenyl)-4-(3,4-dihydroisoquinolin-2(1*H*)-yl)-*N*-methyl-1,3,5-triazin-2-amine (**14**) (336 mg, 0.848 mmol, 25.4%). LC−MS (ESI) m/z: 396.0/398.0 (M, M + 2), 2.329 min.

To a vial charged with *N*-(3-bromophenyl)-4-(3,4-dihydroisoquinolin-2(1*H*)-yl)-*N*-methyl-1,3,5-triazin-2-amine (192 mg, 0.485 mmol) were added acetamide (172 mg, 2.91 mmol), xantphos (56.1 mg,

0.097 mmol), cesium carbonate (631 mg, 1.94 mmol), and pd_2dba_3 (44.4 mg, 0.048 mmol). The vessel was placed under argon prior to the addition of dry 1,4-dioxane (2.42 mL). The vessel was sealed and heated to 110 °C for 16 h. To the mixture was added water, and the material was transferred to a separatory funnel and extracted with CH_2Cl_2 (2×). The combined organics were dried with Na_2SO_4, filtered, and dried under reduced pressure. The crude material was purified by silica gel chromatography (0−100% CH_2Cl_2/CH_3OH/NH_4OH (90:10:1) in CH_2Cl_2), providing the title compound (64 mg, 0.171 mmol, 35.3%) as a yellow solid. LC−MS (ESI) *m/z*: 375.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.97 (s, 1H), 8.15 (s, 1H), 7.60 (t, *J* = 1.86 Hz, 1H), 7.43 (d, *J* = 8.22 Hz, 1H), 7.30 (t, *J* = 8.07 Hz, 1H), 7.22−7.15 (m, 4H), 7.00 (d, *J* = 7.73 Hz, 1H), 4.91−4.73 (m, 2H), 3.99−3.80 (m, 2H), 3.42 (s, 3H), 2.84 (t, *J* = 5.92 Hz, 2H), 2.04 (s, 3H). HRMS calcd for $C_{21}H_{22}N_6O$ $(M + H)^+$ 375.1928, found 375.1931.

***N*-(3-(4-(3,4-Dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-yloxy)phenyl)acetamide (18).** To a flask charged with 2,4-dichloro-1,3,5-triazine (2.00 g, 13.3 mmol) in 10 mL of *N*,*N*-dimethylformamide at 0 °C was added *N*,*N*-diisopropylethylamine (2.32 mL, 13.3 mmol) followed by 1,2,3,4-tetrahydroisoquinoline (1.69 mL, 13.3 mmol). The resulting mixture was stirred at 0 °C for 10 min, then at ambient temperature for 2 h. Water was added to the mixture and the resulting orange slurry stirred for 20 min. Ethyl acetate was added, and the mixture was stirred for 10 min and transferred to separatory funnel. The organic phase was washed with water and brine. The combined aqueous washes were extracted with ethyl acetate (2×). The combined organic washes were dried with Na_2SO_4, filtered, and dried under reduced pressure. The crude residue was adsorbed onto silica gel and purified by silica gel chromatography (0−40% ethyl acetate in hexanes), providing (**16**) 2-(4-chloro-1,3,5-triazin-2-yl)-1,2,3,4-tetrahydroisoquinoline (1.76 g, 54%). LC−MS (ESI) *m/z*: 247.0 (M + 1).

To a flask charged with 3-acetamidophenol (91.9 mg, 608 μmol) in 3 mL *N*,*N*-dimethylformamide at ambient temperature was added sodium hydride (60% in mineral oil) (23.3 mg, 608 μmol). The resulting mixture was stirred at ambient temperature for 30 min, when 2-(4-chloro-1,3,5-triazin-2-yl)-1,2,3,4-tetrahydroisoquinoline (150.0 mg, 608 μmol) was added in one portion. The resulting mixture was stirred at ambient temperature for 17 h. The resulting mixture was quenched with water, diluted with ethyl acetate, and transferred to a separatory funnel. The organic layer was washed water and brine, dried with Na_2SO_4, filtered, and dried under reduced pressure. The crude residue was purified by silica gel chromatography (0−90% ethyl acetate in hexanes), providing the title compound (165 mg, 75%) as a white solid. LC−MS (ESI) *m/z*: 362.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.06 (s, 1H), 8.43 (d, *J* = 4.21 Hz, 1H), 7.56 (br s, 1H), 7.41−7.30 (m, 2H), 7.27−7.14 (m, 4H), 6.88 (m, 1H), 4.91 (s, 1H), 4.76 (s, 1H), 4.06−3.97 (m, 1H), 3.82 (t, *J* = 5.82 Hz, 1H), 2.91−2.81 (m, 2H), 2.05 (s, 3H). HRMS calcd for $C_{20}H_{19}N_5O_2$ $(M + H)^+$ 362.1612, found 362.1618.

***N*-(3-(4-(3,4-dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)phenyl)acetamide (24).** See ref 25 for the procedure.

***N*-(3-(4-(3,4-Dihydroisoquinolin-2(1*H*)-yl)-1,3,5-triazin-2-ylamino)phenyl)-*N*-methylacetamide (25).** To a flask charged with 2,4-dichloro-1,3,5-triazine (91 mg, 0.609 mmol) was added *N*,*N*-dimethylformamide (2.4 mL) followed by *N*,*N*-diisopropylethylamine (319 μL, 1.827 mmol) and *N*-(3-aminophenyl)-*N*-methylacetamide (100 mg, 0.609 mmol). The mixture was stirred at room temperature for 30 min prior to the addition of 1,2,3,4-tetrahydroisoquinoline (77 μL, 0.609 mmol). The resulting solution was stirred for 16 h, then dried under reduced pressure and purified by silica gel chromatography (0−100% CH_2Cl_2/CH_3OH/NH_4OH (90:10:1) in CH_2Cl_2), leading to isolation of the product along with trace impurities. The solid obtained was triturated with MeOH/DCM (1:1) to provide the title compound (12 mg, 0.032 mmol, 5.3% yield) as a white solid. LC−MS (ESI) *m/z*: 375.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.82 (d, *J* = 8.41 Hz, 1H), 8.31 (s, 1H), 7.97−7.74 (m, 1H), 7.66−7.52 (m, 1H), 7.38 (t, *J* = 8.02 Hz, 1H), 7.27−7.12 (m, 4H), 6.97 (d, *J* = 7.53 Hz, 1H), 4.88 (d, *J* = 11.54 Hz, 2H), 4.03−3.93 (m, 2H), 3.18 (t, *J* = 4.89 Hz, 3H), 2.89 (d, *J* = 2.84 Hz, 2H), 1.83 (br s, 3H). HRMS calcd for $C_{21}H_{22}N_6O$ $(M + H)^+$ 374.1928, found 374.1932.

***N*-(3-(4-Amino-1,3,5-triazin-2-ylamino)phenyl)acetamide (26).** The title compound was prepared using a method analogous to the preparation of compound **27** using 2 M NH_3 in MeOH (10 equiv). The crude mixture was dried under reduced pressure and purified by silica gel chromatography (0−100% CH_2Cl_2/CH_3OH (90:10) in CH_2Cl_2) to provide the title compound as a white solid. Yield: 107 mg, 72%. LC−MS (ESI) *m/z*: 245.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.83 (s, 1H), 9.42 (s, 1H), 8.13 (s, 1H), 7.74 (s, 1H), 7.47 (d, *J* = 9.10 Hz, 1H), 7.29 (d, *J* = 8.22 Hz, 1H), 7.16 (t, *J* = 7.80 Hz, 1H), 7.09−6.81 (m, 2H), 2.03 (s, 3H). HRMS calcd for $C_{11}H_{12}N_6O$ $(M + H)^+$ 245.1145, found 245.115.

***N*-(3-(4-(Piperidin-1-yl)-1,3,5-triazin-2-ylamino)phenyl)-acetamide (27).** In a sealed tube charged with *N*-(3-(4-chloro-1,3,5-triazin-2-ylamino)phenyl)acetamide (40 mg, 152 μmol) (**3**) was added *N*,*N*-dimethylformamide (0.600 mL, 152 μmol), *N*,*N*-diisopropylethylamine (0.055 mL, 319 μmol), and piperidine (0.018 mL, 182 μmol), respectively. The tube was sealed and heated to 90 °C. The resulting brown solution was cooled to room temperature and water was added providing a brown precipitate which was collected via vacuum filtration and washed with water, then dried under high vacuum, providing the title compound (39 mg, 82% yield) as a tan solid. LC−MS (ESI) *m/z*: 313.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.86 (s, 1H), 9.54 (br s, 1H), 8.20 (s, 1H), 8.05 (br s, 1H), 7.33−7.28 (m, 1H), 7.20−7.09 (m, 2H), 3.79−3.71 (m, 4H), 2.02 (s, 3H), 1.67−1.59 (m, 2H), 1.52 (br s, 4H). HRMS calcd for $C_{16}H_{20}N_6O$ $(M + H)^+$ 313.1771, found 313.1776.

***N*-(3-(4-(4-Methylpiperidin-1-yl)-1,3,5-triazin-2-ylamino)-phenyl)acetamide (28).** The title compound was prepared using a method analogous to the preparation of **27**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% NH_4OH) in NH_4OH (0.1% aqueous) as the eluent, providing the title compound. Yield: 52 mg, 60%. LC−MS (ESI) *m/z*: 327.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.85 (s, 1H), 9.52 (s, 1H), 8.20 (s, 1H), 8.05 (br s, 1H), 7.30 (d, *J* = 7.34 Hz, 1H), 7.20−7.06 (m, 2H), 4.66 (br s, 2H), 2.91−2.82 (m, 2H), 2.02 (s, 3H), 1.74−1.57 (m, 3H), 1.11−0.97 (m, 2H), 0.92 (d, *J* = 6.26 Hz, 3H). HRMS calcd for $C_{17}H_{22}N_6O$ $(M + H)^+$ 327.1928, found 327.1933.

***N*-(3-(4-(4-Methoxypiperidin-1-yl)-1,3,5-triazin-2-ylamino)-phenyl)acetamide (29).** The title compound was prepared using a method analogous to the preparation of **27**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% NH_4OH) in NH_4OH (0.1% aqueous) as the eluent, providing the title compound. Yield: 42 mg, 64%. LC−MS (ESI) *m/z*: 343.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.84 (s, 1H), 9.54 (s, 1H), 8.21 (s, 1H), 8.07 (br s, 1H), 7.29 (d, *J* = 7.34 Hz, 1H), 7.20−7.09 (m, 2H), 4.21−4.09 (m, 2H), 3.50−3.38 (m, 3H), 3.28 (s, 3H), 2.03 (s, 3H), 1.93−1.78 (m, 2H), 1.48−1.33 (m, 2H). HRMS calcd for $C_{17}H_{22}N_6O_2$ $(M + H)^+$ 343.1877, found 343.1885.

1-(4-(3-Acetamidophenylamino)-1,3,5-triazin-2-yl)piperidine-4-carboxamide (30). The title compound was prepared using a method analogous to the preparation of **27**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% NH_4OH) in NH_4OH (0.1% aqueous) as the eluent, providing the title compound. Yield: 53 mg, 60%. LC−MS (ESI) *m/z*: 356.0 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.88 (s, 1H), 9.59 (s, 1H), 8.22 (s, 1H), 8.10 (br s, 1H), 7.33−7.25 (m, 2H), 7.17 (t, *J* = 7.97 Hz, 1H), 7.09−7.14 (m, 1H), 6.81 (s, 1H), 4.73−4.60 (m, 2H), 2.93 (dt, *J* = 2.64, 12.67 Hz, 2H), 2.39 (tt, *J* = 3.57, 11.44 Hz, 1H), 1.77 (d, *J* = 12.32 Hz, 2H), 1.53−1.38 (m, 2H). HRMS calcd for $C_{17}H_{21}N_7O_2$ $(M + H)^+$ 356.1829, found 356.1833.

***N*-(3-(4-(4-Phenylpiperidin-1-yl)-1,3,5-triazin-2-ylamino)-phenyl)acetamide (31).** The title compound was prepared using a method analogous to the preparation of compound **27**. The crude mixture was dried under reduced pressure and purified by silica gel chromatography (0−100% CH_2Cl_2/CH_3OH (90:10) in CH_2Cl_2) to provide the title compound as a white solid. Yield: 85 mg, 58%. LC−MS (ESI) *m/z*: 389.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.84 (s, 1H), 9.56 (s, 1H), 8.23 (s, 1H), 8.10 (br s, 1H), 7.33−7.24 (m, 5H), 7.22−7.08 (m, 3H), 4.96−4.79 (m, 2H), 2.97 (dt, *J* = 2.45, 12.86 Hz, 2H), 2.84 (tt, *J* = 3.36, 12.09 Hz, 1H), 2.01 (s, 3H), 1.86 (d, *J* = 12.42 Hz, 2H), 1.65−1.52 (m, 2H). HRMS calcd for $C_{22}H_{24}N_6O$ $(M + H)^+$ 389.2084, found 389.2087.

***N*-(3-(4-(4-Benzylpiperidin-1-yl)-1,3,5-triazin-2-ylamino)-phenyl)acetamide (32).** The title compound was prepared using a method analogous to the preparation of **27**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% trifluoroacetic acid) in trifluoroacetic acid (0.1% aqueous) as the eluent, providing the title compound as a trifluoroacetate salt. Yield: 47 mg, 40%. Purity: 92%. LC−MS (ESI) *m/z*: 403.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.86 (s, 1H), 9.70 (s, 1H), 8.24 (s, 1H), 8.06 (s, 1H), 7.31−7.25 (m, 3H), 7.22−7.15 (m, 4H), 7.15−7.09 (m, 1H), 4.74−4.57 (m, 2H), 2.92−2.81 (m, 2H), 2.53 (d, *J* = 6.85 Hz, 2H), 2.01 (s, 3H), 1.88−1.78 (m, 1H), 1.66 (d, *J* = 12.03 Hz, 2H), 1.20−1.06 (m, 2H). HRMS calcd for $C_{23}H_{26}N_6O$ $(M + H)^+$ 403.2241, found 403.2244.

***N*-(3-(4-(4-Hydroxy-4-phenylpiperidin-1-yl)-1,3,5-triazin-2-ylamino)phenyl)acetamide (33).** The title compound was prepared using a method analogous to the preparation of **27**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% trifluoroacetic acid) in trifluoroacetic acid (0.1% aqueous) as the eluent, providing the title compound as a trifluoroacetate salt. Yield: 54 mg, 46%. LC−MS (ESI) *m/z*: 405.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.82 (s, 1H), 9.54 (s, 1H), 8.23 (s, 1H), 8.11 (br s, 1H), 7.50 (dd, *J* = 1.22, 8.36 Hz, 2H), 7.35−7.27 (m, 3H), 7.24−7.14 (m, 2H), 7.14−7.07 (m, 1H), 5.15 (s, 1H), 4.73−4.58 (m, 2H), 3.36−3.26 (m, 2H), 2.01 (s, 3H), 1.96−1.82 (m, 2H), 1.74−1.64 (m, 2H). HRMS calcd for $C_{22}H_{24}N_6O_2$ $(M + H)^+$ 405.2034, found 405.2033.

***N*-(3-(4-(4-Benzoylpiperazin-1-yl)-1,3,5-triazin-2-ylamino)-phenyl)acetamide (34).** The title compound was prepared using a method analogous to the preparation of **27**. The crude residue obtained was purified by silica gel chromatography (0−100% $CH_2Cl_2/CH_3OH/NH_4OH$ (90:10:1) in CH_2Cl_2), providing the title compound as a white solid. Yield: 42 mg, 15%. LC−MS (ESI) *m/z*: 418.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.84 (br s, 1H), 9.65 (s, 1H), 8.30−8.14 (m, 2H), 7.52−7.41 (m, 5H), 7.24 (d, *J* = 8.10 Hz, 1H), 7.17 (t, *J* = 8.02 Hz, 1H), 7.07 (br s, 1H), 3.99−3.76 (m, 4H), 3.74−3,37 (m, 4H), 2.00 (br s, 3H). HRMS calcd for $C_{22}H_{23}N_7O_2$ $(M + H)^+$ 418.1986, found 418.1993.

***N*-(3-(4-(4-Phenoxypiperidin-1-yl)-1,3,5-triazin-2-ylamino)-phenyl)acetamide (35).** The title compound was prepared using a method analogous to the preparation of **27**. Crude solutions were purified with preparative RP-HPLC using CH_3CN (with 0.1% trifluoroacetic acid) in trifluoroacetic acid (0.1% aqueous) as the eluent. Fractions containing the product were combined and washed with saturated $NaHCO_3$. The product was extracted with CH_2Cl_2. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated, providing the title compound as an off-white solid. Yield: 59 mg, 38%. LC−MS (ESI) *m/z*: 405.0 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.85 (s, 1H), 9.59 (s, 1H), 8.23 (s, 1H), 8.10 (br s, 1H), 7.33−7.24 (m, 3H), 7.20−7.08 (m, 2H), 7.03−6.97 (m, 2H), 6.93 (tt, *J* = 1.05, 7.31 Hz, 1H), 4.67 (tt, *J* = 3.83, 7.57 Hz, 1H), 4.27−4.11 (m, 2H), 3.61 (t, *J* = 9.63 Hz, 2H), 2.05−1.93 (m, 5H), 1.70−1.54 (m, 2H). HRMS calcd for $C_{22}H_{24}N_6O_2$ $(M + H)^+$ 405.2034, found 405.2036.

***N*-(3-(4-(4-(Benzyloxy)piperidin-1-yl)-1,3,5-triazin-2-ylamino)-phenyl)acetamide (36).** The title compound was prepared using a method analogous to the preparation of compound **27**. The crude mixture was dried under reduced pressure and purified by silica gel chromatography (0−100% ethyl acetate in hexanes) to provide the title compound as a white solid. Yield: 954 mg, 62%. LC−MS (ESI) *m/z*: 419.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.85 (s, 1H), 9.56 (s, 1H), 8.22 (s, 1H), 8.08 (br s, 1H), 7.37−7.34 (m, 4H), 7.33−7.25 (m, 2H), 7.20−7.10 (m, 2H), 4.56 (s, 2H), 4.24−4.11 (m, 2H), 3.68 (tt, *J* = 3.85, 7.79 Hz, 1H), 3.52−3.42 (m, 2H), 2.03 (s, 3H), 1.96−1.86 (m, 2H), 1.58−1.45 (m, 2H). HRMS calcd for $C_{23}H_{26}N_6O_2$ $(M + H)^+$ 419.219, found 419.2194.

***N*-(3-(4-(4-(Benzyloxy)piperidin-1-yl)-1,3,5-triazin-2-ylamino)-2-methylphenyl)acetamide (37).** The title compound was prepared using a method analogous to the preparation of **21** using precursor **22**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% NH_4OH) in NH_4OH (0.1% aqueous) as the eluent, providing the title compound. Yield: 110 mg, 70.6%. LC−MS (ESI) *m/z*: 433.0 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.96 (s, 1H), 8.11 (s, 1H), 7.37−7.32 (m, 4H), 7.31−7.24 (m, 1H), 7.20−7.08 (m, 3H), 4.53 (s, 2H), 4.19−3.94 (m, 2H), 3.65 (tt, *J* = 3.72, 7.87 Hz, 1H), 3.48−3.32 (m, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 1.91−1.82 (m, 2H), 1.52−1.41 (m, 2H). HRMS calcd for $C_{24}H_{28}N_6O_2$ $(M + H)^+$ 433.2347, found 433.2352.

***N*-(2-Methyl-3-(4-(4-phenylpiperidin-1-yl)-1,3,5-triazin-2-ylamino)phenyl)acetamide (38).** The title compound was prepared using a method analogous to the preparation of **21** using precursor **22**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% NH_4OH) in NH_4OH (0.1% aqueous) as the eluent, providing the title compound. Yield: 17 mg, 17%. LC−MS (ESI) *m/z*: 403.2 (M + 1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.35 (s, 1H), 9.02 (s, 1H), 8.13 (s, 1H), 7.28 (d, *J* = 8.55 Hz, 2H), 7.20−7.14 (m, 2H), 7.13−7.05 (m, 4H), 4.69−4.61 (m, 1H), 4.22−3.95 (m, 2H), 3.59−3.41 (m, 2H), 2.06−2.00 (m, 6H), 2.00−1.91 (m, 2H), 1.63−1.52 (m, 2H). HRMS calcd for $C_{22}H_{26}N_6ONa$ $(M + Na)^+$ 425.206, found 425.2066.

***N*-(2-Methyl-3-(4-(4-phenoxypiperidin-1-yl)-1,3,5-triazin-2-ylamino)phenyl)acetamide (39).** The title compound was prepared using a method analogous to the preparation of **21** using precursor **22**. Crude solutions were purified with preparative RP-HPLC using CH_3CN (with 0.1% trifluoroacetic acid) in trifluoroacetic acid (0.1% aqueous) as the eluent. Fractions containing the product were combined and washed with saturated $NaHCO_3$. The product was extracted with CH_2Cl_2. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and concentrated, providing the title compound. Yield: 53 mg, 35%. LC−MS (ESI) *m/z*: 419.0 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (s, 1H), 9.02 (s, 1H), 8.13 (s, 1H), 7.32−7.23 (m, 2H), 7.21−7.14 (m, 2H), 7.13−7.07 (m, 1H), 6.98 (d, *J* = 7.82 Hz, 2H), 6.93 (t, *J* = 7.29 Hz, 1H), 4.68−4.58 (m, 1H), 4.23−3.95 (m, 2H), 3.63−3.38 (m, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 1.99−1.90 (m, 2H), 1.64−1.52 (m, 2H). HRMS calcd for $C_{23}H_{26}N_6O_2$ $(M + H)^+$ 419.219, found 419.2193.

***N*-(2-Methyl-3-(4-(4-(1-phenylethoxy)piperidin-1-yl)-1,3,5-triazin-2-ylamino)phenyl)acetamide (40).** To a solution of *tert*-butyl 4-hydroxy-1-piperidinecarboxylate (369 μL, 2.5 mmol) in *N*,*N*-dimethylformamide (8.3 mL, 2484 μmol) cooled in an ice−water bath was added sodium hydride (60% in mineral oil) (100 mg, 4.2 mmol). The bath was removed and the suspension stirred at room temperature. The suspension was stirred for 1 h at room temperature prior to the addition of (1-bromoethyl)benzene (460 μL, 2.5 mmol) via syringe. The resulting solution was stirred for 1 h at room temperature and heated at 90 °C for 16 h. The mixture was cooled to room temperature, diluted with water, and extracted with ethyl acetate. The organic layer was dried with Na_2SO_4, filtered, and dried under reduced pressure, providing crude *tert*-butyl 4-(1-phenylethoxy)piperidine-1-carboxylate. LC−MS (ESI) *m/z*: 398.2 (M + 23).

The crude material was dissolved in CH_2Cl_2 (10 mL), and TFA was added (1 mL). The mixture was stirred at room temperature for 18 h. The mixture was dried under reduced pressure and purified using catch

and release with a 5 g SCX-2 column, washing first with CH_3OH, then with 2 M NH_3 in CH_3OH to elute basic materials. The light brown oil isolated contained small quantities of product, 4-(1-phenylethoxy)-piperidine, along with multiple impurities. It was used in the subsequent step without further purification. LC−MS (ESI) m/z: 206.2 (M + 1).

To a solution of 4-(1-phenylethoxy)piperidine (240 mg, 1169 μmol) in *N*,*N*-dimethylformamide (3.9 mL, 1169 μmol) was added *N*-(3-(4-chloro-1,3,5-triazin-2-ylamino)-2-methylphenyl)acetamide (325 mg, 1169 μmol) followed by *N*,*N*-diisopropylethylamine (204 μL, 1169 μmol). The resulting solution was shaken at room temperature for 3 h. The mixture was dried under reduced pressure and purified by silica gel chromatography (0−100% $CH_2Cl_2/CH_3OH/NH_4OH$ (90:10:1) in CH_2Cl_2), providing product along with 25% impurity. The material was repurified with preparative RP-HPLC using CH_3CN (with 0.1% trifluoroacetic acid) in trifluoroacetic acid (0.1% aqueous) as the eluent. Fractions containing the product were combined and washed with saturated $NaHCO_3$. The product was extracted with ethyl acetate (2×). The organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated, providing the title compound. The oil obtained was lyophilized from CH_3OH/H_2O to provide the title compound (1.2 mg, 0.2%) as a white powder. LC−MS (ESI) m/z: 447.2 (M + 23). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.96 (s, 1H), 8.09 (s, 1H), 7.38−7.31 (m, 4H), 7.29−7.22 (m, 1H), 7.20−7.06 (m, 3H), 4.66 (q, J = 6.42 Hz, 1H), 4.22−3.86 (m, 2H), 3.52−3.38 (m, 1H), 3.26 (d, J = 5.97 Hz, 2H), 2.47−2.43 (m, 1H), 2.04 (s, 3H), 2.02 (s, 3H), 1.92−1.81 (m, 1H), 1.72−1.60 (m, 1H), 1.52−1.37 (m, 1H), 1.32 (s, 3H).

***N*-(3-(4-(4-(2-Fluorobenzyloxy)piperidin-1-yl)-1,3,5-triazin-2-ylamino)-2-methylphenyl)acetamide (41).** The title compound was prepared using a method analogous to the preparation of **52**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% NH_4OH) in NH_4OH (0.1% aqueous) as the eluent, providing the title compound. Yield: 66 mg, 52%. LC−MS (ESI) m/z: 451.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.96 (s, 1H), 8.11 (s, 1H), 7.47 (dt, J = 1.76, 7.63 Hz, 1H), 7.42−7.32 (m, 1H), 7.22−7.05 (m, 5H), 4.61−4.53 (m, 2H), 4.18−3.92 (m, 2H), 3.72−3.60 (m, 1H), 3.48−3.31 (m, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 1.93−1.81 (m, 2H), 1.53−1.39 (m, 2H). HRMS calcd for $C_{24}H_{27}FN_6O_2$ $(M + H)^+$ 451.2252, found 451.2255.

***N*-(3-(4-(4-(3-Fluorobenzyloxy)piperidin-1-yl)-1,3,5-triazin-2-ylamino)-2-methylphenyl)acetamide (42).** The title compound was prepared using a method analogous to the preparation of **52**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% NH_4OH) in NH_4OH (0.1% aqueous) as the eluent, providing the title compound. Yield: 90 mg, 69%. LC−MS (ESI) m/z: 451.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.96 (s, 1H), 8.11 (s, 1H), 7.42−7.35 (m, 1H), 7.22−7.13 (m, 4H), 7.13−7.04 (m, 2H), 4.56 (s, 2H), 3.93−4.19 (m, 2H), 3.70−3.60 (m, 1H), 3.52−3.32 (m, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 1.92−1.82 (m, 2H), 1.55−1.38 (m, 2H). HRMS calcd for $C_{24}H_{27}FN_6O_2$ $(M + H)^+$ 451.2252, found 451.2253.

***N*-(3-(4-(4-(4-Fluorobenzyloxy)piperidin-1-yl)-1,3,5-triazin-2-ylamino)-2-methylphenyl)acetamide (43).** The title compound was prepared using a method analogous to the preparation of **52**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% NH_4OH) in NH_4OH (0.1% aqueous) as the eluent, providing the title compound. Yield: 75 mg, 58%. LC−MS (ESI) m/z: 451.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.96 (s, 1H), 8.11 (s, 1H), 7.43−7.32 (m, 2H), 7.22−7.04 (m, 5H), 4.52 (s, 2H), 4.19−3.89 (m, 2H), 3.69−3.58 (m, 1H), 3.48−3.32 (m, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 1.91−1.78 (m, 2H), 1.53−1.34 (m, 2H). HRMS calcd for $C_{24}H_{27}FN_6O_2$ $(M + H)^+$ 451.2252, found 451.2254.

***N*-(2-Methyl-3-(4-(4-(2-(trifluoromethyl)benzyloxy)piperidin-1-yl)-1,3,5-triazin-2-ylamino)phenyl)acetamide (44).** The title compound was prepared using a method analogous to the preparation of **52**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% NH_4OH) in NH_4OH (0.1% aqueous) as the eluent, providing the title compound. Yield: 20 mg, 10%. LC−MS (ESI) m/z: 501.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.97 (s, 1H), 8.12 (s, 1H), 7.77−7.64 (m, 3H), 7.55−7.47 (m, 1H), 7.21−7.04 (m, 3H), 4.70 (s, 2H), 4.21−3.92 (m, 2H), 3.71 (tt, J = 3.81, 7.73 Hz, 1H), 3.47−3.32 (m, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 1.94−1.83 (m, 2H), 1.54−1.42 (m, 2H). HRMS calcd for $C_{25}H_{27}F_3N_6O_2$ $(M + H)^+$ 501.222, found 501.2226.

***N*-(2-Methyl-3-(4-(4-(3-(trifluoromethyl)benzyloxy)piperidin-1-yl)-1,3,5-triazin-2-ylamino)phenyl)acetamide (45).** The title compound was prepared using a method analogous to the preparation of **52**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% NH_4OH) in NH_4OH (0.1% aqueous) as the eluent, providing the title compound. Yield: 47 mg, 23%. Purity: 94%. LC−MS (ESI) m/z: 501.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (s, 1H), 8.98 (s, 1H), 8.12 (s, 1H), 7.72−7.50 (m, 4H), 7.22−7.03 (m, 3H), 4.64 (s, 2H), 4.22−3.91 (m, 2H), 3.72−3.64 (m, 1H), 3.48−3.34 (m, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 1.95−1.82 (m, 2H), 1.56−1.37 (m, 2H). HRMS calcd for $C_{25}H_{27}F_3N_6O_2$ $(M + H)^+$ 501.222, found 501.2224.

***N*-(2-Methyl-3-(4-(4-(4-(trifluoromethyl)benzyloxy)piperidin-1-yl)-1,3,5-triazin-2-ylamino)phenyl)acetamide (46).** The title compound was prepared using a method analogous to the preparation of **52**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% NH_4OH) in NH_4OH (0.1% aqueous) as the eluent, providing the title compound. Yield: 634 mg, 62%. LC−MS (ESI) m/z: 501.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.37 (s, 1H), 9.01 (s, 1H), 8.12 (s, 1H), 7.71 (d, J = 8.02 Hz, 2H), 7.57 (d, J = 7.92 Hz, 2H), 7.20−7.06 (m, 3H), 4.65 (s, 2H), 4.22−3.92 (m, 2H), 3.72−3.59 (m, 1H), 3.52−3.34 (m, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 1.96−1.79 (m, 2H), 1.56−1.39 (m, 2H). HRMS calcd for $C_{25}H_{27}F_3N_6O_2$ $(M + H)^+$ 501.222, found 501.2221.

***N*-(2-Methyl-3-(4-(4-(4-methylbenzyloxy)piperidin-1-yl)-1,3,5-triazin-2-ylamino)phenyl)acetamide (47).** The title compound was prepared using a method analogous to the preparation of **52**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% NH_4OH) in NH_4OH (0.1% aqueous) as the eluent, providing the title compound. Yield: 45 mg, 19%. LC−MS (ESI) m/z: 447.2 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (s, 1H), 8.97 (s, 1H), 8.11 (s, 1H), 7.26−7.20 (m, 2H), 7.19−7.06 (m, 5H), 4.48 (s, 2H), 4.17−3.92 (m, 2H), 3.66−3.56 (m, 1H), 3.34 (br s, 2H), 2.32−2.25 (m, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.93−1.78 (m, 2H), 1.54−1.36 (m, 2H). HRMS calcd for $C_{25}H_{30}N_6O_2$ $(M + H)^+$ 447.2503, found 447.2504.

***N*-(3-(4-(4-(4-Isopropylbenzyloxy)piperidin-1-yl)-1,3,5-triazin-2-ylamino)-2-methylphenyl)acetamide (48).** The title compound was prepared using a method analogous to the preparation of **52**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% NH_4OH) in NH_4OH (0.1% aqueous) as the eluent, providing the title compound. Yield: 69 mg, 27%. LC−MS (ESI) m/z: 475.3 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (s, 1H), 8.97 (s, 1H), 8.11 (s, 1H), 7.29−7.07 (m, 7H), 4.52 (s, 2H), 4.17−3.88 (m, 2H), 3.67−3.58 (m, 1H), 3.47−3.34 (m, 2H), 2.87 (spt, J = 6.90 Hz, 1H), 2.04 (s, 3H), 2.03 (s, 3H), 1.91−1.80 (m, 2H), 1.50−1.40 (m, 2H), 1.19 (d, J = 6.94 Hz, 6H). HRMS calcd for $C_{27}H_{34}N_6O_2$ $(M + H)^+$ 475.2816, found 475.2817.

***N*-(3-(4-(4-(4-*tert*-Butylbenzyloxy)piperidin-1-yl)-1,3,5-triazin-2-ylamino)-2-methylphenyl)acetamide (49).** The title compound was prepared using a method analogous to the preparation of **52**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% NH_4OH) in NH_4OH (0.1% aqueous) as the eluent, providing the title compound. Yield: 109 mg, 56%. LC−MS (ESI) m/z: 489.4 (M + 1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.35 (s,

1H), 8.97 (s, 1H), 8.11 (s, 1H), 7.38−7.33 (m, 2H), 7.31−7.22 (m, J = 8.30 Hz, 2H), 7.21−7.05 (m, 3H), 4.52 (s, 2H), 4.17−3.93 (m, 2H), 3.63 (tt, J = 3.79, 7.75 Hz, 1H), 3.47−3.33 (m, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 1.90−1.81 (m, 2H), 1.52−1.40 (m, 2H), 1.29 (s, 9H). HRMS calcd for $C_{28}H_{36}N_6O_2$ $(M + H)^+$ 489.2973, found 489.2973.

4-((1-(4-(3-Acetamido-2-methylphenylamino)-1,3,5-triazin-2-yl)piperidin-4-yloxy)methyl)benzamide (50). The title compound was prepared using a method analogous to the preparation of **52**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% NH_4OH) in NH_4OH (0.1% aqueous) as the eluent, providing the title compound. Yield: 1.2 mg, 0.5%. LC−MS (ESI) m/z: 475.3 (M^+). ^1H NMR (400 MHz, DMSO-d_6) δ 9.33 (s, 1H), 8.95 (s, 1H), 8.11 (s, 1H), 7.90 (br s, 1H), 7.85 (d, J = 8.22 Hz, 2H), 7.40 (d, J = 8.31 Hz, 2H), 7.32−7.25 (m, 1H), 7.20−7.14 (m, 2H), 7.14−7.08 (m, 1H), 4.59 (s, 2H), 4.19−3.94 (m, 2H), 3.69−3.61 (m, 1H), 3.44−3.34 (m, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 1.93−1.82 (m, 2H), 1.54−1.41 (m, 2H). HRMS calcd for $C_{25}H_{29}N_7O_3$ $(M + H)^+$ 476.2405, found 476.2406.

***N*-(3-(4-(4-(4-Methoxybenzyloxy)piperidin-1-yl)-1,3,5-triazin-2-ylamino)-2-methylphenyl)acetamide (51).** To a solution of *tert*-butyl 4-hydroxypiperidine-1-carboxylate (1.00 g, 4.97 mmol) in *N*,*N*-dimethylformamide (19.9 mL) cooled in an ice−water bath was added NaH (0.219 g, 5.47 mmol). The bath was removed and the mixture stirred for 2 h at room temp, providing a cloudy yellow suspension. The resulting yellow suspension was cooled in an ice−water bath, and 1-(chloromethyl)-4-methoxybenzene (1.01 mL, 7.45 mmol) was added over 15 min. The mixture was allowed to stir for 4 h while slowly warming to room temperature. To the turbid yellow solution was carefully added water, and the resulting mixture was extracted with ethyl acetate (2×). The combined organic washes were dried with Na_2SO_4, filtered, and dried under reduced pressure. The crude yellow oil obtained was purified by silica gel chromatography (0−100% ethyl acetate in hexanes), providing *tert*-butyl 4-(4-methoxybenzyloxy)piperidine-1-carboxylate (935 mg, 2.91 mmol, 58.5% yield) as a colorless oil. LC−MS (ESI) m/z: 344.4 (M + 23).

To a solution of *tert*-butyl 4-(4-methoxybenzyloxy)piperidine-1-carboxylate (300 mg, 0.933 mmol) in CH_3OH (1.9 mL) was added 2 N NaOH (1.9 mL) in a 20 mL microwave vial. The vessel was sealed and irradiated for 40 min at 165 °C. The resulting mixture was diluted with water and extracted with ethyl acetate (2×). The combined organics were dried with Na_2SO_4, filtered, and dried under reduced pressure. The crude oil was filtered through a 5 g SCX-2 column, washing first with MeOH, then with 2 M NH_3 in MeOH, providing 4-(4-methoxybenzyloxy)piperidine (180 mg, 0.813 mmol, 87% yield) as a yellow oil. LC−MS (ESI) m/z: 222.2 (M + 1).

To a flask charged with *N*-(3-(4-chloro-1,3,5-triazin-2-ylamino)-2-methylphenyl)acetamide (226 mg, 0.813 mmol) was added *N*,*N*-dimethylformamide (3.25 mL) followed by *N*,*N*-diisopropylethylamine (156 μL, 0.895 mmol) and 4-(4-methoxybenzyloxy)piperidine (180 mg, 0.813 mmol). The mixture was stirred for 16 h at room temperature. The mixture was dried under reduced pressure and the residue triturated with CH_2Cl_2, providing a yellow solid which was collected by vacuum filtration and washed with excess CH_2Cl_2. The filtrate was dried under reduced pressure and the oil obtained and purified by silica gel chromatography (0−100% CH_2Cl_2/CH_3OH/NH_4OH (90:10:1) in CH_2Cl_2), leading to the isolation mixture of two compounds primarily by TLC, confirmed by LC−MS. The solid obtained (∼65 mg) was repurified with preparative RP-HPLC using CH_3CN (with 0.1% trifluoroacetic acid) in trifluoroacetic acid (0.1% aqueous) as the eluent. A small portion of the material was obtained because of instrument error. The material obtained was free-based using a 2 g SCX-2 column, washing first with CH_3OH, then with 2 M NH_3 in CH_3OH. The basic wash was collected, dried under reduced pressure, and the oil obtained was lyophilized from CH_3OH/water to provide the title compound (5 mg, 1.3% yield) as a white powder. LC−MS (ESI) m/z: 463.2 (M + 1). ^1H NMR (400 MHz, DMSO-d_6) δ 9.34 (s, 1H), 8.96 (s, 1H), 8.11 (s, 1H), 7.29−7.23 (m, 2H), 7.20−7.14 (m, 2H), 7.13−7.07 (m, 1H), 6.93−6.86 (m, 2H), 4.45 (s, 2H), 4.18−3.92 (m, 2H), 3.74 (s, 3H), 3.66−3.56 (m, 1H), 3.44−3.34 (m, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 1.89−1.79 (m, 2H), 1.52−1.34 (m, 2H). HRMS calcd for $C_{25}H_{30}N_6O_3$ $(M + H)^+$ 463.2452, found 463.2457.

***N*-(2-Methyl-3-(4-(4-(4-(trifluoromethoxy)benzyloxy)-piperidin-1-yl)-1,3,5-triazin-2-ylamino)phenyl)acetamide (52).** To a dried flask charged with *tert*-butyl 4-hydroxypiperidine-1-carboxylate (3.60 g, 17.9 mmol) was added *N*,*N*-dimethylformamide (40 mL), and the resulting solution was cooled in an ice−water bath prior to the addition of sodium hydride (60%) (748 mg, 18.9 mmol) in two portions 5 min apart. The mixture was stirred for 1 h and allowed to warm to room temperature over that time. The resulting suspension was cooled in an ice−water bath prior to the addition of a solution of 1-(bromomethyl)-4-(trifluoromethoxy)benzene (5.018 g, 19.7 mmol) in DMF (5 mL) via syringe. The resulting mixture was allowed to slowly warm to room temperature while stirring for 15 h. The resulting pale yellow suspension was carefully diluted with water (∼150 mL) and extracted with ethyl acetate (2×). The combined organics were dried with Na_2SO_4, filtered, and dried under reduced pressure. The material was purified by silica gel chromatography (0−50% ethyl acetate in hexanes), providing *tert*-butyl 4-(4-(trifluoromethoxy)benzyloxy)-piperidine-1-carboxylate a white crystalline solid upon drying (5 g, 13.3 mmol). LC−MS (ESI) m/z: 398.2 (M + 23).

tert-Butyl 4-(4-(trifluoromethoxy)benzyloxy)piperidine-1-carboxylate (5 g, 13.3 mmol) was dissolved in CH_2Cl_2 (40 mL), and trifluoroacetic acid (4 mL) was added. The light yellow solution was stirred at room temperature for 20 h. The solution was dried under reduced pressure, providing 4-(4-(trifluoromethoxy)benzyloxy)piperidine trifluoroacetate as a pale yellow oil (5.89 g, 85% (two steps)). LC−MS (ESI) m/z: 276.2 (M + 1).

A solution of 4-(4-(trifluoromethoxy)benzyloxy)piperidine trifluoroacetate (1.90 g, 4.9 mmol) in *N*,*N*-dimethylformamide (12.0 mL, 3.60 mmol) was cooled in an ice−water bath prior to the addition of *N*,*N*-diisopropylethylamine (2.08 mL, 12.0 mmol) and *N*-(3-(4-chloro-1,3,5-triazin-2-ylamino)-2-methylphenyl)acetamide (1.00 g, 3.60 mmol), respectively. The mixture was allowed to warm slowly to room temperature and was stirred for 4 h. The material was dried under reduced pressure and the resulting residue triturated with CH_2Cl_2/MeOH (∼1:1), providing a pale yellow solid which contained a minor impurity. The material was dissolved in 20 mL of 1:1 MeOH/DCM and sonicated for approximately 1 min. Purification was performed on a Prep SFC-2 using the following conditions: 25:75:0.2 CH_3OH/CO_2/diethylamine on a 20 mm × 250 mm, 5 μm ChiralPak AD-H column at 80 mL/min and 100 bar system pressure. Thirty injections were automated based on the 0.75 mL pilot injection, providing the title compound (1.45 g, 78.0% yield) as a white solid. LC−MS (ESI) m/z: 517.2 (M + 1). ^1H NMR (400 MHz, DMSO-d_6) δ 9.37 (s, 1H), 9.01 (s, 1H), 8.12 (s, 1H), 7.47 (d, J = 8.80 Hz, 2H), 7.33 (d, J = 7.73 Hz, 2H), 7.21−7.14 (m, 2H), 7.12−7.06 (m, 1H), 4.57 (s, 2H), 4.19−3.91 (m, 2H), 3.72−3.60 (m, 1H), 3.48−3.36 (m, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 1.95−1.80 (m, 2H), 1.55−1.36 (m, 2H). HRMS calcd for $C_{25}H_{27}F_3N_6O_3$ $(M + H)^+$ 517.2169, found 517.2182. See Supporting Information for peak assignments and proton and carbon spectra.

***N*-(2-Methyl-3-(4-(4-(4-(trifluoromethyl)phenoxy)piperidin-1-yl)-1,3,5-triazin-2-ylamino)phenyl)acetamide (53).** The title compound was prepared using a method analogous to the preparation of **21** using precursor **22**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% NH_4OH) in NH_4OH (0.1% aqueous) as the eluent, providing the title compound. Yield: 77 mg, 32%. LC−MS (ESI) m/z: 487.2 (M + 1). ^1H NMR (400 MHz, DMSO-d_6) δ 9.35 (s, 1H), 9.03 (s, 1H), 8.13 (s, 1H), 7.64 (d, J = 8.61 Hz, 2H), 7.23−7.14 (m, 4H), 7.13−7.07 (m, 1H), 4.82−4.74 (m, 1H),

4.23−3.95 (m, 2H), 3.63−3.41 (m, 2H), 2.04 (s, 3H), 2.03 (s, 3H), 2.01−1.93 (m, 2H), 1.65−1.54 (m, 2H). HRMS calcd for $C_{24}H_{25}F_3N_6O_2$ $(M + H)^+$ 487.2064, found 487.2064.

***N*-(2-Methyl-3-(4-(4-(4-(trifluoromethoxy)phenoxy)piperidin-1-yl)-1,3,5-triazin-2-ylamino)phenyl)acetamide (54).** The title compound was prepared using a method analogous to the preparation of **21** using precursor **22**. Crude solutions were purified with preparative mass triggered HPLC using CH_3CN (with 0.1% NH_4OH) in NH_4OH (0.1% aqueous) as the eluent, providing the title compound. Yield: 58 mg, 64%. LC−MS (ESI) m/z: 503.2 $(M + 1)$. ^1H NMR (400 MHz, DMSO-d_6) δ 9.36 (s, 1H), 9.03 (s, 1H), 8.14 (s, 1H), 7.28 (dd, J = 0.68, 9.10 Hz, 2H), 7.21−7.14 (m, 2H), 7.14−7.05 (m, 3H), 4.65 (tt, J = 3.61, 7.64 Hz, 1H), 4.20−4.00 (m, 2H), 3.61−3.41 (m, 2H), 2.04 (s, 6H), 2.00−1.90 (m, 2H), 1.63−1.52 (m, 2H). HRMS calcd for $C_{24}H_{25}F_3N_6O_3$ $(M + H)^+$ 503.2013, found 503.2015.

***N*-(2-Methyl-3-(6-(4-(4-(trifluoromethoxy)benzyloxy)-piperidin-1-yl)pyrimidin-4-ylamino)phenyl)acetamide (55).** To 4,6-dichloropyrimidine (1523 mg, 1.02 mmol) in 2-propanol (3 mL) at ambient temperature was added *N*,*N*-diisopropylethylamine (0.266 mL, 1.53 mmol) followed by 4-(4-(trifluoromethoxy)benzyloxy)-piperidine (280 mg, 1.02 mmol). The resulting mixture was stirred at room temperature for 3.5 h. The reaction mixture was concentrated under reduced pressure and purified by silica gel chromatography (0−40% ethyl acetate in hexanes), providing 4-chloro-6-(4-(4-(trifluoromethoxy)-benzyloxy)piperidin-1-yl)pyrimidine (275 mg, 70%). LC−MS (ESI) m/z: 388.2 $(M + 1)$.

To a vial charged with 4-chloro-6-(4-(4-(trifluoromethoxy)benzyloxy)-piperidin-1-yl)pyrimidine (105.6 mg, 0.272 mmol) and *N*-(3-amino-2-methylphenyl)acetamide (52.2 mg, 0.300 mmol) were added 2-propanol (2.5 mL) and trifluoroacetic acid (0.063 mL, 0.817 mmol). The resulting mixture was heated at 73 °C for 3 days, then at 90 °C for 16 days. The reaction mixture was cooled to room temperature, dried under reduced pressure, and purified with silica gel chromatography (0−80% $CH_2Cl_2/CH_3OH/NH_4OH$ (90:10:1) in CH_2Cl_2), providing the title compound as a white solid. Yield: 22.7 mg, 16%. LC−MS (ESI) m/z: 516.2 $(M + 1)$. ^1H NMR (400 MHz, DMSO-d_6) δ 9.38 (s, 1H), 8.48 (br s, 1H), 8.07 (s, 1H), 7.47 (d, J = 8.80 Hz, 2H), 7.33 (d, J = 7.92 Hz, 2H), 7.22−7.09 (m, 3H), 5.67 (s, 1H), 4.57 (s, 2H), 3.89−3.76 (m, 2H), 3.65 (tt, J = 4.08, 8.00 Hz, 1H), 3.24−3.13 (m, 2H), 2.05 (s, 3H), 2.03 (s, 3H), 1.93−1.83 (m, 2H), 1.54−1.42 (m, 2H). HRMS calcd for $C_{26}H_{28}F_3N_5O_3$ $(M + H)^+$ 516.2217, found 516.222.

^{3}H-36. To a flask charged with 2,4-dichloro-1,3,5-triazine (5.0 g, 32 mmol) was added *N*,*N*-dimethylformamide (40 mL), and the mixture was cooled to 0 °C. To this solution were added *N*,*N*-diisopropylethylamine (12 mL, 70 mmol) and 4-(benzyloxy)piperidine hydrochloride (7.2 g, 32 mmol). The resulting mix was allowed to slowly warm for over 4 h. To the reaction mixture was added excess water, providing a yellow precipitate which was collected via vacuum filtration and washed with excess water followed by methanol, providing 2-(4-(benzyloxy)piperidin-1-yl)-4-chloro-1,3,5-triazine (7.25 g, 75%). LC−MS (ESI) m/z: 305.1 $(M + 1)$.

To a mixture of 2-(4-(benzyloxy)piperidin-1-yl)-4-chloro-1,3,5-triazine (4.72 g, 15.5 mmol) and benzene-1,3-diamine (2.09 g, 19.3 mmol) in 2-propanol (50 mL) was added *N*,*N*-diisopropylethylamine (5.39 mL, 31 mmol). The resulting mixture was heated to 35 °C for 29 h leading to the formation of a precipitate which was collected via vacuum filtration and washed with excess 2-propanol, providing N^1-(4-(4-(benzyloxy)-piperidin-1-yl)-1,3,5-triazin-2-yl)benzene-1,3-diamine (4.12 g, 71% yield) as light brown solid upon drying under high vacuum.

Radiolabel Incorporation Step. A solution of ^{3}T-acetic acid (CT_3COOT, 100 μmol) in *N*,*N*-dimethylformamide (100 μL) was treated with 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) (3.8 mg, 100 μmol). The resulting mixture was then added to a solution of N^1-(4-(4-(benzyloxy)piperidin-1-yl)-1,3,5-triazin-2-yl)benzene-1,3-diamine (4 mg, 10.6 mmol) and *N*,*N*-diisopropylethylamine (17 μL, 0.13 mmol) in *N*,*N*-dimethylformamide (0.5 mL). The resulting mixture was stirred at room temperature for 30 min, then dried under reduced pressure. The crude material was purified with reverse phase HPLC (C18, mobile phase = 40% CH_3CN, UV detection = 241 nm, flow = 6 mL/min), providing product with specific activity of 54.4 Ci/mmol, 99% purity.

■ ASSOCIATED CONTENT

Supporting Information. Additional diagrams and tables illustrating details of state dependence, formalin, open field activity, radiolabeling studies, and NMR peak assignments for **52**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

Nav1.7, voltage-gated sodium channel type 7; TTX, tetrodotoxin; SCN9A, sodium channel, voltage-gated, type IX, α subunit; SAR, structure−activity relationship; DIEA, *N*,*N*-diisopropylethylamine; TFA, trifluoroacetic acid; DMF, *N*,*N*-dimethylformamide; Boc, *tert*-butoxycarbonyl; SCX-2, strong cation exchange 2 (functionalized with propylsulfonic acid); hERG, human ether-a-go-go-related gene; PK, pharmacokinetic; Cl_{int}, in vitro intrinsic clearance; RLM, rat liver microsome; HLM, human liver microsome; *F*, oral bioavailability, PKDM, pharmacokinetics and drug metabolism; K_R, affinity for resting channels; KI, affinity for inactivated channel; DRG, dorsal root ganglion; K_V1.7, voltage-gated potassium channel type 7; TRPM8, transient receptor potential cation channel subfamily M, member 8; TRPV3, transient receptor potential cation channel, subfamily V, member 3; TRPV4, transient receptor potential cation channel, subfamily V, member 4; P_2X_7, purinergic receptor P2X, ligand-gated ion channel 7; GPCR, G-protein-coupled receptor; PX, PatchXpress; BTX, batrachotoxin

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