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Discovery of potent and use-dependent sodium channel blockers for treatment of chronic pain

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Abstract—A new series of voltage-gated sodium channel blockers with potential for treatment of chronic pain is reported. Systematic structure–activity relationship studies, starting with compound **1**, led to identification of potent analogs that displayed use-dependent block of sodium channels, were efficacious in pain models in vivo, and most importantly, were devoid of activity against the cardiac potassium channel hERG.

1. Introduction

It is widely recognized that chronic pain encompasses a number of heterogeneous symptoms, which can generally be characterized as inflammatory or neuropathic, the latter representing a category for which few therapies currently exist. Injuries or diseases of the peripheral nervous system can lead to neuropathic pain.[1] Experimentally and clinically, such pain states are associated with hyper-excitability and spontaneous action potential firing in peripheral sensory neurons.

Voltage-gated sodium channels (Na_v1) underlie the initiation and propagation of action potentials in peripheral neurons, and are therefore appealing molecular targets for treating chronic pain.[2] Proof of concept for Na_v1 channels in the clinic has been suggested from the use of blockers such as carbamazepine, lamotrigine, and lidocaine.[3] A shared feature among these treatments for chronic pain is the ability to block sodium channels, and, at therapeutic concentrations, sodium channel block is the only known effect of lidocaine. Several subtypes of sodium channels have been identified, including $Na_v1.7$ (PN1)[4] and $Na_v1.8$ (PN3),[5] which are predominantly expressed in the peripheral nervous system and dorsal root ganglion (DRG),[6] and have been implicated in pain transmission pathways.[7] Sodium channel blockers used to treat chronic pain do not discriminate between Na_v1 subtypes and derive their therapeutic index from their use-dependent properties. The objective of this study was to identify potent, use-dependent, and orally bioavailable sodium channel blockers with good therapeutic windows, that could be developed for treatment of chronic pain in patients.

2. Results and discussion

Compound **1** (Fig. 1) was first disclosed by scientists at Ciba-Geigy as a highly potent blocker of cardiac sodium channels.[8] This compound was prepared and found to be active against $Na_v1.7$ in a functional, Voltage/Ion Probe Reader (VIPR) assay that measures veratridine-induced depolarization in HEK-293 cells stably transfected with $Na_v1.7$ channels[9] (IC_{50} 4.0 μM). Compound **1** was also tested in a rat formalin paw model[10] as previously described.[9b] In this model, injection of formalin into the hind paw induces two phases of spontaneous pain behavior. Compound **1** reduced phase II (10–60 min) formalin-induced flinching in a dose-dependent manner when injected locally into

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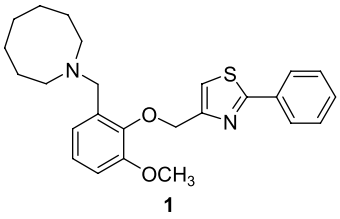

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Figure 1. Structure of compound **1**.

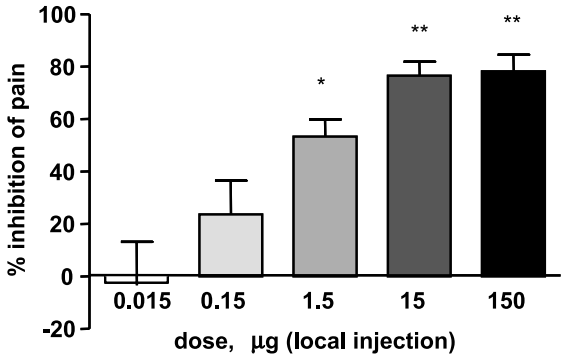


Figure 2. In vivo activity of **1** in a rat model of pain.

the paw (Fig. 2); however it was inactive when dosed intravenously at 3 mg/kg (13% inhibition). Further evaluation of the compound indicated a number of off-target activities. Of these, the effect on the cardiac potassium channel hERG[11] (determined as displacement of ^{35}S-MK-499 binding to hERG channels stably expressed in HEK293 cells, IC_{50} 0.18 µM) was considered the greatest liability.

Compound **1** was administered directly into a single hind paw prior to formalin injection into the same site. Behavioral assessment consisted of counting the number of formalin-evoked hind paw flinches for 1 h post-injection. Data are presented as mean percent inhibition from 10–60 min post-formalin relative to vehicle control (*$p < 0.05$, **$p < 0.01$; ANOVA with Bonferroni's Multiple Comparison Test compared to vehicle treatment; $n = 4$–10 rats/group).

With compound **1** as a lead, an effort was directed to develop potent, use-dependent $Na_v1.7$ sodium channel blockers, with minimal hERG activity. Herein is reported the synthesis as well as in vitro and in vivo activities of novel voltage-gated sodium channel blockers.

3. Removal of the basic amino group reduced hERG activity

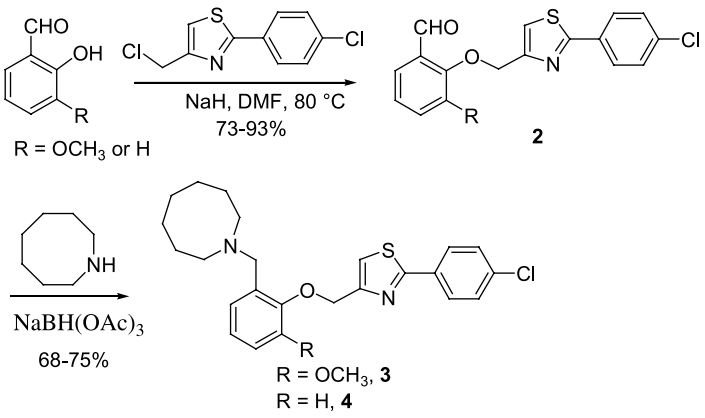
A convergent two-step synthesis was designed that afforded the *p*-chloro analogs of **1** (Scheme 1), taking advantage of the commercially available *p*-chlorophenylthiazole methylchloride. The C3-desmethoxy analog **4** was also synthesized in a similar manner for comparison. Both compounds had in vitro activities similar to **1** (Table 1), suggesting that the *p*-chloro substitution was well tolerated. Not unexpectedly, significant hERG (MK-499) activity was present in both compounds.

It was suspected that the potent hERG activity of **1** (IC_{50} 0.18 µM) could be attributed to its basic tertiary amino group.[11] This hypothesis was verified in a series of cyclic amides that rendered the amine non-basic. The amide analogs were prepared by oxidation of the aldehyde **2** to the carboxylic acid, followed by amide formation with various cyclic amines. Indeed, hERG activities were diminished in these amide analogs (Table 2), especially as the ring size of cyclic amide decreased. The affinities of these amides for sodium channels were largely retained, especially for the pyrrolidine analogs **11** and **12**.

Both **11** and **12** were studied in detail in vitro and in vivo in a rat formalin paw model (Table 3). Whole cell electrophysiology[9b] was used to evaluate the state dependence of block, and results suggested that both analogs blocked the inactivated state much more

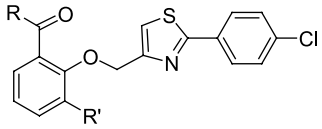
Table 1. VIPR and MK-499 activities

	1	3	4
VIPR ($NA_v1.7$, IC_{50}, µM)	4.0	3.8	2.9
MK-499 (IC_{50}, µM)	0.18	0.04	0.03



Scheme 1.

Table 2. VIPR and hERG activities of cyclic amides



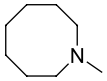
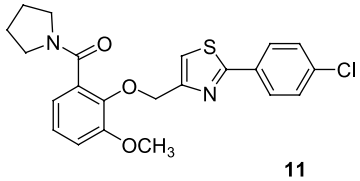
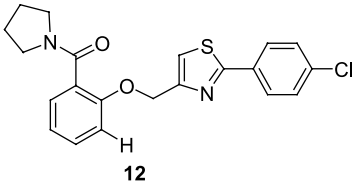
Compound	R	R′	VIPR $Na_v1.7$ (IC_{50}, μM)	MK-499 (inh. at 10 μM) (%)
5		OCH_3	19.4	88
6		H	29	90
7		OCH_3	6.5	85
8		H	40	90
9		OCH_3	3.7	63
10		H	7.4	89
11		OCH_3	1.3	50
12		H	1.0	63

Table 3. In vitro and in vivo profiles of analogs **11** and **12**



11



12

	11	**12**
VIPR, $Na_v1.7$, IC_{50} (μM):	1.3	1.0
$Na_v1.7$, ephys. K_R/K_I (μM):	10/0.14	6.7/0.16
MK-499, IC_{50} (μM):	7.7	9.8
Cl_p (mL/min/kg):	101	627
$t_{1/2}$ (h):	0.48	0.44
F%	9.4	17
Formalin paw response (10–40 min, 3 mg/kg, iv):	68%	65%

potently than the resting state (Table 3). Unlike **1** which was ineffective during the first 45 min post-dose when given intravenously (19% inhibition), **11** and **12** were compounds equally efficacious in the rat formalin paw model (68% and 65%, respectively; $p < 0.01$, unpaired *t*-test vs vehicle). Duration of action was brief (<60 min), but consistent with rat pharmacokinetic data for both compounds showing high clearance rates and short half-lives.

4. Exploring the C2-*O*-alkyl side chain

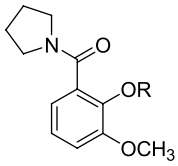
Replacement of the *p*-chlorophenylthiazole with other side chains was studied (Table 4). In most cases, analogs were synthesized via *O*-alkylation of the C2-phenol with commercially available electrophiles. It is noteworthy that compounds with biaryl groups tend to be more active than those with only one phenyl ring. The bithiophene analog **19** was slightly more active than the compounds with a phenyl thiazole side chain ($Na_v1.7$: IC_{50} 0.55 vs 1.3 μM for **11**). However, this compound was not examined in animal models due to concerns regarding the well-documented metabolism issues of thiophenes in vivo.[12]

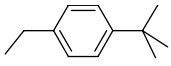
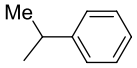
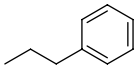
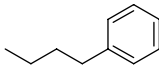
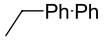
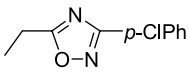
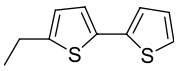
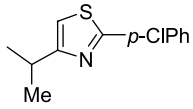
A methyl group was introduced at the benzylic position, in an attempt to reduce the potential *O*-dealkylation pathway. The in vitro activity of **20** was comparable to that of **11** ($Na_v1.7$: IC_{50} 2.2 vs 1.3 μM); however, a moderate improvement in the rat PK parameters was noted (**20**: Cl_p 73 mL/min/kg; $t_{1/2}$ 0.8 h).

5. Probing the C3-Substituent on the phenyl ring

The role of the C3-substituent on the phenyl ring was investigated (Table 5). A series of compounds with different substituents at the C3-position were prepared. A variety of substituents were tolerated at this position;

Table 4. In vitro activity of different *O*-alkyl analogs

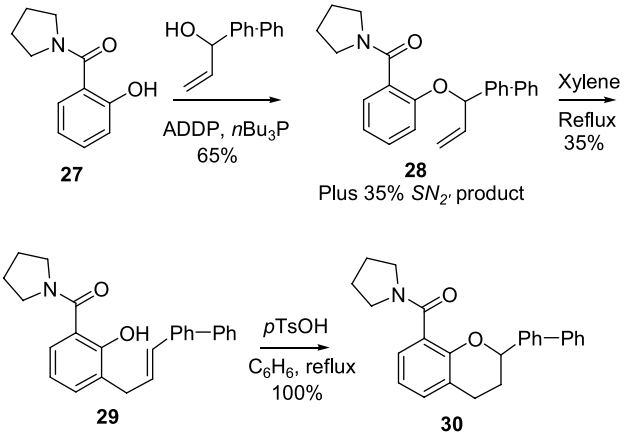


R				
Compd.	**13**	**14**	**15**	**16**
VIPR $Na_v1.7$, IC_{50} (μM)	9.0	>30	>30	21
R				
Compd.	**17**	**18**	**19**	**20**
VIPR $Na_v1.7$, IC_{50} (μM)	4.0	0.86	0.55	2.2

however, the more polar hydroxyl group and bulky substituents (i.e., *O*-*tert*-butyl) displayed markedly diminished potency against the $Na_v1.7$ channel.

6. A conformationally restricted analog of 11

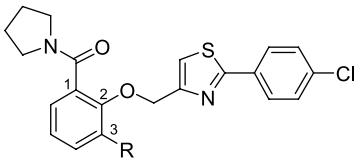
To assess the effect of conformational rigidity, a conformationally constrained analog in which the C3-substituent was tied to the 2-*O*-alkyl side chain within a dihydropyran ring, was prepared. It was envisioned that removing the metabolically labile C3-methoxy and C2-*O*-benzyl methylene groups might improve the PK profile, while preventing any unfavorable steric interactions that could compromise potency.



Scheme 2. Synthesis of a conformationally constrained analog.

A short synthesis to **30** was developed (Scheme 2), with a biphenyl group replacing the *p*-chlorophenylthiazole in **11**. With the pyrrolidine amide **27** as starting material, it was noted that a significant amount of a byproduct, arising from an $SN_{2'}$ displacement pathway, was formed in the Mitsunobu reaction.[13] Nevertheless, the desired SN_2 product **28** was obtained in 65% yield. **28** was heated to reflux in xylene for 24 h to effect the thermal Claisen rearrangement.[14] Subsequent Prins cyclization[15] in the presence of catalytic amount of *p*-toluenesulfonic acid in refluxing benzene afforded **30**.[16] It is noteworthy that the competing 5-*exo*-trig cyclization was not observed, presumably due to the favored formation of the more stable benzylic carbenium ion. The conformationally constrained analog **30** displayed comparable in vitro activity ($Na_v1.7$ VIPR 3.6 μM; ephys. K_R/K_I 4.3 μM/0.21 μM), was reasonably clean in hERG activity (IC_{50} 5.4 μM), while its clearance rate was reduced (Cl_p 40 mL/min/kg) and half-life in vivo was doubled ($t_{1/2}$ 0.88 h).

Table 5. In vitro activity of C3-substituted analogs



R	OCH_3	H	OH	CH_3	Cl	OEt	O*i*Pr	O*t*Bu
Compd.	**11**	**12**	**21**	**22**	**23**	**24**	**25**	**26**
VIPR $Na_v1.7$, IC_{50} (μM)	1.3	1.0	>30	0.55	0.73	6.6	7.5	22

7. Conclusion

Voltage-gated sodium channels have recently emerged as attractive targets for treatment of chronic pain. Potent and state-dependent blockers of sodium channels have been identified with minimal hERG activity and acceptable pharmacokinetic properties. Such compounds are also found to be efficacious in animal pain models, suggesting that they could be developed as novel analgesics to relieve chronic pain in patients.

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16. Spectra data for compound **30**: ^{1}H NMR ($CDCl_3$) δ 7.62 (d, 4H, J = 7.2 Hz), 7.47 (d, 4H, J = 7.3 Hz), 7.38 (t, 1H, J = 5.2 Hz), 7.18 (1H, dt, J = 5.2 Hz), 7.12 (1H, dt, J = 5.1 Hz), 5.20 (dd, 1H, J = 7.8, 2.4 Hz), 3.55 (m, 2H), 3.41 (br s, 1H), 3.32 (m, 1H), 3.04 (m, 1H), 2.84 (m, 1H), 2.36 (m, 1H), 2.11 (m, 1H), 1.91 (m, 4H); ^{13}C NMR ($CDCl_3$) 168.0, 150.8, 140.9, 140.7, 140.6, 130.5, 129.0, 127.6, 127.4, 127.3, 127.2, 126.2, 125.9, 122.7, 120.7, 47.9, 45.7, 29.9, 26.1, 25.2, 24.9.