State-Dependent Inhibitors of the Nav1.3 Sodium Channel For Treating Neuropathic Pain

Xention

Marc Rogers, Richard Davies, Jenny Wilson, Lam Tang, Simon Jones, Dave Madge & John Ford
Xention Ltd, Iconix Park, London Rd, Pampisford, Cambridge CB22 3EG U.K.

Xention

1. Introduction

Neuropathic pain is characterized by ectopic firing and increased action potential spiking in response to normal stimuli, electrophysiological correlates of the hallmark behavioural responses known as allodynia and hyperalgesia. Although several different peripheral and central signaling pathways are altered during the initiation and maintenance of neuropathic pain (e.g. P2X receptors, acid-sensing channels, Ca^{2+} channels, neurotrophin receptors, noradrenergic and glutamanergic receptors), most interest has focused on voltage-dependent sodium channels (Navs), as they are responsible for transmitting nociceptive and neuropathic pain signals, and are the common target of most clinically effective oral treatments.

It would appear that both TTX-sensitive and TTX-resistant Navs are involved in neuropathic pain, with differences in their contribution depending on the animal model used, as well as the species (Priest & Kaczorowski, 2007). In the rat, Nav1.3 and Nav1.8 are the major TTX-S and TTX-R channel candidates, respectively, but recent genetic evidence suggests that mutations in the TTX-S Nav1.7 channel are also involved in several rare forms of human neuropathic pain (Waxman & Dib-Hajj, 2005).

Table 1: Classification of Sodium Channel α -Subunits

Name	Further Names	Gene	TTX Sensitive	Localisation	Disease link/ Change in NP
Nav1.1	Type 1	*SCN1A*	Yes	CNS, DRG	Epilepsy
Nav1.2	Type 2	*SCN2A2*	Yes	CNS	Epilepsy
Nav1.3	Type III	*SCN3A*	Yes	Embryonic CNS, injured DRG	Ectopic discharge in NP Upregulated
Nav1.4	Skm1	*SCN4A*	Yes	Skeletal muscle	Contractility problems
Nav1.5	H1, Skm2	*SCN5A*	Low	Heart, embryonic CNS	Cardiac arrhythmias
Nav1.6	PN4, CerIII	*SCN8A*	Yes	DRG, motor neurons, primarily on axons	Neurological dysfunction
Nav1.7	PN1, hNe,	*SCN9A*	Yes	DRG, low in CNS	Peripheral transmission mutations in human NP
Nav1.8	PN3, SNS	*SCN10A*	No	DRG	Sensory hypersensitivity Downregulated
Nav1.9	NaN, SNS-2	*SCN11A*	No	DRG, low levels in hippocampus	Modulates resting potential Downregulated

We have chosen hNav1.3 as our main screening target because:

1. Nav1.3 is upregulated throughout the nociceptive signaling pathway in most neuropathic pain models, as well as in human patients (reviewed in Rogers *et al*., 2006).
2. Low concentrations of TTX reduce neuropathic pain without affecting normal signaling, and Nav1.3 is the major TTX-S channel in the nociceptive system of neuropathic animals.
3. The rapid repriming kinetics of Nav1.3 match those of Navs in injured DRG neurons.
4. Antisense knockdown of Nav1.3 abrogates hyperexcitability and neuropathic pain in animal models (Waxman & Hains, 2006).

2. Xention and other development compounds

Xention Lead Series	Nav1.3 IC50 μ M	Nav1.5 IC50 μ M	Solubility μ M	Microsome stability
LS-07	2.5	4.8	164	79%
LS-08	6.5	12	18	100%
LS-13	4.1	14.3	116	85%
LS-14	0.7	1.5	2	48%
LS-16	3.9	24	98	100%

Competitor Lead Cmpds	Nav1.3 IC50 μ M	Nav1.5 IC50 μ M	Solubility μ M	Microsome stability
Comp-A	2.5	7.5	<2	nd
Comp-B	7	30	<2	nd
Comp-C	> 5	8.2	22	4%
Comp-D	10.8	10	<2	nd
Comp-E	1.4	1.5	10	nd
Comp-F	3.5	nd	0.3	nd

Table 2: Summary of *in vitro* Nav1.x activity and ADME properties for Xention and competitor compounds. Where possible, all competitor data is from material synthesized in-house and tested alongside Xention compounds in the same assays. All Nav1.x data is from automated patch clamp experiments; most ADME data was generated using the same protocol, with some competitor data obtained from the public domain. Microsome stability measures drug remaining after 60 min for rat tissue.

3. Mechanism of Action

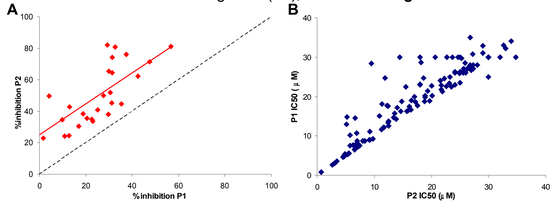
Several different mechanisms of action have been described for Nav blockers, the two most important being use-dependent block (e.g. local anaesthetics), and stabilization of the inactivated state (anticonvulsants, TCAs). We use a complex voltage clamp protocol to detect various types of block during a single sweep, speeding up drug discovery and enhancing the information acquired during routine electrophysiological screening:

Peak 1 (P1) represents the resting state of the Nav1.3 channel;
Peak 2 (P2) is sensitive to use-dependent block;
Peak 3 (P3) measures the inactivated state.

a. Use-dependence

Use-dependent block occurs when a compound accesses a binding site (or channel state) revealed during repeated cycling between closed and open states. This mechanism of block could target Navs exhibiting heightened activity, as occurs during ectopic firing, while sparing action potential signaling in unaffected nerves or during normal synaptic transmission.

We identified a subset of Xention compounds showing use-dependent block of Nav1.3 during two HTS electrophysiological screens. In the first screen of ~800 compounds, nearly 30 exhibited ≥1.5 fold selectivity for P2 over P1 (**Fig. 2a**). During subsequent Lead Series expansion and SAR screening, ~10% of active compounds exhibited a P2 IC50 value ≥1.5 fold lower than that for the resting state (P1), as shown in **Fig. 2b**.



The complex voltage clamp protocol underestimates the full amount of use-dependent block, but is useful for early identification of compounds with this mechanism of action. Compounds with a use-dependent profile were investigated in more detail using longer pulse trains and varying frequencies on a manual patch clamp rig, as shown below for the 5 Xention compounds.

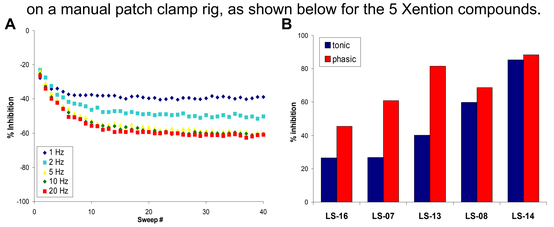


Fig. 3: Xention LS compounds exhibit a range of use-dependent block. All compounds tested at 3 μM, close to their IC_{50} value against P3. **A:** Steady-state (tonic) block of P1 is indicated by the reduction in peak amplitude of the 1st sweep in the series; additional use- and frequency-dependent (phasic) block develops during the 40 pulse train delivered at 1-20 Hz. Data for LS-07 compound. **B**: A comparison of tonic *vs* phasic block (20 Hz data shown for clarity) for all compounds.

b. Stabilization of the inactivated state

Many drugs showing greatest oral efficacy in treating neuropathic pain, such as anticonvulsants and TCAs, stabilize the inactivated state of the Nav1.3 channel during periods of high spiking activity and/or prolonged membrane depolarization. This is also true of recent preclinical candidates (Table 2), as well as the majority of Xention compounds (**Fig. 4**).

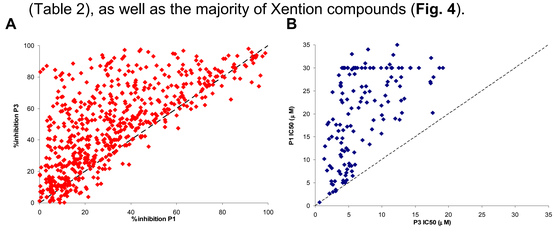


Fig. 4: The majority of Nav1.3 hits exhibit a preference for blocking the inactivated state. **A:** Data from a HTS survey of 800 Xention compounds on an APC machine, showing the enrichment of compounds with greater block of the inactivated peak (P3), compared to the resting state (P1). **B:** Compounds developed during the subsequent SAR campaign also exhibit a preference for the inactivated state of the Nav1.3 channel, indicated by the lower IC_{50} value of P3 versus P1.

4. Formalin Pain Assay

There are many different types of animal models to study acute and chronic neuropathic pain, but the formalin assay offers several advantages as an early proof-of-concept test:

a. It has been claimed that chemical stimulation is the best model of human clinical pain, owing to its inescapable nature and slow, persistent stimulus (Le Bars *et al*., 2001).

b. The nociceptive behaviours elicited during the assay reflect aspects in the development of peripheral and central neuropathic pain signaling, and it has proven to be highly predictive of efficacy in more protracted, and expensive, surgical models (Blackburn-Munro & Erichsen, 2005).

c. The formalin assay is quick, returning results within a day, whereas chronic surgical models require weeks to establish and measure pain efficacy.

Nociceptive behaviour (paw licking, flinching, biting, etc) occurs in two phases after injection of 5% formalin; the 1st phase lasts 0-5 min and represents acute activation of nociceptors, probably with an inflammatory pain component, while the 2nd phase (15-45 min) is associated with early peripheral and central sensitization processes associated with the development of neuropathic pain.

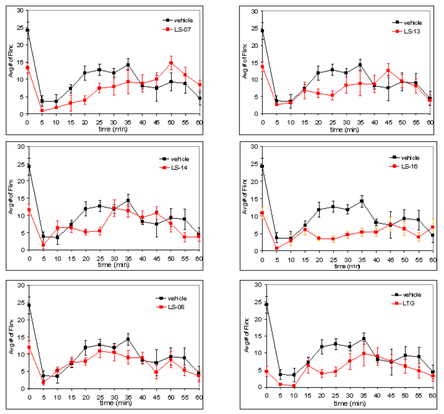


Fig. 5: Effect of Xention compounds on nociceptive responses during time-course of formalin pain assay. Note the transient early phase (0-5 min) and sustained 2nd phase (15-45 min). Xention compounds administered i.v. at 5 mg/kg 30 min prior to injection of formalin; the positive control, Lamotrigine (LTG), administered p.o. at 20 mg/kg. Same control data in each graph (black).

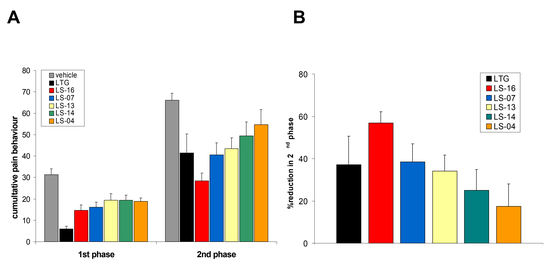
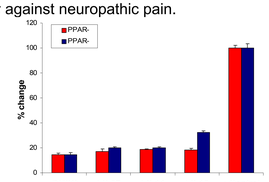


Fig. 6: Reduction of pain behaviour during the 2 phases of the formalin assay. **A:** Cumulative total of behavioural responses (paw licks, flinching) during the 1st (0-5 min) and 2nd phase (15-45 min) are shown for vehicle, positive control (Lamotrigine, LTG) and 5 Xention compounds. LTG greatly reduced responses during the early (inflammatory) phase as well as the later neuropathic phase. Xention compounds had much less effect during the 1st phase, but all reduced late phase behavior. **B**: Percentage reduction in 2nd phase nociceptive behaviour compared to vehicle. Only LS-08 failed to significantly decrease neuropathic pain responses.

5. Xention Nav1.3 compounds are selective

a. PPAR agonists

Agonists at the a Peroxisome Proliferation Activator Receptor (PPAR) are potent analgesics in rat models of neuropathic pain, possibly *via* an effect on ion channels (LoVerme *et al*., 2006). We tested the active Nav1.3 compounds for such off-target activity using luciferase reporter assays specific for the α and γ forms of PPAR. None of them exhibited any significant PPAR agonist activity (**Fig. 7**), arguing against this mechanism having a role in their efficacy against neuropathic pain.



b. Ca^{2+} channels

Voltage-dependent Ca^{2+} channels are also implicated in neuropathic pain, with several compounds targeting this class already in the marketplace (e.g. Gabapentin, Ziconotide). Several "selective" Nav1.x preclinical compounds also block Ca^{2+} channels (Benjamin *et al*., 2006; Brochu *et al*., 2006; Martinborough *et al*., 2006), so we determined if our Lead Series compounds shared this activity, and whether this might explain their efficacy in the formalin pain assay, or potentially contribute to unwanted side effects.

Neuronal Ca^{2+} channels were studied in differentiated NG108-15 neuroblastoma cells, which express a mixture of low voltage-activated (LVA) T-type and high voltage-activated (HVA) N- and L-type channels (Lukyanetz *et al*., 1998). Most Xention POC compounds had a similar pattern of activity, reducing the amplitude of the LVA current (~30%) slightly more than the peak of the HVA current (~25%), albeit at a concentration 10 μM (**Fig. 9**).

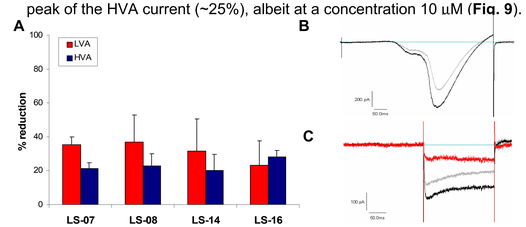


Fig. 9: Ca^{2+} channel inhibition by Xention LS candidates (10 μM). **A:** All except the LS-16 compound affected T-type LVA current slightly more than the HVA current. **B:** Voltage ramp from -90 mV to +60 mV reveals the LVA T-type current activating at -30 mV, followed by the larger HVA current (peak ~0 mV). Both components are reduced by the LS-14 exemplar compound (gray race). **C:** An example of an HVA current (voltage step to 0 mV from Vh of -90 mV), showing control amplitude (black trace), current in the presence of the LS-16 candidate (gray trace), and the digitally subtracted difference current (red trace), which resembles a slowly activating, sustained L-type like current.

6. Conclusions

1. A single multivariate voltage clamp protocol can identify hits with various state-dependent Nav blocking mechanisms during routine, high throughput electrophysiological screening.
2. The majority of hits exhibit a preference for the inactivated state, with a subset also showing use-dependence. Compounds exhibiting these mechanisms of action have the potential to effectively treat allodynia and hyperalgesia associated with neuropathic pain.
3. The potency, cardiac safety, and ADME properties of Xention Lead Series compounds meets or exceeds those of candidates in development for neuropathic pain by other companies.
4. Exemplar compounds from 4 out of 5 Xention Lead Series significantly reduce neuropathic pain, as measured in the formalin assay.
5. Active Xention LS candidates have 5-30 fold selectivity over PPARα and voltage-dependent Ca^{2+} channels, suggesting that their efficacy against neuropathic pain is due to state-dependent inhibition of Nav1.3 channels.

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