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ORIGINAL RESEARCH

Pharmacology and Mechanism of Action of Suzetrigine, a Potent and Selective $Na_V1.8$ Pain Signal Inhibitor for the Treatment of Moderate to Severe Pain

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ABSTRACT

Introduction: There is a high unmet need for safe and effective non-opioid medicines to treat moderate to severe pain without risk of addiction. Voltage-gated sodium channel 1.8 ($Na_V1.8$) is a genetically and pharmacologically validated pain target that is selectively expressed in peripheral pain-sensing neurons and not in the central nervous system (CNS). Suzetrigine (VX-548) is a potent and selective inhibitor of $Na_V1.8$, which has demonstrated clinical efficacy and safety in multiple acute pain studies. Our study was designed to characterize the mechanism of action of suzetrigine and assess both nonclinical and clinical data to test the hypothesis that selective $Na_V1.8$ inhibition translates into clinical efficacy and safety, including lack of addictive potential.

Methods: Preclinical pharmacology and mechanism of action studies were performed in vitro using electrophysiology and radiolabeled binding methods in cells recombinantly expressing human Na_V channels, human proteins, and primary human dorsal root ganglion (DRG) sensory neurons. Safety and addictive potential assessments included in vitro secondary pharmacology studies, nonclinical repeat-dose toxicity and dependence studies in rats and/or monkeys, and a systematic analysis of adverse event data generated from 2447 participants from phase 3 acute pain studies of suzetrigine.

Results: Suzetrigine is selective against all other Na_V subtypes (≥ 31,000-fold) and 180 other molecular targets. Suzetrigine inhibits $Na_V1.8$ by binding to the protein's second voltage sensing domain (VSD2) to stabilize the closed state of the channel. This novel allosteric mechanism results in tonic inhibition of $Na_V1.8$ and reduces pain signals in primary human DRG sensory neurons. Nonclinical and clinical safety assessments with suzetrigine demonstrate no adverse

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CNS, cardiovascular or behavioral effects and no evidence of addictive potential or dependence. ***Conclusions***: The comprehensive pharmacology assessment presented here indicates that suzetrigine represents the first in a new class of non-opioid analgesics that are selective $Na_V1.8$ pain signal inhibitors acting in the peripheral nervous system to safely treat pain without addictive potential.

Keywords: Moderate to severe pain; $Na_V1.8$; Non-opioid analgesic; Selective pain signal inhibitor; Suzetrigine; VX-548

Key Summary Points

Why carry out this study?

There is a high unmet need for safe and effective non-opioid medicines to treat moderate to severe pain without the risk of addiction.

Suzetrigine is a potent and highly selective inhibitor of the voltage-gated sodium channel 1.8 ($Na_V1.8$), which has demonstrated clinical efficacy and safety in multiple acute pain studies.

This study was designed to characterize the mechanism of action of suzetrigine and determine if selective $Na_V1.8$ inhibition translates into clinical efficacy and safety, including lack of addictive potential.

What was learned from this study?

$Na_V1.8$ is not expressed in the central nervous system (CNS).

Suzetrigine acts by a novel allosteric mechanism to stabilize the closed state of the channel and reduce pain signals in primary human dorsal root ganglion sensory neurons.

The pharmacology and mechanism of action of suzetrigine translate into clinical efficacy and safety, including no adverse CNS, cardiovascular, or behavioral effects and no evidence of addictive potential or dependence.

INTRODUCTION

Pain is a natural part of the human experience and can be beneficial when it serves a protective function or signals tissue damage and injury. However, moderate to severe pain can lead to unnecessary suffering that requires effective pain management [1–4]. Current treatments include several analgesics and central nervous system (CNS) agents such as acetaminophen, nonsteroidal anti-inflammatory drugs, local anesthetics, antidepressants, anticonvulsants, and opioids. These agents act via mechanisms that are not specific to pain sensation, signal transmission, or perception and often have side effects that can result in inadequate pain control because they affect multiple targets and organs [5–13]. For example, opioids are efficacious and widely used analgesics but have well-known tolerability issues and carry a risk of dependence and addiction due to their effects on the CNS [14, 15]. Misuse of prescription opioids contributes to the continuing public health opioid crisis in the United States [16, 17]. Due to these liabilities, new classes of non-opioid analgesics are needed that specifically inhibit pain to provide safe and effective pain relief without addictive potential [18]. While many targets have been explored to address this unmet need, they have largely failed to translate into clinical efficacy [19, 20].

Selective inhibition of specific Na_V channels is a promising new approach to treat peripheral pain signaling without affecting other biological processes [21, 22]. Na_Vs are membrane proteins that conduct sodium ions across cell membranes and are responsible for electrical signals known as action potentials (APs). There are nine mammalian Na_V subtypes ($Na_V1.1$–$Na_V1.9$) with distinct roles in certain cell types and tissues, including in the nervous system, heart, and skeletal and smooth muscle tissues [23]. Local anesthetics and certain anticonvulsants act by nonselectively blocking permeation of sodium through the pore of all Na_V channels [23–25], and while they are used to treat pain, they exhibit side effects due to their nonspecific block of multiple Na_Vs [13].

Of the nine mammalian Na_V subtypes, $Na_V1.7$, $Na_V1.8$, and $Na_V1.9$ have been identified as

potential pain targets based on their predominant expression and functional roles in peripheral pain-sensing neurons (nociceptors) [26–28], and human genetic mutations that result in altered pain sensation [29–35]. Of these three potential targets, $Na_V1.8$ is the most selectively expressed in nociceptors where its role is to transmit pain signals (action potentials) in peripheral sensory nerves [36–38].

We and others hypothesized that a therapy acting through selective inhibition of $Na_V1.8$ channels could specifically reduce transmission of pain signals in peripheral sensory nerves without affecting functions mediated by other members of the Na_V family. $Na_V1.8$ is not expressed in the human brain or spinal cord [39–43]. Therefore, highly selective $Na_V1.8$ inhibitors should not exhibit CNS side effects associated with nonselective Na_V blockers or result in the tolerability issues and addictive potential associated with opioids.

Despite the rationale for $Na_V1.8$ as a target, discovery and advancement of highly selective inhibitors has proven challenging, due in part to the high sequence homology between Na_V subtypes [44]. Over the last 20 years, we have used cell-based high-throughput screening and medicinal chemistry to identify potent and selective $Na_V1.8$ inhibitors. Pharmacological validation of selective $Na_V1.8$ inhibition was first demonstrated with an oral small molecule, VX-150 [45], which reduced pain in phase 2 studies of participants with acute (postoperative) and chronic (neuropathic) pain [46, 47]. Following proof-of-concept, we discovered suzetrigine as a potent and selective $Na_V1.8$ inhibitor with favorable drug-like properties. Suzetrigine has demonstrated clinical efficacy and safety in multiple acute pain studies [48–50]; suzetrigine is also being evaluated in neuropathic pain [51].

Here, we describe the pharmacology and mechanism of action of suzetrigine and expand upon the reported clinical data [48–50] to provide a comprehensive assessment of suzetrigine's nonclinical and clinical safety.

MATERIALS AND METHODS

Analysis of *SCN10A* Expression in Human Central Nervous System

The expression of *SCN10A*, the gene that encodes the $Na_V1.8$ protein, in the CNS (brain and spinal cord) was evaluated from two independent comprehensive public reference databases: the Human Protein Atlas (HPA) [39–41] and the Genotype-Tissue Expression project (GTEx) [42, 43]. The HPA database evaluated human gene expression by highly sensitive ribonucleic acid sequencing (RNA-seq) on 193 CNS tissues from 966 postmortem samples, and the GTEx database evaluated human gene expression using similar methods on 13 different CNS tissues from each of $n = 139$–255 postmortem samples. The HPA database reported mean *SCN10A* transcripts per million (TPM) of 0.0 in all human brain and spinal cord regions evaluated. To distinguish signal (an expressed gene) from noise in the GTEx data, we used the threshold standard for an expressed gene defined by the GTEx project: > 0.1 TPM in at least 20% of samples and ≥ 6 reads in at least 20% of samples.

In Vitro Electrophysiology

In vitro electrophysiology experiments were performed using automated platforms (Sophion Q-Patch, Sophion Qube, IonWorks Quattro, IonWorks Barracuda) with human embryonic kidney (HEK), Chinese hamster ovary (CHO), or rodent neuroblastoma fusion (ND7/23) cell lines transiently or stably overexpressing ion channel constructs of interest. For Na_V subtype selectivity assessments, voltage clamp protocols evaluated potency and selectivity across closed and inactivated states. Potency was defined as the concentration resulting in 50% of the maximum inhibition of suzetrigine for the Na_V channel evaluated (IC_{50}). To determine selectivity, the most potent off-target Na_V measurement from any endpoint was used to generate a selectivity assessment for suzetrigine against that channel compared to its first pulse IC_{50} on $Na_V1.8$. Chimera experiments

utilized a simple voltage pulse from –90 mV to +20 mV to assess inhibition by suzetrigine.

Manual patch voltage clamp experiments utilized dissociated primary dorsal root ganglion (DRG) neurons isolated from humans and other species and cultured on glass coverslips. Tetrodotoxin (TTX)-resistant currents were recorded using a simple voltage protocol stepping from –90 mV to –10 or 0 mV to assess inhibition by suzetrigine.

Manual patch current clamp experiments used the same preparation of primary human DRG neurons as for voltage clamp experiments. Currents were injected in increasing steps until AP firing was first observed (rheobase). To assess inhibition of excitability by suzetrigine, 100 current injections at 110% rheobase were injected at 1 Hz, and the number of resulting APs was assessed before and during application of suzetrigine.

For all experiments, suzetrigine was diluted into extracellular buffers from a 10 mM stock solution. Details on buffers used in electrophysiology experiments can be found in the Supplementary Materials and Methods.

Protein Expression and Purification

In order to allow for sufficient protein expression and purification yield, voltage-sensing domain (VSD) 2 of $Na_V1.8$ (accession number NP_006505.3) and $Na_V1.2$ (accession number NP_001035232.1) were cloned into a Na_VPaS backbone (accession number D0E0C2.1) with N-terminal FLAG and Twin-Strep purification tags, similar to the strategy described for VSD4 [52]. The chimeric proteins were expressed by transient transfection of HEK Expi293 cells and ExpiFectamine (Thermo Fisher) according to the manufacturer's protocol. Proteins were purified using methods similar to those described previously [52, 53]. Protein purity and monodispersity were assessed using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and fluorescent size exclusion chromatography on an Agilent high-performance liquid chromatography (HPLC) system. Details on detergent extraction and purification protocols can be found in the Supplementary Materials and Methods.

^{3}H Suzetrigine Binding to Purified Protein

Tritiated suzetrigine (4-[[(2*R*,3*S*,4*S*,5*R*)-3-(3,4-difluoro-2-methoxy-5-tritio-phenyl)-4,5-dimethyl-5-(trifluoromethyl)tetrahydrofuran-2-carbonyl]amino]pyridine-2-carboxamide) was generated by ViTrax (Placentia, CA). Binding experiments were conducted at ambient temperature by incubating purified protein with a range of ^{3}H suzetrigine concentrations in the absence or presence of unlabeled suzetrigine for 30 min. Bound probe was then separated from unbound probe, and bound probe was quantitated using liquid scintillation counting. Specific counts per minute (CPM) bound were then calculated by subtracting the nonspecific CPM (measured in the presence of excess unlabeled suzetrigine) from the total CPM (in the absence of excess unlabeled suzetrigine). Specific CPM were then plotted and fit to a one-site-specific binding model in GraphPad Prism using the equation $y=B_{max}*x/(K_d+x)$, where B_{max} is the extrapolated maximal CPM and K_d is the apparent affinity. Details on binding assay protocols can be found in the Supplementary Materials and Methods.

Tanimoto Similarity Analysis

The chemical similarity of suzetrigine was compared to more than 500 Schedule I through V controlled substances in the United States. Each compound was assigned a fingerprint based on its two-dimensional molecular structure using 166-bit MACCS keys. The similarity between any two fingerprints was computed using the Tanimoto coefficient (T_c), as implemented in OpenEye Toolkit 2022.1.1 [54]. A cutoff of $T_c \geq 0.85$ was used to group similar compounds, as this value has been shown to identify molecules likely to have similar on-target activity among diverse sets of molecules [55, 56]. The maximum T_c between suzetrigine and any Schedule I compound (those with the highest abuse potential) is 0.57.

In Vitro Pharmacology Modeling

Synergy analysis was conducted using the BIGL package in R version 4.4.1 [57, 58] using last pulse potency measurements. Details on pharmacology modeling can be found in the Supplementary Materials and Methods.

Secondary Pharmacology Assays

Secondary pharmacology assays included in vitro ligand binding assays and functional assays available from Eurofins. The Eurofins Spectrum Screen (174 targets; Eurofins catalog #PP16), and the Drug Abuse Potential Safety Screen Panel (44 targets; catalog #P293) were conducted at a screening concentration of 10 µM. Follow-up assays to determine binding IC_{50}s or functional effects were conducted at concentrations ranging from 1 nM to 30 µM.

Repeat-Dose Toxicity Studies

Repeat-dose toxicity studies were conducted in Sprague Dawley (CD®[Crl:CD®(SD)]) rats (up to 26 weeks in duration) and cynomolgus monkeys (up to 39 weeks in duration) using daily administration of suzetrigine by oral gavage at doses resulting in exposures that were multiples of the estimated human exposure ($AUC_{0–24\ h}$) at steady-state (up to 23-fold in male rats, 54-fold in female rats, and 2.1-fold in monkeys). The studies included a vehicle control arm and three dose levels of suzetrigine. Details on animal sources, ages, and numbers used in the studies can be found in the Supplementary Materials and Methods.

Clinical observations were conducted daily; animals were observed in and out of their home cage for morbidity, mortality, injury, and availability of food and water. Observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, nervous system effects including tremors, convulsions, reactivity to handling, and unusual behavior.

The toxicity studies also evaluated mortality, body weight, food consumption, ophthalmology assessment, electrocardiograms (ECGs; monkeys only), clinical pathology (hematology, clinical chemistry, urinalysis, and urine chemistry), anatomic pathology, and the toxicokinetics of suzetrigine. The anatomic pathology evaluation included organ weights, gross pathology, and microscopic evaluation of organs, including all major organ systems.

Nonclinical CNS Evaluation

A functional observational battery (FOB) was included on day 2 of a 28-day monkey study ($n = 4$ animals/sex/group). The doses evaluated resulted in exposures that were multiples of the estimated human exposure (free C_{max}) at steady-state (up to 15-fold in monkeys). FOB evaluations were conducted by two independent raters for all occasions and consisted of a detailed home cage and open area neurobehavioral evaluation [59]. Each technician scored the monkey independently (without sharing the results with each other) for each home cage and out-of-cage observational score, and then the individual scores were assessed for agreement with their partner's score after the completion of the testing. FOB evaluations were conducted on each animal on day −1 to establish baseline differences, on day 2 at 4 h (±1 h), and at 24 h post-dose (before day 3 dose). The observations included, but were not limited to, evaluation of activity level, posture, lacrimation, salivation, tremors, convulsions, fasciculations, stereotypic behavior, facial muscle movement, palpebral closure, pupil response, response to stimuli (visual, auditory, and food), body temperature, Chaddock and Babinski reflexes, proprioception, paresis, ataxia, dysmetria, and slope assessment.

ECG Evaluations

ECG evaluations were performed in repeat-dose monkey studies. All animals in all groups received an ECG examination twice pretest, and at the approximate suzetrigine maximum

observed concentration (C_{max}) during the final week of each study (e.g., weeks 4, 13, 39). Care was taken to avoid causing undue excitement of the animals before the recording of ECGs to minimize extreme fluctuations or artifacts in the measurements. Standard ECGs (10-lead) were recorded at 50 mm/sec. The RR, PR, and QT intervals, and QRS duration were measured and recorded using an appropriate lead. The ECGs were interpreted by a board-certified veterinary cardiologist.

Cardiovascular and Respiratory Telemetry Study

Potential cardiovascular and respiratory effects of suzetrigine were evaluated in conscious, freely moving cynomolgus monkeys using a single-dose Latin square design with a 7-day washout between treatments. The same four male animals received the vehicle control and three dose levels of suzetrigine via oral gavage. The doses evaluated resulted in exposures that were multiples of the estimated human exposure (free C_{max}) at steady-state (up to 15-fold in monkeys). Mortality, clinical observations, body weight, body temperature, systolic, diastolic, and mean arterial blood pressures, pulse pressure, heart rate, and effects on the ECG were evaluated in the study. Telemetry monitoring was conducted from at least 2 h before dosing through at least 24 h post-dose.

Details on animal sources, ages, and numbers used in the studies can be found in the Supplementary Materials and Methods.

Rat Physical Dependence Study

The physical dependence study was conducted in female Sprague Dawley (CD®[Crl:CD®(SD)]) rats with a vehicle control group, three suzetrigine dose groups at dose levels representing multiples of the human exposure (up to 52-fold the human C_{max} at steady-state), and a morphine-positive control group. Details on animal sources, ages, and numbers used in the studies can be found in the Supplementary Materials and Methods.

Animals were dosed for 30 consecutive days. Vehicle and suzetrigine-treated animals received a dose once daily in the morning and were subjected to a sham dosing procedure (during which no dose was administered) in the evening on days 1 to 30 to coincide with dosing of the positive control group to diminish the effects of the possible classical conditioning confounds. The positive control group received morphine twice per day (bid) via oral (gavage) administration. Morphine was administered in escalating doses over the first 2 weeks starting at 20 mg/kg/dose (40 mg/kg/day) and then fixed doses of morphine (150 mg/kg/dose; 300 mg/kg/day) were administered over the last 2 weeks of the study. On the evening of day 30, morphine-treated rats received a sham dose of sterile saline.

FOBs were conducted by testers without knowledge of the treatment groups that each animal belonged to, and the animals were evaluated using the neurobehavioral evaluations per testing facility standard operating procedure. The FOBs were conducted once pretest, then at 2 h post-dose (±20 min) following the first dose of the day on days 1, 15, and 30 during the dosing phase. During the withdrawal phase on days 31 through 39, the FOBs were scheduled at approximately the same time of day as was conducted on days 1, 15, and 30. The FOB in the rats included evaluation of activity level, excitability, motor activity, neuromuscular assessments, autonomic function, and physiologic measurements. Further details of the parameters evaluated and procedures used have been described previously [60–63].

Clinical Abuse Potential Assessment

To assess for evidence of clinical abuse potential, a systematic analysis of adverse events (AEs) was conducted across all phase 3 acute pain studies (VX-548-104, VX-548-105, VX-548-107; total of 2447 participants, with 1130 treated with suzetrigine for up to 14 days). Two of these studies were double-blind, randomized placebo- and active-controlled studies (one in participants with acute pain after abdominoplasty and the other after bunionectomy), and the third was an open-label safety

and effectiveness study (included a broad range of surgical and nonsurgical acute pain conditions) [49, 50]. In accordance with guidelines on assessment of abuse potential of drugs [64], this assessment included an analysis of all AEs in the studies using an expansive list of nearly 200 screening preferred terms (MedDRA version 26.1) that may be associated with abuse potential. A customized MedDRA query (CMQ) with these 200 screening preferred terms was created by using the four categories of abuse-related terms provided in the guidance, which are (1) euphoria, (2) effects on mood, cognition, or attention, (3) dissociative or psychotic reactions, and (4) any additional terms that might be indicative of abuse potential that were not included in one of the above categories. Within MedDRA, relevant system organ class (SOC) and high-level group terms (HLGT) were evaluated, and terms consistent with the above categories were included in the CMQ. These preferred terms are also relevant for assessing addictive potential [65, 66]. This CMQ was used to evaluate the phase 3 trials, and the number of participants in each treatment group with AEs coded to preferred terms contained in the screening list was summarized. These results were analyzed to determine whether suzetrigine showed any pattern or evidence of abuse or addictive potential.

Ethical Approval

Animal studies were conducted in Sprague Dawley (CD®[Crl:CD®(SD)]) rats and cynomolgus monkeys. Animals were sourced from Charles River Laboratories (USA). Each study in animals was approved by the laboratory's institutional animal care and use committee (IACUC). Animal care conformed to applicable national/international guidelines, and the studies were conducted in accordance with Good Laboratory Practice (GLP).

The clinical trial protocols were approved by a central institutional review board (Advarra Institutional Review Board, Columbia, MD, USA), and all sites accepted this central approval. The trials were conducted according to the International Council for Harmonization Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. All participants provided written informed consent.

Statistical Analysis

In vitro data are presented as mean ± standard error of the mean (SE). For experiments reported in Fig. 1d, errors are presented as 95% confidence intervals (CI) based on reported errors from Hill equation fits in GraphPad Prism software. In vivo data are presented as mean ± standard deviation (SD) or SE. Significance tests were performed as described in the figure legends (analysis of variance [ANOVA], Kruskal–Wallis, or generalized linear model with Dunnett's or Dunn's post hoc significance testing). The minimum significance level was $p > 0.05$.

RESULTS

$Na_V1.8$ is Not Expressed in the Human CNS

Formal analysis of the RNA-seq of more than 1000 postmortem samples and over 190 CNS tissues from the HPA [39–41] and GTEx project [42, 43] databases did not identify any expression of *SCN10A* in CNS tissues meeting the threshold defined by the database investigators (see analysis of *SCN10A* expression from the GTEx database in Supplementary Fig. S1).

Suzetrigine Is a Potent and Highly Selective Small Molecule Inhibitor of $Na_V1.8$

The chemical structure (4-[[(2*R*,3*S*,4*S*,5*R*)-3-(3,4-difluoro-2-methoxy-phenyl)-4,5-dimethyl-5-(trifluoromethyl)tetrahydrofuran-2-carbonyl]amino]pyridine-2-carboxamide) of suzetrigine is shown in Fig. 1a. The potency and selectivity of suzetrigine against the channels of the human Na_V family ($Na_V1.1$-$Na_V1.9$) are shown in Fig. 1b and c. Figure 1b shows whole-cell sodium currents from a $Na_V1.8$-expressing HEK cell before and 10 min after application of 10 nM suzetrigine, indicating the drug acts rapidly to produce nearly complete inhibition of both the peak and sustained phase of current

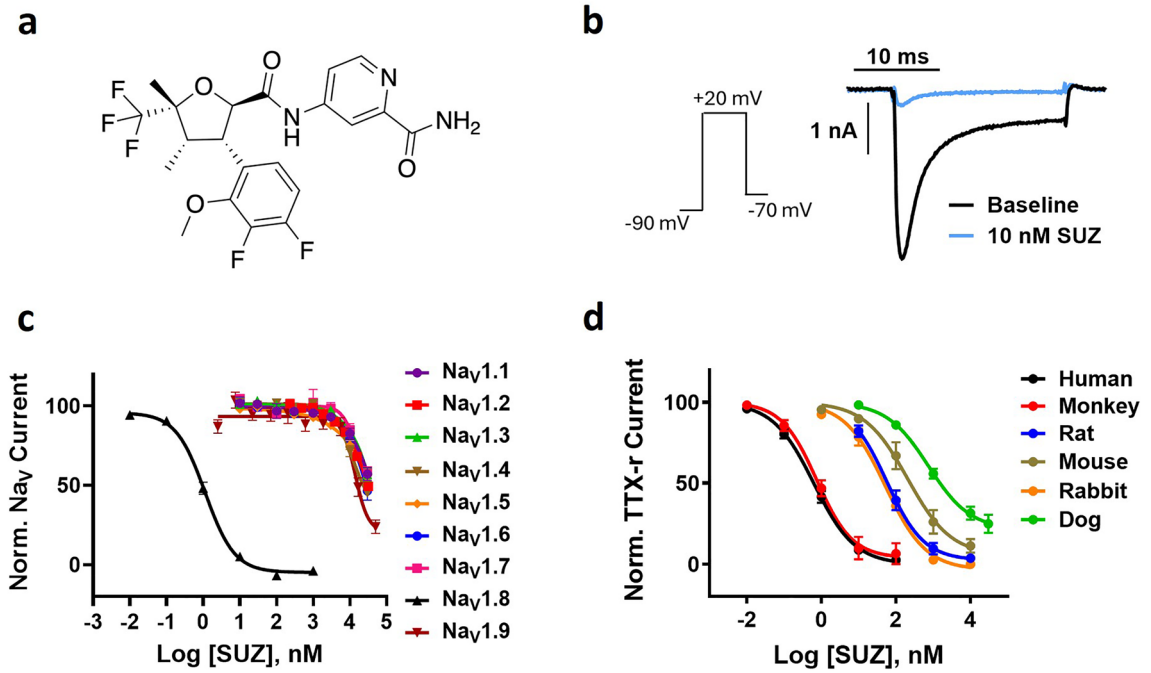

Fig. 1 Suzetrigine (SUZ) is a potent and selective inhibitor of human $Na_V1.8$ over other human Na_V subtypes and other $Na_V1.8$ orthologs. **a** Chemical structure of SUZ. **b** Inhibition of $Na_V1.8$ current by 10 nM SUZ in HEK cells expressing human $Na_V1.8$ and using automated electrophysiology techniques. Inset shows the voltage protocol used to generate the $Na_V1.8$ current trace shown (**c**). Inhibition of human Na_V subtypes 1.1–1.9 expressed in HEK or CHO cells and recorded using automated electrophysiology platforms. SUZ is ≥ 31,000-fold selective for $Na_V1.8$ over other human Na_V subtypes [48]. **d** Normalized inhibition of TTX-r $Na_V1.8$ current in DRG neurons using manual patch clamp electrophysiology. Average of data taken from $n = 3$ cells isolated from four (human) or one (monkey, rat, mouse, rabbit, and dog) tissue samples. Human $IC_{50} = 0.68 \pm 0.16$ nM across four biological replicates, monkey $IC_{50} = 0.75$ nM (95% CI of IC_{50} 0.44–1.4 nM), rat $IC_{50} = 56$ nM (95% CI of IC_{50} 36–93 nM), mouse $IC_{50} = 210$ nM (95% CI of IC_{50} 98–860 nM), rabbit $IC_{50} = 52$ nM (95% CI of IC_{50} 37–75 nM), dog $IC_{50} = 740$ nM (95% CI of IC_{50} 420–2100 nM)

at this concentration. We therefore aimed to achieve clinical concentrations in this range [48–50]. The concentration–response curves for suzetrigine on $Na_V1.1$–1.9 shown in Fig. 1c demonstrate that suzetrigine shows sub-nanomolar potency on $Na_V1.8$ and has a ≥ 31,000-fold selectivity ratio for $Na_V1.8$. Based on these data, at clinically relevant concentrations of suzetrigine, there is < 0.1% inhibition of any other Na_V. In addition, suzetrigine was selective against 180 other human non-Na_V targets including 44 targets associated with abuse potential with a > 600-fold margin to estimated clinical concentrations (Supplementary Table S1). The lack of any binding of suzetrigine to any known target (opioid-like or other) is consistent with a structural similarity analysis that showed suzetrigine is not similar to opioids or any known drug of abuse (Table 1).

The potency of suzetrigine for $Na_V1.8$ across species using primary neurons isolated from DRG from each species is shown in Fig. 1c. Suzetrigine is most potent in humans ($IC_{50} = 0.68 \pm 0.16$ nM) and monkeys ($IC_{50} = 0.75$ nM; 95% CI of 0.44–1.4 nM) compared to other species, with rats having intermediate sensitivity (IC_{50} of 56 nM; 95% CI 36–93 nM) and dogs being the least potent (IC_{50} of 740 nM; 95% CI 420–2100 nM) (Fig. 1d). Based on high sensitivity in monkeys and intermediate sensitivity in rats, these

Table 1 Evidence for lack of abuse potential with suzetrigine

In silico evaluations	
Tanimoto similarity scores to drugs of abuse	Not similar
In vitro studies	
Selectivity against other Na_V channels	≥ 31,000-fold selective for $Na_V1.8$ against other Na_Vs
Receptor binding studies with G protein-coupled receptors (GPCRs), ion channels, transporters	No off-target activity for over 180 targets at > 600-fold margin to estimated clinical concentrations
Receptor binding studies with targets associated with abuse potential	
Follow-up functional assays (antagonism/agonism)	
In vivo studies	
CNS evaluation in monkeys following a suzetrigine dose (Functional observational battery)	No stimulant or sedative effects
Clinical observations in rats (up to 6 months) and monkeys (9 months)	No stimulant or sedative effects
Physical dependence study in rats w/morphine-positive control	No dependence at concentrations up to 52-fold over the estimated human exposure

In silico, in vitro, and in vivo evaluations determined that there is no evidence for abuse potential with suzetrigine

species were selected for in vivo safety pharmacology and toxicity studies.

Suzetrigine Inhibits $Na_V1.8$ by Binding to VSD2 to Stabilize the Closed State of the Channel Under Physiologically Relevant Conditions

The binding location of suzetrigine on the $Na_V1.8$ channel was determined using a domain-swapping strategy which introduced each of the four homologous $Na_V1.8$ voltage-sensing domains (VSD1–4) into $Na_V1.2$, which is not inhibited by suzetrigine. This allowed us to assess whether sensitivity to suzetrigine inhibition was conferred by any of the $Na_V1.8$ VSDs.

As shown in Fig. 2a and c, suzetrigine sensitivity was only conferred when VSD2 of $Na_V1.8$ was introduced to $Na_V1.2$. Further refinement of the swapped regions indicated that the introduction of the VSD2 subregion containing transmembrane segments S3 and S4 of $Na_V1.8$ into $Na_V1.2$ was sufficient to confer sensitivity to suzetrigine (Fig. 2a and c). Sequence alignment in this region (Fig. 2b) suggested a KKGS sequence unique to $Na_V1.8$ that was confirmed experimentally to confer sensitivity to suzetrigine (Fig. 2a and c). Direct binding of suzetrigine to the VSD2 domain of $Na_V1.8$ was confirmed using radiolabeled suzetrigine (Fig. 2d, Supplementary Fig. S2).

As shown in Fig. 3a, Na_V channels transition through distinct conformational states during their gating cycle that include (1) a closed state that exists at hyperpolarized membrane voltages, (2) an open state that exists briefly when the membrane is depolarized, and (3) an inactivated state that is nonconducting and during which it cannot be opened again. Suzetrigine inhibited $Na_V1.8$ channels in a dose-dependent manner following hyperpolarized pre-pulses (designed to place the $Na_V1.8$ channel into the closed state) but was less efficacious following depolarized pre-pulses (designed to place the $Na_V1.8$ channel into the inactivated state) (Fig. 3b and c). This indicated that suzetrigine

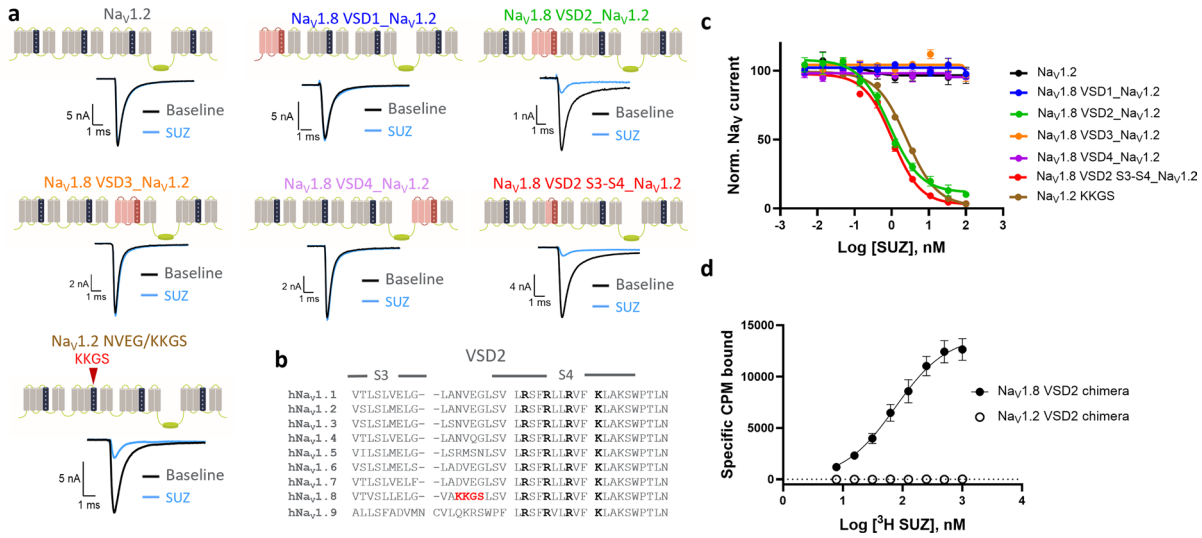

Fig. 2 Suzetrigine (SUZ) inhibits Na$_V$1.8 through interactions with VSD2. **a** Schematic and sample electrophysiological traces showing effect of 11 nM SUZ on different Na$_V$1.8_Na$_V$1.2 chimeras and mutations. The domains in Na$_V$1.2 replaced with the corresponding Na$_V$1.8 region are shown in red in the schematic. **b** VSD2 sequence alignment highlighting unique motif (red) in S3–S4 loop in Na$_V$1.8. Conserved amino acids comprising the basic voltage sensing residues are in bold. **c** Representative SUZ concentration response curves in different Na$_V$1.8_Na$_V$1.2 chimeras. **d** SUZ binds directly to purified Na$_V$1.8 VSD2 chimera protein ($K_d = 65 \pm 10$ nM) but not Na$_V$1.2 VSD2 protein. Representative curves from one experiment are shown in (**c** and **d**). Three independent experiments were conducted with a minimum of three technical replicates in each experiment

stabilizes the closed state, in contrast to local anesthetics, which are more efficacious following depolarizations, indicating block of the open and inactivated states [23–25, 67] (Supplementary Fig. S3).

We next assessed the pharmacology of suzetrigine using a voltage protocol designed to simulate a physiological AP at different frequencies (1, 5, and 10 Hz; Fig. 3d and Supplementary Fig. S4a and d). Under these conditions, suzetrigine demonstrated tonic inhibition of Na$_V$1.8 activity. 10 nM suzetrigine fully inhibited Na$_V$1.8 current across the full physiologic range of voltages and AP frequencies (Fig. 3e and Supplementary Fig. S4b and e) and its potency was consistent at first and last (50th) pulse, confirming the relevance of this target clinical exposure (Fig. 3f and Supplementary Fig. S4c and f).

Suzetrigine Inhibits Pain Signals in Sensory Neurons Isolated from Human DRG

Using manual patch current clamp methods in primary human pain-sensing neurons isolated from the DRG of human donors, suzetrigine significantly inhibited APs in 10/17 DRG neurons recorded from three donor tissues at concentrations ≥ 1 nM (Fig. 4). Maximal pain signal inhibition was observed at 10 nM, providing further support for targeting this concentration in clinical studies.

Suzetrigine Is Additive to, and Does Not Interfere with, Local Anesthetics that Also Inhibit Na$_V$1.8

Anesthetics are nonselective Na$_V$ blockers that are frequently used in clinical practice and could in principle be administered together with suzetrigine [68]. Suzetrigine in combination with either of the local anesthetics, bupivacaine

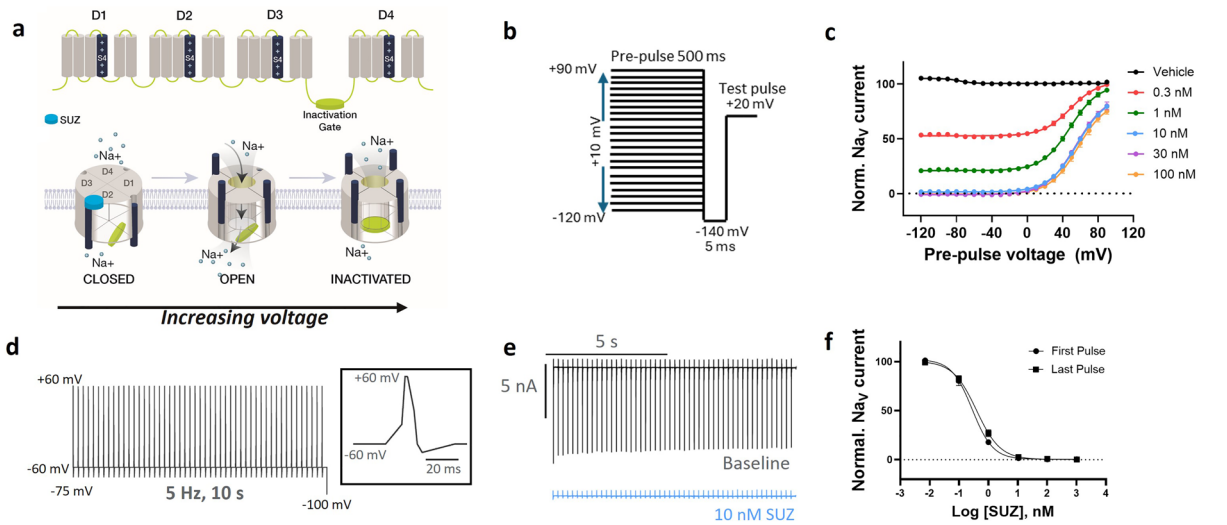

Fig. 3 Suzetrigine (SUZ) inhibits $Na_V1.8$ through stabilization of the closed state resulting in tonic inhibition. **a** Twenty-four membrane-spanning helices from four homologous domains of the $Na_V1.8$ protein are shown as cylinders (top). $Na_V1.8$ channels form around a central ion-conducting pore domain, which can adopt closed, open and inactivated conformations influenced by the position of peripheral voltage sensors shown in black (bottom). Adapted from [22]. SUZ binding to the closed state is represented by a blue oval (**b** and **c**) Pre-pulse voltages are applied to drive the $Na_V1.8$ channel to different states in the presence of SUZ. After a brief recovery pulse, inhibition of $Na_V1.8$ at different concentrations of SUZ is assessed and plotted against pre-pulse voltage. **d** Protocol to probe repetitive stimulation using a physiologically relevant voltage waveform. Inset shows expanded view of voltage protocol for each pulse in the 5 Hz train. **e** Representative current traces from 5 Hz repetitive stimulation experiment showing control and 10 nM SUZ. **f** Potency of SUZ is constant from first to last pulse measurements, consistent with a tonic mechanism of inhibition. Representative curves from one experimental replicate are shown in (**c** and **f**). Three independent experiments were conducted with a minimum of three technical replicates per experiment

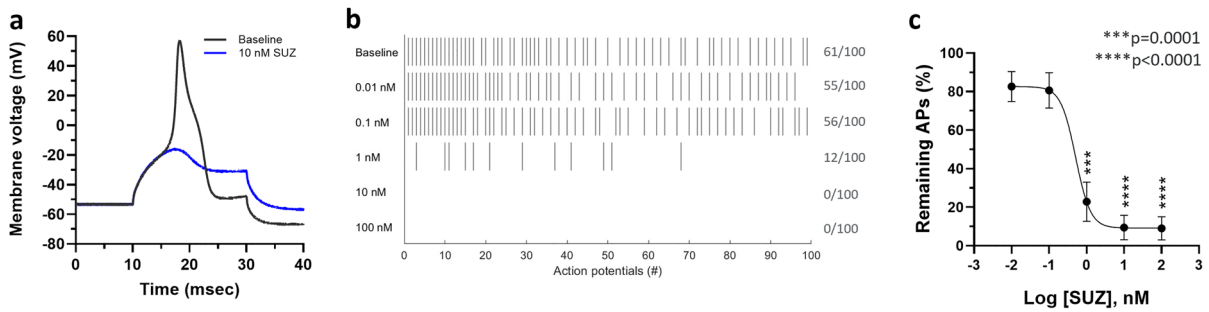

Fig. 4 Suzetrigine (SUZ) inhibits pain signals in human DRG neurons. **a** Representative single AP from a human DRG neuron elicited by a 20 ms (from 10 to 30 ms), 1.1 × rheobase current injection before (black) and after (blue) application of 10 nM SUZ. **b** Raster plot of evoked action potentials fired in response to 100, 1 Hz current injections at 1.1 × rheobase in the absence (baseline) and presence of increasing concentrations of SUZ. **c** Concentration–response relationship showing APs remaining following application of increasing concentrations of SUZ in 10 cells that responded (greater than 5% reduction in APs) out of 17 total cells recorded from 3 human donors. *** Denotes $p = 0.0001$; **** denotes $p < 0.0001$ (repeated-measures one-way ANOVA with Dunnett's multiple comparisons)

or ropivacaine, exhibited additive inhibition of $Na_V1.8$ across a dose matrix of varying concentrations of each of the agents (neither synergistic nor antagonistic) according to the Loewe generalized additivity model. $Na_V1.8$ IC_{50} measurements of these agents in combination are shown in Supplementary Fig. S5. As expected, suzetrigine did not show additive pharmacology with bupivacaine or ropivacaine on $Na_V1.2$ (Supplementary Fig. S6), since suzetrigine does not inhibit and is not expected to bind to other Na_Vs (Fig. 1c).

Nonclinical Animal Studies with Suzetrigine Show No Evidence of Safety Liabilities or Addictive Potential

A comprehensive safety assessment of suzetrigine was conducted including in vitro and in vivo studies in rats and monkeys at pharmacologically relevant exposures based on the measured IC_{50}s for $Na_V1.8$ across species (Fig. 1d); these studies are summarized in Table 1 and Supplementary Table S1.

In a CNS safety study in monkeys, we did not observe any neurobehavioral effects, including stimulant or sedative effects. There was no evidence of CNS effects associated with abuse potential or dependence in the rat and monkey repeat-dose toxicity studies at concentrations that exceeded intended therapeutic exposures. In a rat physical dependence study, abrupt withdrawal of suzetrigine did not produce any signs of dependence at pharmacologically relevant exposures (up to 52-fold the human C_{max} at steady-state and $>IC_{80}$ for $Na_V1.8$ inhibition [rat]), while the positive control article morphine did have effects on body temperature, body weight, and motor activity in the hours and days following cessation of treatment (Fig. 5).

Na_V channels are also present throughout the cardiovascular system, and nonselective Na_V blockers have cardiovascular side effects [69]. Further demonstrating the specificity of suzetrigine pharmacology, no effects were noted in cardiovascular assessments (including evaluation of effects on blood pressure and quantitative and qualitative changes on the ECG) or in respiratory assessments in telemetered monkeys after a single dose of suzetrigine. In repeat-dose monkey studies dosed for up to 9 months at exposures greater than or equal to those at the recommended human dose, no quantitative or qualitative changes were observed on surface ECGs.

Phase 3 Clinical Trials with Suzetrigine Show No Evidence of Addictive Potential

Three phase 3 trials, comprising 2447 participants, were previously conducted to assess the efficacy and safety of suzetrigine in moderate to severe acute pain with up to 14 days of treatment [49, 50]. A systematic analysis of AEs from a list of nearly 200 screening preferred terms that may be associated with abuse potential is summarized in Supplementary Tables S2 and S3. We found that the incidence of these abuse-related AEs was low and similar between suzetrigine and placebo in the two randomized trials. Dizziness was the most common AE and was observed at a numerically lower rate in participants who received suzetrigine ($n=33$, 3.8%) than in participants who received placebo ($n=28$, 6.4%) and those who received hydrocodone/acetaminophen ($n=47$, 5.3%). All other AEs related to these preferred terms were observed in ≤ 2 participants in each treatment group and were also similar between suzetrigine and placebo groups. In the open-label phase 3 study, AEs from the screening list of preferred terms were uncommon, occurring in only four (1.6%) participants. The most common AE was dizziness, which occurred in three (1.2%) participants.

DISCUSSION

The evidence presented here indicates that suzetrigine is a highly potent and selective inhibitor of $Na_V1.8$ with a novel mechanism of action and no addictive potential. Specifically, we demonstrate that (1) $Na_V1.8$ is expressed in the peripheral nervous system but not the brain; (2) suzetrigine inhibits $Na_V1.8$, but not other voltage-gated sodium channels; (3) suzetrigine

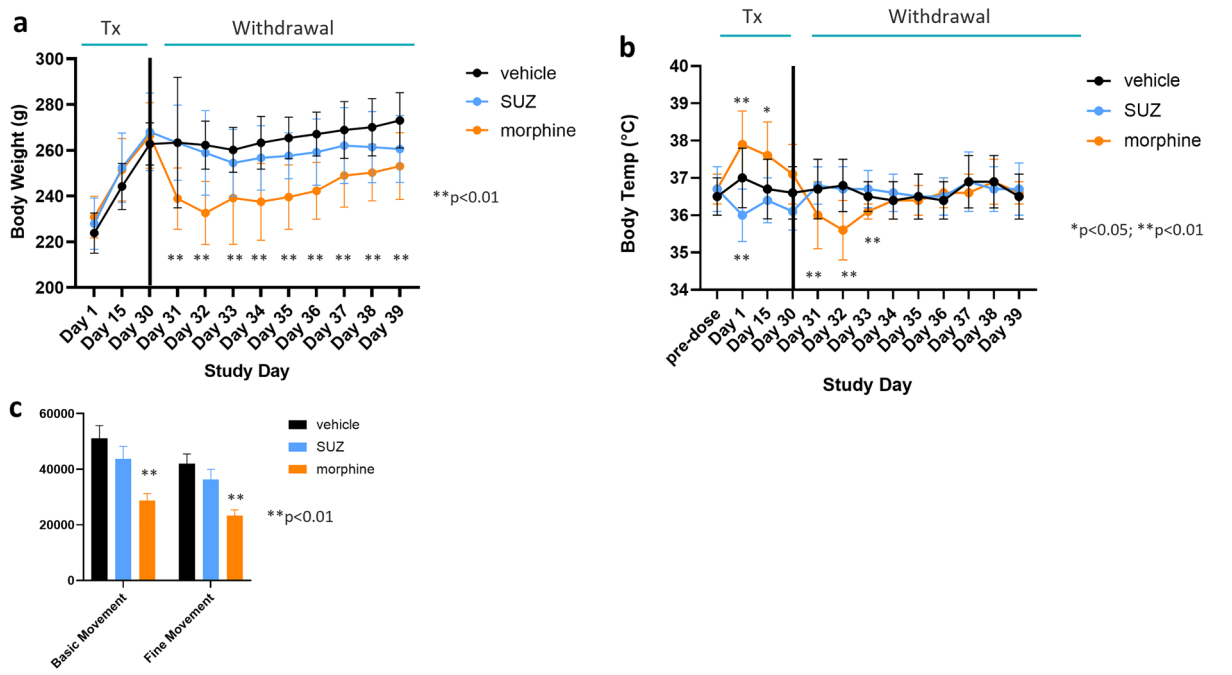

Fig. 5 Suzetrigine (SUZ) administration to female rats for 30 days followed by abrupt cessation did not produce signs of withdrawal. **a** Mean body weight (±SD). Rat body weight was measured on days 1, 15, and 30 during the treatment (Tx) period and daily during the withdrawal period. Body weight loss was observed in the morphine-positive control group beginning immediately during the withdrawal period but was not observed with SUZ. Significance was based on Kruskal–Wallis with post hoc Dunn's significance testing. **b** Mean body temperature (±SD). Morphine treatment was associated with hyperthermia during treatment, and hypothermia in the period following withdrawal of treatment. SUZ did not display any effects on body temperature during the withdrawal period. Significance was evaluated with ANOVA using post hoc Dunnett's test. **c** Least squares mean motor activity (±SE). In the 14 h following abrupt withdrawal of treatment, motor activity of the animals was monitored. Both basic and fine movements were decreased in the morphine-positive control group during the overnight hours (when rats are most active), while motor activity in the SUZ group was comparable to the vehicle control group

binds to a unique site on $Na_V1.8$ and has a novel allosteric mechanism of action that is different than nonspecific local anesthetics; (4) suzetrigine does not bind to or inhibit known targets for other CNS acting agents; (5) in vivo studies in monkeys and rats showed no findings indicating CNS activity or addictive potential; and (6) clinical trial data from over 2400 people did not show evidence of abuse or addictive potential [48–50]. Suzetrigine has demonstrated a statistically significant and clinically meaningful reduction in moderate to severe acute pain in human clinical trials with a favorable tolerability profile [48–50]. In addition, suzetrigine exhibited comparable efficacy to a commonly prescribed opioid [48, 49]. These data indicate that suzetrigine may be a promising non-opioid option to treat moderate to severe pain. Therefore, we conclude that a preclinical strategy focused on $Na_V1.8$ subtype selectivity and inhibition of pain signaling in human primary sensory neurons provides a model for preclinical to clinical translation in pain.

$Na_V1.8$ is selectively expressed in human peripheral pain-sensing neurons where its role is to transmit pain signals in the peripheral sensory nerve. The absence of $Na_V1.8$ expression in the CNS is fundamental evidence supporting the lack of addictive potential associated with highly selective $Na_V1.8$ inhibition. While gene expression data are available for $Na_V1.8$ in various human tissues, a formal analysis for selective

expression based on established statistical thresholds in the CNS has not been presented. Our evaluation of published gene expression data confirmed and extended that $Na_V1.8$ is not expressed in any of the > 190 regions sampled in the human brain or spinal cord. Based on validation of the role of $Na_V1.8$ in peripheral pain signaling by human genetics and clinical trials, and because there is no expression of $Na_V1.8$ detected in the CNS, a selective $Na_V1.8$ inhibitor would be expected to reduce peripheral pain signaling without addictive potential or affecting other physiological functions.

Suzetrigine is highly selective for $Na_V1.8$ over other Na_V subtypes because it binds to a specific site on VSD2 of $Na_V1.8$. While Na_Vs share high homology with each other [44], especially in the pore region where sodium ions permeate the channel, the voltage-sensing domains exhibit some sequence variation which have evolved to support their specific roles in different tissues. The suzetrigine binding site on $Na_V1.8$ depends on the extracellular loop of the S3–S4 segment of VSD2 and is similar to the site recently reported for a VX-150 ([4-[[2-(4-fluoro-2-methyl-phenoxy)-4-(trifluoromethyl)benzoyl] amino]-2-oxo-1-pyridyl]methyl dihydrogen phosphate [45] analogue [70]. While our results indicate that suzetrigine binding depends on the KKGS sequence unique to $Na_V1.8$ among human Na_V subtypes, it may not be the only region of the protein important for conferring $Na_V1.8$-selectivity of suzetrigine. Indeed, the species selectivity of suzetrigine cannot be explained only by an interaction with the KKGS sequence since this motif is conserved in other species and Gilchrist et al. identified additional residues within the S3–S4 region of VSD2 that are important for the species specificity of a related $Na_V1.8$ inhibitor [71]. We can speculate that the unique sequence of the $Na_V1.8$ VSD2 site may have evolved to support the distinct biophysics of $Na_V1.8$ relative to other Na_Vs, including its high voltage threshold for activation and its slow kinetics of inactivation, which have been linked to its physiological role [22]. The preference of suzetrigine for human and nonhuman primates was considered when conducting trials with suzetrigine in different species.

By binding to the VSD2 site located away from the pore and stabilizing the channel in a closed state, suzetrigine is an allosteric channel inhibitor not a channel blocker. Moreover, the suzetrigine binding site is a novel drug binding site that is distinct from nonselective Na_V channel blockers or other analgesics which bind nonselectively to a site within the pore region and block permeation of sodium through the channel [23–25, 67]. The ≥ 31,000-fold selectivity that suzetrigine exhibits for $Na_V1.8$ over other Na_Vs in vitro is a product of both the unique binding site and the high specificity of suzetrigine for this site. It has not been determined how binding of suzetrigine to VSD2 stabilizes the closed state, but it is likely that drug binding to the closed state of $Na_V1.8$ impedes the movement of VSD2, thereby preventing the channel from opening following depolarization. Whether other $Na_V1.8$ binding sites exist with the potential for highly selective inhibition also remains to be determined.

Our results show that the binding of suzetrigine to VSD2 of $Na_V1.8$ stabilizes the channel in the closed state which is consistent with recent reports on the mechanism of action of suzetrigine and other related $Na_V1.8$ inhibitors [70, 71]. Protocols designed to demonstrate this mechanism of action require strong and extended depolarization of cells to observe relief of inhibition, a phenomenon referred to as reverse use-dependence [70]. While this mechanism of action, in principle, could result in reduced efficacy under repetitive firing conditions, the data presented here show that suzetrigine maintains inhibition under repetitive depolarizations that mimic APs (pain signals) in pain-sensing neurons. Therefore, suzetrigine can be considered a tonic inhibitor of $Na_V1.8$ because it does not require the channel to open and maintains consistent inhibition over a wide range of voltages and AP frequencies that could occur across pain states. In contrast, the potency of Na_V blockers currently in clinical use differs depending on AP frequency [23–25].

Traditional rodent behavioral pain models have had limited success in assessing potential for clinical efficacy [19, 20]. To assess the potential for suzetrigine to demonstrate clinical pain reduction, we evaluated the effect of suzetrigine

in primary sensory neuronal cultures isolated from human DRG containing both pain-sensing nociceptors and non-nociceptors [26–28]. Suzetrigine significantly inhibited pain signals in the majority (~60%) of DRG neurons in a dose-dependent manner. Near maximal pain signal inhibition was observed at high levels of target coverage (10 nM, corresponding to predicted target coverage > IC_{90}). Based on these data, we aimed to achieve free plasma concentrations equal to or greater than 10 nM in clinical trials.

In one phase 2 and two phase 3 randomized controlled trials following bunionectomy and abdominoplasty surgeries, suzetrigine demonstrated a statistically significant and clinically meaningful reduction in moderate to severe pain compared to placebo at a 100 mg loading dose followed by a 50 mg maintenance dose every 12 h [48, 49]. This reduction in pain was further supported by a phase 3 single-arm safety and effectiveness study with suzetrigine at the same dose administered for up to 14 days in participants with a range of surgical and non-surgical, moderate to severe acute pain conditions. In this study, most participants rated the effectiveness of suzetrigine for treating pain on a patient global assessment (PGA) as good, very good, or excellent at the end of treatment [50]. The clinical results suggest that the preclinical pain signaling assay in human DRG neurons was generally predictive of clinical efficacy. It will be of interest to determine the quantitative relationship between the level of pain signal inhibition in vitro and clinical efficacy seen with suzetrigine, including the potential contribution of its active metabolite. This analysis will be aided by clinical trials that demonstrate saturation of effect in vivo, which has yet to be established [48]. It also remains to be established whether selective inhibition of additional Na_Vs would further increase the percentage of responsive neurons and/or clinical efficacy.

Because pain is often treated using a multimodal approach that combines different analgesic classes, it is of interest to assess potential additivity or interference between analgesic classes. As local anesthetics can block all Na_Vs, including $Na_V1.8$, and might be administered along with suzetrigine, we conducted in vitro dose matrix studies of two local anesthetics (bupivacaine and ropivacaine) and suzetrigine to assess the effects of co-application on $Na_V1.8$. These studies indicated that the local anesthetics and suzetrigine showed simple additive pharmacology and are consistent with the distinct mechanism of action described above. The practical implication of this is that continuous inhibition of $Na_V1.8$ would be expected if suzetrigine was present as the anesthetic block wears off. Although not studied here, it is also expected that suzetrigine could be administered with other nonselective Na_V blockers, such as anticonvulsants (carbamazepine) or antiarrhythmics (mexiletine), as well as other analgesics, without interfering with their mechanisms of action.

A key conclusion from this report is that the high selectivity of suzetrigine for $Na_V1.8$ and its mechanism of action translated to a well-tolerated profile in vivo, with no evidence of addictive potential. This conclusion is supported by three sets of data presented here. First, in vitro profiling showed that suzetrigine was selective against an extensive panel of non-Na_V targets including 44 targets associated with abuse potential, with a > 600-fold margin to estimated clinical concentrations. Second, suzetrigine was well-tolerated in nonclinical in vivo safety studies in rats and monkeys and exhibited no cardiovascular or behavioral effects, including stimulant or sedative effects at exposure multiples greater than the estimated human exposure. There was no evidence of withdrawal in a rat physical dependence study at pharmacologically relevant doses. Importantly, two phase 2 and three phase 3 trials showed that suzetrigine was well-tolerated and effective in patients with moderate to severe acute pain [48–50]. Lastly, in these phase 3 human clinical trials, suzetrigine showed no evidence of abuse or addictive potential based on an analysis of relevant AEs.

This assessment has several limitations as noted throughout the discussion. Of note, the in vitro data presented demonstrate that potent and selective inhibition of $Na_V1.8$ by suzetrigine results in inhibition of pain signals in primary human DRG neurons and this translated to clinical efficacy. More data will be needed to fully characterize the quantitative relationship

between the level of pain signal inhibition in vitro and clinical efficacy. In addition, the clinical safety assessments provided here are derived from trials of acute pain with dosing up to 14 days. Longer term clinical trials will further inform the long-term safety profile of suzetrigine and future compounds within this novel class.

CONCLUSIONS

The in vitro and in vivo data presented here expand upon previously reported results for suzetrigine by further characterizing its mechanism of action and summarizing nonclinical and clinical data that support the translation of selective $Na_V1.8$ inhibition into clinical efficacy and safety, including lack of addictive potential. Suzetrigine represents a potential new class of non-opioid analgesics that are selective $Na_V1.8$ pain signal inhibitors. By acting in the peripheral nervous system to safely treat pain without addictive potential, selective pain signal inhibitors could alter the paradigm of pain management.

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Data Availability. The datasets generated during and/or analyzed during the current study are available on reasonable request. Vertex Pharmaceuticals is committed to advancing medical science and improving patient health. This includes the responsible sharing of data with qualified researchers. Proposals for the use of these data will be reviewed by a scientific board. Approvals are at the discretion of Vertex and will be dependent on the nature of the request, the merit of the research proposed, and the intended use of the data. Please contact CTDS@vrtx.com if you would like to submit a proposal or need more information.

Declarations

Conflict of interest. All authors (Jeremiah D. Osteen, Swapna Immani, Tim L. Tapley, Tim Indersmitten, Nicole W. Hurst, Tiffany Healey, Kathleen Aertgeerts, Paul A. Negulescu, Sandra M. Lechner) are employees of Vertex Pharmaceuticals and own stock and/or options in the company.

Ethical Approval. Animal studies were conducted in Sprague Dawley (CD®[Crl:CD®(SD)]) rats and cynomolgus monkeys. Animals were sourced from Charles River Laboratories (USA).

Each study in animals was approved by the laboratory's Institutional Animal Care and Use Committee (IACUC). Animal care conformed to applicable national/international guidelines and the studies were conducted in accordance with Good Laboratory Practice (GLP). The clinical trial protocols were approved by a central institutional review board (Advarra Institutional Review Board, Columbia, MD, USA) and all sites accepted this central approval. The trials were conducted according to the International Council for Harmonization Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. All participants provided written informed consent.

REFERENCES

1. Sinatra R. Causes and consequence of inadequate management of acute pain. Pain Med. 2010;11:1859–71.
2. Kent ML, Tighe PJ, Belfer I, et al. The ACTTION-APS-AAPM pain taxonomy (AAAPT) multidimensional approach to classifying acute pain conditions. J Pain. 2017;18:479–89.
3. Yong RJ, Mullins PM, Bhattacharyya N. Prevalence of chronic pain among adults in the United States. Pain. 2022;163(2):e328–32.
4. Fillingim RB, Bruehl S, Dworkin RH, et al. The ACTTION-American Pain Society Pain Taxonomy (AAPT): an evidence-based and multidimensional approach to classifying chronic pain conditions. J Pain. 2014;5:241–9.
5. Chou R, Gordon DB, de Leon-Casasola OA, et al. Management of postoperative pain: a clinical practice guideline from the American Pain Society, the American Society of Regional Anesthesia in Pain Medicine, and the American Society of Anesthesiologists' Committee on Regional Anesthesia, Executive Committee, and Administrative Council. J Pain. 2016;17:131–57.
6. Gilron I, Baron R, Jensen T. Neuropathic pain: principles of diagnosis and treatment. Mayo Clin Proc. 2015;90(4):532–45.
7. Finnerup NB, Attal N, Haroutounian S, et al. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. Lancet Neurol. 2015;14:162–73.
8. Lunn MP, Hughes RA, Wiffen PJ. Duloxetine for treating painful neuropathy, chronic pain or fibromyalgia. Cochrane Database Syst Rev. 2014;1: CD007115.
9. Wiffen PJ, Derry S, Bell RF, Rice ASC, Tölle TR, Phillips T, et al. Gabapentin for chronic neuropathic pain in adults. Cochrane Database Syst Rev. 2017;6: CD007938.
10. Derry S, Bell RF, Straube S, Wiffen PJ, Aldington D, Moore RA, et al. Pregabalin for neuropathic pain in adults. Cochrane Database Syst Rev. 2019;1: CD007076.
11. Russo M, Graham B, Santarelli DM. Gabapentin-friend or foe? Pain Pract. 2023;1:63–9.
12. Ishitsuka Y, Kondo Y, Kadowaki D. Toxicological property of acetaminophen: the dark side of a safe antipyretic/analgesic drug? Biol Pharm Bull. 2020;43(2):195–206.
13. Maher AJ, Metcalfe SA, Parr S. Local anaesthetic toxicity. Foot (Edinb). 2008;18(4):192–7.
14. Kurz A, Sessler DI. Opioid-induced bowel dysfunction: pathophysiology and potential new therapies. Drugs. 2003;63:649–71.
15. Machelska H, Celik MO. Advances in achieving opioid analgesia without side effects. Front Pharmacol. 2018;9:1388.
16. Tyndale RF, Sellers EM. Opioids: the painful public health reality. Clin Pharmacol Ther. 2018;103(6):924–35.

17. Coussens NP, Sittampalam S, Jonson SG, et al. The opioid crisis and the future of addiction and pain therapeutics. J Pharmacol Exper Ther. 2019;371(2):396–408.

18. Kissin I. The development of new analgesics over the past 50 years: a lack of real breakthrough drugs. Anesth Analg. 2010;110:780–9.

19. Eagles DA, Chow CY, King GF. Fifteen years of NaV1.7 channels as an analgesic target: Why has excellent in vitro pharmacology not translated into in vivo analgesic efficacy? Br J Pharm. 2022;179:3592–611.

20. Mogil JS. Animal models of pain: progress and challenges. Nat Rev Neurosci. 2009;10:283–94.

21. Krafte D, Bannon A. Sodium channels and nociception: recent concepts and therapeutic opportunities. Curr Opin Pharmacol. 2008;8(1):50–6.

22. Bennett DL, Clark AJ, Huang J, Waxman SG, Dib-Hajj SD. The role of voltage-gated sodium channels in pain signaling. Physiol Rev. 2019;99(2):1079–151.

23. Catterall WA. Voltage-gated sodium channels at 60: structure, function and pathophysiology. J Physiol. 2012;590(11):2577–89.

24. Ahern CA, Payandeh J, Bosmans F, Chanda B. The hitchhiker's guide to the voltage-gated sodium channel galaxy. J Gen Physiol. 2016;147:1–24.

25. Wu Q, Huang J, Fan X, et al. Structural mapping of Nav1.7 antagonists. Nat Commun. 2023;14:3224.

26. Shiers S, Klein RM, Price TJ. Quantitative differences in neuronal subpopulations between mouse and human dorsal root ganglia demonstrated with RNAscope in situ hybridization. Pain. 2020;161(10):2410–24.

27. Ray P, Torck A, Quigley L, et al. Comparative transcriptome profiling of the human and mouse dorsal root ganglia: an RNA-seq-based resource for pain and sensory neuroscience research. Pain. 2018;159(7):1325–45.

28. The University of Texas at Dallas, Center for Advanced Pain Studies. Available at: https://painc enter.utdallas.edu/sensoryomics/.

29. Yang Y, Wang Y, Li S, et al. Mutations in SCN9A encoding a sodium channel alpha subunit, in patients with primary erythermalgia. J Med Genet. 2004;41(3):171–4.

30. Fertleman CR, Baker MD, Parker KA, et al. SCN9A mutations in paroxysmal extreme pain disorder: allelic variants underlie distinct channel defects and phenotypes. Neuron. 2006;52(5):767–74.

31. Cox JJ, Reimann F, Nicholas AK, et al. An SCN9A channelopathy causes congenital inability to experience pain. Nature. 2006;444(7121):894–8.

32. Faber CG, Lauria G, Merkies ISJ, et al. Gain-of-function Nav1.8 mutations in painful neuropathy. Proc Natl Acad Sci U S A. 2012;109(47):19444–9.

33. Han C, Vasylyev D, Macala LJ, et al. The G1662S NaV1.8 mutation in small fibre neuropathy: impaired inactivation underlying DRG neuron hyperexcitability. J Neurol Neurosurg Psychiatry. 2014;85(5):499–505.

34. Leipold E, Liebmann L, Korenke GC, et al. A de novo gain-of-function mutation in SCN11A causes loss of pain perception. Nat Genet. 2013;45(11):1399–404.

35. Leipold E, Hanson-Kahn A, Frick M, et al. Cold-aggravated pain in humans caused by a hyperactive NaV1.9 channel mutant. Nat Commun. 2015;6:10049.

36. Akopian AN, Sivilotti L, Wood JN. A tetrodotoxin-resistant voltage-gated sodium channel expressed by sensory neurons. Nature. 1996;379(6562):257–62.

37. Hameed S. Na(v)1.7 and Na(v)1.8: role in the pathophysiology of pain. Mol Pain. 2019;15:1744806919858801.

38. Han C, Estacion M, Huang J, et al. Human Na(v)1.8: enhanced persistent and ramp currents contribute to distinct firing properties of human DRG neurons. J Neurophysiol. 2015;113(9):3172–85.

39. Uhlén M, et al. Tissue-based map of the human proteome. Science. 2015;347:1260419.

40. Digre A, Lindskog C. The human protein atlas - Integrated omics for single cell mapping of the human proteome. Protein Sci. 2023;32(2): e4562.

41. The Human Protein Atlas Project. Human Protein Atlas: SCN10A gene page. 2023. Available at: https://www.proteinatlas.org/search/scn10a. Accessed 10 Sept 2024.

42. The GTEx Consortium. The GTEx Consortium atlas of genetic regulatory effects across human tissues. Science. 2020;369:1318–30.

43. Broad Institute. GTEx portal: SCN10A gene page. 2024. Available at: https://www.proteinatlas.org/search/scn10a. Accessed 13 May 2024.

44. Catterall WA, Goldin AL, Waxman SG. International Union of Pharmacology. XLVII. Nomenclature and structure-function relationships of voltage-gated sodium channels. Pharmacol Rev. 2005;57(4):397–409.

45. WO/2015/089361: Prodrugs of Pyridone Amides Useful as Modulators of Sodium Channels.

46. NCT03206749: A Study to Evaluate Efficacy and Safety of VX-150 in Subjects with Acute Pain Following Bunionectomy. VX16-150-103. https://clinicaltrials.gov/study/NCT03206749?intr=vx-150&rank=6&tab=results.

47. NCT03304522: A Study to Evaluate the Efficacy and Safety of VX-150 in Treating Subjects With Pain Caused by Small Fiber Neuropathy. VX16-150-102. https://clinicaltrials.gov/study/NCT03304522?intr=VX-150&rank=4&tab=results.

48. Jones J, Correll DJ, Lechner SM, et al. Selective inhibition of NaV1.8 with VX-548 for acute pain. N Engl J Med. 2023;389(5):393–405.

49. Bertoch T, D'Aunno D, McCoun J, et al. Randomized, placebo-controlled, phase 3 trials of suzetrigine, a non-opioid, pain signal inhibitor for treatment of acute pain after abdominoplasty or bunionectomy. American Society of Anesthesiologists Annual Meeting. 2024; Presentation A1187.

50. McCoun J, Winkle P, Solanki D, et al. A Phase 3, single-arm study of suzetrigine, a non-opioid, pain signal inhibitor for treatment of acute pain from surgical and non-surgical conditions. American Society of Anesthesiologists Annual Meeting. 2024; Presentation A2074, Poster 74.

51. NCT05660538: Evaluation of Efficacy and Safety of VX-548 for Painful Diabetic Peripheral Neuropathy (DPN). VX21-548-103. https://clinicaltrials.gov/study/NCT05660538?intr=vx-548&page=2&rank=12.

52. Clairfeuille T, Cloake A, Infield DT, et al. Structural basis of α-scorpion toxin on Na_V channels. Science. 2019;363(6433): eaav8573.

53. Shen H, Zhou Q, Pan X, et al. Structure of a eukaryotic voltage-gated sodium channel at near-atomic resolution. Science. 2017;355(6328): eaal4326.

54. OpenEye Toolkits 2022.11. OpenEye, Cadence Molecular Sciences, Santa Fe, NM. https://www.eyesopen.com/.

55. Jasial S, Hu Y, Vogt M, Bajorath J. Activity-relevant similarity values for fingerprints and implications for similarity searching. Chem Inf Sci. 2016;5:591.

56. Patterson DE, Cramer RD, Ferguson AM, Clark RD, Weinberger LE. Neighborhood behavior: a useful concept for validation of "molecular diversity" descriptors. J Med Chem. 1996;39(16):3049–59.

57. BIGL package in R version 4.4.1: https://www.nature.com/articles/s41598-017-10868-5.

58. R Core Team 2024. R: A language and Environment for Statistical Computing. Vienna, Austria. https://www.r-project.org/.

59. Moser VC, Ross JF. US EPA/AIHC training video and reference manual for a functional observational batter. Washington, DC: Environmental Protection Agency; 1996.

60. Moser VC, McCormick JP, Creason JP, MacPhail RC. Comparison of chlordimeform and carbaryl using a functional observational battery. Fund Appl Toxicol. 1988;11:189–206.

61. Meyer OA, Tilson HA, Byrd WC, Riley MT. A method for the routine assessment of fore and hindlimb grip strength of rats and mice. Neurobehav Toxicol. 1979;1:233–6.

62. Edwards PM, Parker VH. A simple, sensitive and objective method for early assessment of acrylamide neuropathy in rats. Toxicol Appl Pharmacol. 1977;40:589–91.

63. Ankier SI. New hot plate tests to quantify antinociceptive and narcotic antagonist activities. Eur J Pharmacol. 1974;27:1–4.

64. U.S. Department of Health and Human Services Food and Drug Administration. Assessment of Abuse Potential of Drugs: Guidance for Industry. 2017. https://www.fda.gov/drugs/guidance-compliance-regulatory-information/guidances-drugs.

65. Hasin DS, O'Brien CP, Auriacombe M, et al. DSM-5 criteria for substance use disorders: recommendation and rationale. Am J Psychiatry. 2013;170(8):834–51.

66. Olsen Y. What is addiction? History, terminology, and core concepts. Med Clin N Amer. 2022;106:1–12.

67. Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. Neuron. 2000;26:13–25.

68. Becker DE, Reed KL. Local anesthetics: review of pharmacological considerations. Anesth Prog. 2012;59:90–102.

69. El-Boghdadly K, Pawa A, Chin KJ. Local anesthetic systemic toxicity: current perspectives. Local Reg Anesth. 2018;11:35–44.

70. Vaelli P, Fujita A, Jo S, et al. State-dependent inhibition of NaV1.8 channels by VX-150 and VX-548. Mol Pharm. 2024. https://doi.org/10.1124/molpharm.124.000944.

71. Gilchrist JM, Yang N-D, Jiang V, Moyer BD. Pharmacologic characterization of LTGO-33, a selective small molecule inhibitor of the voltage-gated sodium channel NaV1.8 with a unique mechanism of action. Mol Pharmacol. 2024;105:233–49.