



Towards Structure-Guided Development of Pain Therapeutics Targeting Voltage-Gated Sodium Channels

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Voltage-gated sodium (Na_v) channels are critical molecular determinants of action potential generation and propagation in excitable cells. Normal Na_v channel function disruption can affect physiological neuronal signaling and lead to increased sensitivity to pain, congenital indifference to pain, uncoordinated movement, seizures, or paralysis. Human genetic studies have identified human Na_v1.7 (hNa_v1.7), hNa_v1.8, and hNa_v1.9 channel subtypes as crucial players in pain signaling. The premise that subtype selective Na_v inhibitors can reduce pain has been reinforced through intensive target validation and therapeutic development efforts. However, an ideal therapeutic has yet to emerge. This review is focused on recent progress, current challenges, and future opportunities to develop Na_v channel targeting small molecules and peptides as non-addictive therapeutics to treat pain.

Keywords: sodium channel, peptide toxin, local anesthetic, voltage sensor, pain therapeutic

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INTRODUCTION

Voltage-gated sodium (Na_v) channels play vital roles in initiating and propagating action potentials in excitable cells, conduct pain signals in primary afferents, and emerged as attractive targets for developing non-addictive pain therapeutics (Mulcahy et al., 2019; Alsaloum et al., 2020). The human genome contains nine Na_v channel subtypes expressed in various cell types and tissues (Na_v1.1 to Na_v1.9) (Catterall et al., 2005). Na_v1.7, Na_v1.8, and Na_v1.9 channels are predominantly expressed in the peripheral nervous system and linked to various pain disorders in humans (Cheng et al., 2021). Genetic and functional studies have identified and validated Na_v1.7, Na_v1.8, and Na_v1.9 channels as targets for pain treatment. This review briefly summarizes the recent progress, current challenges, and discusses the potential use of advanced computational methods for structure-guided development of selective Na_v channel inhibitors as therapeutics to treat pain.

CURRENT EFFORTS IN THE DEVELOPMENT OF PAIN THERAPEUTICS TARGETING SPECIFIC BINDING SITES ON Na_v CHANNELS

The structure of eukaryotic Na_v channel α subunit comprises four homologous domains I, II, III, IV, each containing six transmembrane segments S1 to S6. The segments S1 to S4 form four distinct voltage sensor domains (VSD I, VSD II, VSD III, and VSD IV) that sense membrane voltage and trigger conformational changes (Ahern et al., 2016) that couple to the pore, which is formed by the segments S5 and S6 from each

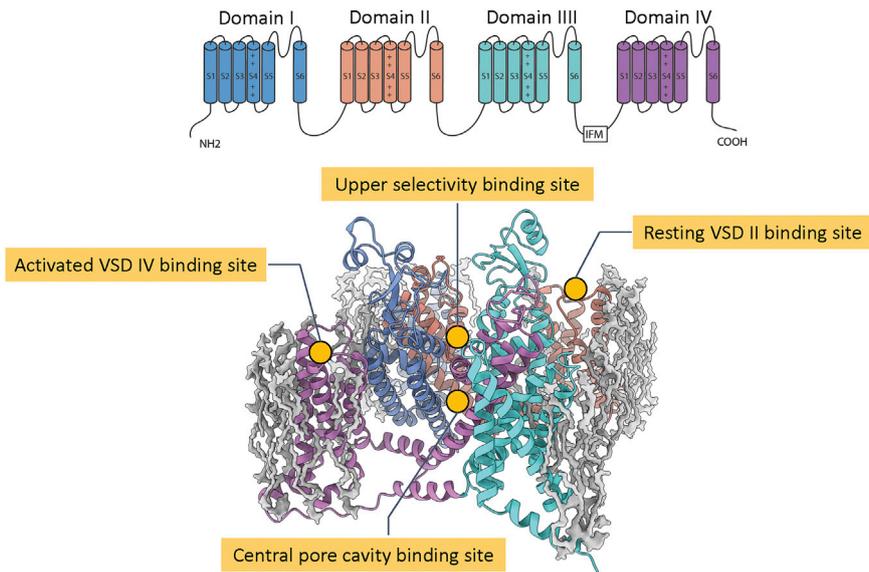


FIGURE 1 | Notable pharmacological binding sites on Na_v channels. The top panel shows the transmembrane topology of a eukaryotic Na_v channel with four homologous domains and six transmembrane segments S1 to S6. The bottom panel shows a structure of a Na_v channel (colored ribbons) with four common pharmacological binding sites (labeled) embedded in a lipid patch (gray blobs).

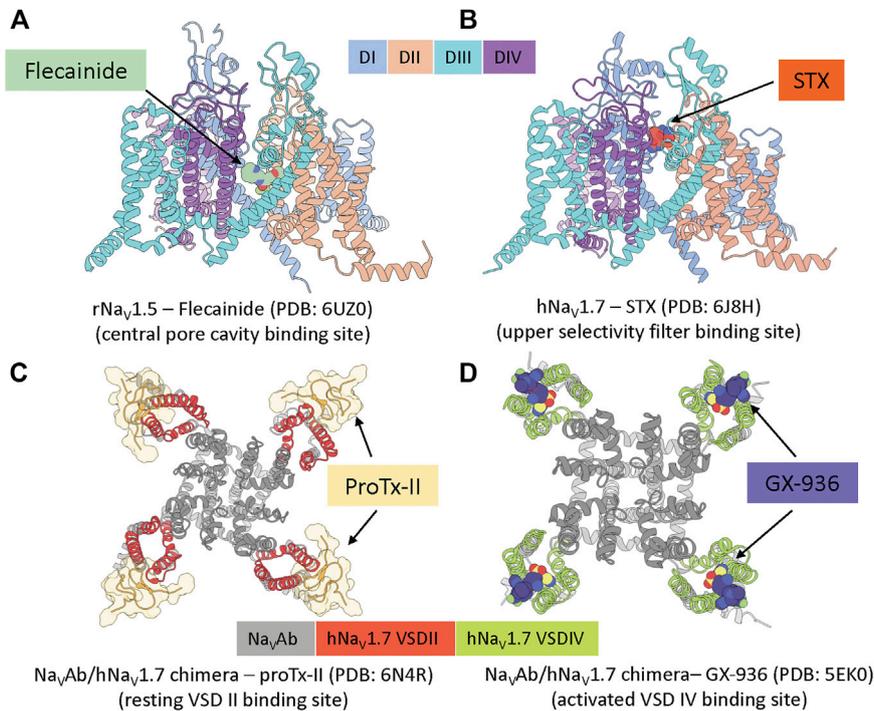


FIGURE 2 | Example structures of small molecules and peptide toxins binding to the four common pharmacological binding sites. **(A)**, rat Na_v1.5 channel in complex with antiarrhythmic drug flecainide (pdb: 6uz0) (Jiang et al., 2020). **(B)**, human Na_v1.7 channel in complex with saxitoxin STX (pdb: 6j8h) (Shen et al., 2019). **(C)**, bacterial Na_vAb/human Na_v1.7(VSDII) chimeric channel in complex with protoxin II (pdb: 6n4r) (Xu et al., 2019). **(D)**, bacterial Na_vAb/human Na_v1.7(VSDIV) chimeric channel in complex with investigational compound GX-936 (pdb: 5ek0) (Ahuja et al., 2015).

domain. The membrane depolarization activates VSD I, VSD II, and VSD III to open the channel pore and the subsequent activation of VSD IV leads to the fast inactivation of the channel (Ahern et al., 2016). Biophysical and pharmacological characterization identified multiple binding sites for neurotoxins and drugs on Na_v channels (Stevens et al., 2011). Grouping them by location and conformational state, there are four binding sites that have attracted significant interests in the development of pain therapeutics: the central pore cavity, the upper selectivity filter, the resting VSD II, and the activated VSD IV (Figure 1).

The Central Pore Cavity

The central pore cavity binding site is formed by transmembrane segments S5 and S6 (Figure 1). Drugs are thought to access this binding site either from the intracellular side *via* the S6 gate or from the lipid membrane through inter-domain fenestrations (Hille, 1977), although recent evidence has suggested direct access from the extracellular side is possible (Lee et al., 2019; Nguyen et al., 2019). This location is a hub of channel interactions with drugs and neurotoxins. Notably, local anesthetics, such as flecainide (see Figure 2A) and lidocaine, bind to this region and directly block ion conduction or trap the channel in non-conducting states (Hille, 1977). Neurotoxins such as batrachotoxin and veratridine could engage in an interesting dual-action mechanism that can stabilize the open state while partially blocking ion conduction (Wang et al., 2007) (Craig et al., 2020). Binding at this location often exhibits state-dependent and use-dependent effects in which binding affinity is highly dependent on the membrane potentials and frequencies of stimulation protocols.

This binding site has drawn significant interest due to the clinical use of broad-spectrum small molecule sodium channel inhibitors like local anesthetics, antiarrhythmics, and antiepileptics without adverse effects. For example, FDA has approved lacosamide for partial-onset seizures (Lattanzi et al., 2015), lamotrigine for epilepsy (Nolan et al., 2016), and carbamazepine for the treatment of trigeminal neuralgia (Cruccu et al., 2008). Because these compounds are non-selective, the use cases are limited, highlighting the need for improved therapeutics. However, the high sequence homology among Na_v isoforms in the central pore cavity has created challenges for developing selective compounds targeting this site. Investigational compounds such as Vixotrigine (Convergence/Biogen) targeting Na_v1.7 (Hinckley et al., 2021), currently in phase II clinical trials for trigeminal neuralgia and small fiber neuropathy or PF-01247324 (Pfizer) (Payne et al., 2015), PF-04531083 (Pfizer) (Bagal et al., 2014) targeting Na_v1.8 are non-selective or have modest selectivity against the on-target channel. Another Na_v1.8 targeting compound, A-803467 (Abbott/Icagen) also binds to the T-type calcium channel in low micromolar affinity, within the range of therapeutic concentration (Bladen and Zamponi, 2012). Nevertheless, the central pore cavity binding site is still a tractable target even though achieving subtype selectivity could be challenging. Perhaps, this is because broad-spectrum and state-dependent compounds could still be effective and safe for local administrations or conditions with elevated neural activities

such as neuropathic pain, as demonstrated by previously approved Na_v inhibitor drugs.

The Upper Selectivity Filter Binding Site

This binding site is located at the entrance of the selectivity filter, mainly formed by residues from the P2 helices in each Na_v channel domain (Figure 1). The binding site is well-known for different classes of marine toxins such as tetrodotoxin (TTX), saxitoxin (STX) (see Figure 2B), and μ -conotoxin (Stevens et al., 2011) that directly block sodium ions from passing through the channel. TTX has classically been used to classify Na_v channels based on their sensitivity to the toxin: Na_v1.5, Na_v1.8, Na_v1.9 are TTX resistant (TTX-R) while the others are TTX sensitive (TTX-S). Because this binding site is broadly accessible at all membrane potentials, toxin binding here exhibits minimal state-dependent effect, distinguishing this site from all other binding sites mentioned. Mutagenesis and structural studies showed TTX and STX are located deeper in the selectivity filter while μ -conotoxins interact more dominantly with residues at the entrance of the selectivity filter and the extracellular loops of the pore (Walker et al., 2012; Pan et al., 2019). Interestingly, two residues near the binding site for these toxins located on the Na_v1.7 DIII P2 helix, T1398/I1399 (hNa_v1.7 numbering), are unique among human Na_v channels making this site attractive for subtype selectivity optimization targeting Na_v1.7.

The development of selective inhibitors for this binding site has been pioneered by SiteOne Therapeutics. Using structure-activity relationship (SAR) study interrogating the T1398/I1399 positions, SiteOne Therapeutics first obtained several selective STX analogs that have >1,000-fold selectivity for Na_v1.7 over Na_v1.4 and Na_v1.6 (Mulcahy et al., 2018). Recently, a compound named ST-2262 was reported to be potent (IC₅₀ = 72 nM) with >200-fold subtype selectivity over hNa_v1.6 and >900-fold over all other hNa_v isoforms (except for hNa_v1.9, which was not tested) (Pajouhesh et al., 2020). ST-2262 was tested on non-human primate pain models and exhibited reduced sensitivity to noxious heat. More recently, the company disclosed another saxitoxin analog named ST-2530 that is potent (IC₅₀ = 25 nM) and highly selective with >500-fold over hNa_v1.1–hNa_v1.6 and hNa_v1.8 (Beckley et al., 2021). Testing in mice, the ST-2530 was analgesic in acute pain models using noxious thermal, mechanical, and chemical stimuli when administered subcutaneously. The compound also showed reversal of thermal hypersensitivity after a surgical incision on the plantar surface of the hind paw and transiently reversed mechanical allodynia in the spared nerve injury model of neuropathic pain. Another compound from SiteOne Therapeutics, ST-2427 is currently in Phase I clinical trial.

The Resting VSD II

The resting VSD II binding site is formed by the extracellular S1-S2 and S3-S4 regions on VSD II (Figure 1). Notably, this site is well-known for binding of several cysteine knot spider toxins, including ProTx-II (see Figure 2C), ProTx-III, HwTx-IV, GpTx-1, and JzTx-V (Schmalhofer et al., 2008; Xiao et al., 2008; Cardoso et al., 2015; Murray et al., 2016; Moyer et al., 2018). These peptide toxins bind with a higher affinity to the resting state of VSD II and

can bind with a lower affinity to the activated state of VSD II. High-affinity binding to the resting state of VSD II prevents the voltage sensor from activation upon depolarization and traps the channel in a closed state.

Several peptide toxins targeting resting VSD II binding site are at least partially $\text{Na}_V1.7$ selective, making them attractive for pain therapeutic developments. However, several reports have pointed out that they have limited analgesic efficacy in animal pain models, likely caused by a narrow therapeutic window (Schmalhofer et al., 2008; Liu et al., 2014; Deuis et al., 2017). The development of pain therapeutics for this binding site focuses on engineering peptides to enhance subtype selectivity targeting $\text{Na}_V1.7$. Using SAR and multiattribute positional scan (MAPS), Amgen used GpTx-1, a 34-residue tarantula peptide toxin that has potent activity on $\text{Na}_V1.7$ ($\text{IC}_{50} = 10 \text{ nM}$) and promising selectivity against important off-target Na_V subtypes (20-fold over $\text{Na}_V1.4$ (skeletal muscle) and 1,000-fold over $\text{Na}_V1.5$ (cardiac)) to engineer a GpTx-1 analog (named compound 71) that is nearly 10-fold more potent than the wild-type GpTx-1 ($\text{IC}_{50} = 1.6 \text{ nM}$) and has >1,000-fold selectivity over $\text{Na}_V1.4$ and $\text{Na}_V1.5$ (Murray et al., 2015). However, Amgen did not advance this compound for further evaluation *in vivo*. Amgen also used another tarantula peptide toxin, 29-residue JzTx-V, to engineer several highly selective analogs (Wu et al., 2018). The final lead peptide (named compound AM-6120) has six modifications from the wild-type toxin, including three non-canonical amino acids. AM-6120 is highly potent ($\text{IC}_{50} = 0.8 \text{ nM}$), has ~ 100 folds selectivity over $\text{Na}_V1.4$ and >750 folds over $\text{Na}_V1.5$, $\text{Na}_V1.6$ and $\text{Na}_V1.8$. Testing in animal pain models showed that the compound robustly blocked histamine-induced scratching in mice after subcutaneous administration. Notably, high plasma drug concentration (100-fold over the *in vitro* IC_{50}) was essential to establish the pharmacological effect. Additionally, AM-6120 had no effect in a capsaicin-induced nociception pain model. The developers suggested that AM-6120 may need high exposure in plasma to achieve effective target entanglement, a problem that has been previously characterized for $\text{Na}_V1.7$ small molecule inhibitors. Amgen has filed related patents for GpTx-1 and JzTx-V analogs, but no further development has been disclosed.

Using a thorough positional scanning approach, Janssen produced a library of 1,500 analogs of ProTx-II, a highly potent tarantula toxin ($\text{IC}_{50} < 1 \text{ nM}$) and $\text{Na}_V1.7$ selective (>30 folds) to identify the lead compound JNJ63955918 (Flinspach et al., 2017). The compound is less potent ($\text{IC}_{50} \sim 10 \text{ nM}$) than the wild-type but has a better selectivity profile, with 100- to >1,000-fold selectivity over $\text{Na}_V1.1$, $\text{Na}_V1.2$, $\text{Na}_V1.4$, $\text{Na}_V1.5$, and $\text{Na}_V1.6$. In animal models, central and peripheral administration of JNJ63955918 not only cause a pharmacological insensitivity to chemical and thermal pain and induces itch, which resemble behaviors observed in $\text{Na}_V1.7$ knock-out in adult mice (Gingras et al., 2014). Janssen has filed a patent for ProTx-II analogs but no further development has been disclosed.

The efforts by Amgen and Janssen were mainly performed with limited structural guidance. Recently, Genentech solved the structure of $\text{Na}_V1.7/\text{Na}_V\text{Ab}$ chimera in complex with ProTx-II in resting and activated VSD II states, revealing structural determinants of toxin binding at this site (Xu et al., 2019)

(Figure 2C). The structure has revealed the channel-toxin interactions in detail and confirmed the role of F813 ($\text{hNa}_V1.7$) in the S3 segment of VSD II as important determinant of $\text{hNa}_V1.7$ selectivity (Schmalhofer et al., 2008). It also revealed a minimum contribution of the S1-S2 region to the interaction with ProTx-II, leaving room for future optimization of peptides targeting this region for subtype selectivity.

The Activated VSD IV

The activated VSD IV binding site is formed by the extracellular S1-S2 and S3-S4 regions on VSD IV (Figure 1). A considerable development in recent years is the discovery of sulfonamide-based small molecule compounds binding to this site that have resulted in multiple selective inhibitors against $\text{Na}_V1.7$. These compounds bind to activated conformations of VSD IV and trap the channel in an inactivated state. Since the activation of VSD IV is required, this binding site is available at depolarized membrane potentials, thus creating state-dependent effects of binding.

Pfizer and Icagen first discovered the binding site in the effort that led to the development of selective sulfonamide compounds ICA-121431 and PF-04856264 targeting $\text{Na}_V1.3$ and $\text{Na}_V1.7$, respectively (McCormack et al., 2013). PF-04856264 is a state-dependent modulator of $\text{Na}_V1.7$ with a binding preference for an inactivated state. The potency of PF-04856264 measured with protocols favoring inactivated states was 28 nM for $\text{hNa}_V1.7$ and >10 μM for $\text{Na}_V1.3$ and $\text{Na}_V1.5$, showing promise of this binding site for Na_V channel subtype selectivity optimization (McCormack et al., 2013). Pfizer later advanced a subsequently optimized version of PF-04856264 named PF-05089771 into clinical trials. The potency of PF-05089771 was 15 nM for $\text{hNa}_V1.7$ with >600 fold selectivity over $\text{Na}_V1.3$, $\text{Na}_V1.4$, $\text{Na}_V1.5$, $\text{Na}_V1.8$, and modest selectivity over $\text{Na}_V1.2$ (~ 8 folds) and $\text{Na}_V1.6$ (~ 11 folds) (Swain et al., 2017). PF-05089771 was studied most extensively with multiple clinical trials completed (Mulcahy et al., 2019). Notably, in a phase II randomized, double-blind study for diabetic peripheral neuropathy, PF-05089771 was less effective than the control using pregabalin, a common drug used for the disease and did not show a significant difference to the placebo (McDonnell et al., 2018). In another phase II study to assess the efficacy of increasing oral daily doses of PF-05089771 for the treatment of postoperative dental pain, the compound did not show superiority over ibuprofen which used as a control although adverse effects were similar (US National Library of Medicine, 2021). The predefined efficacy criteria were not met and these studies did not proceed further. Several studies on similar compounds including PF-06456384 (Pfizer) and AMG8379 (Amgen) suggested that the lack of efficacy is possibly from high binding to plasma proteins, thus leading to low concentrations at the target binding site. Specifically, high unbound plasma exposure relative to *in vitro* potency against $\text{Na}_V1.7$ was necessary to achieve pharmacodynamic effects in these studies (Kornecook et al., 2017; Pero et al., 2017; Storer et al., 2017; Sun et al., 2019).

More recently, Genentech developed and optimized other sulfonamide compounds to target $\text{Na}_V1.7$. Notably, Genentech

captured the X-ray structure of a $\text{Na}_V1.7/\text{Na}_V\text{Ab}$ chimera in complex with sulfonamide, GX-936, revealing the structural basis for sulfonamide interaction with the activated VSD IV binding site (Ahuja et al., 2015) (Figure 2D). The $\text{Na}_V1.7/\text{Na}_V\text{Ab}$ —GX-936 structure complex, together with physiological characterizations of another sibling compound, GX-674 on $\text{Na}_V1.7$ VSD IV, revealed critical roles of R4 gating charge on S4 and YWxxV motif on S2 for potency and selectivity, respectively. Genentech in collaboration with Xenon Pharmaceuticals then advanced two highly potent acyl sulfonamide candidates targeting $\text{Na}_V1.7$, GDC-0276 ($\text{IC}_{50} = 0.4 \text{ nM}$) and GDC-0310 ($\text{IC}_{50} = 0.6 \text{ nM}$) for clinical developments (Safina et al., 2021). GDC-0276 has > 21 folds selectivity over $\text{hNa}_V1.1$, $\text{hNa}_V1.2$, $\text{hNa}_V1.4$, $\text{hNa}_V1.5$, $\text{hNa}_V1.6$ with the highest selectivity against $\text{hNa}_V1.6$ (~ 1,200 fold) and the lowest selectivity against $\text{hNa}_V1.4$ (~ 21 fold). GDC-0310 has > 63 fold selectivity over $\text{hNa}_V1.1$, $\text{hNa}_V1.2$, $\text{hNa}_V1.5$, ~ 330 fold over $\text{Na}_V1.6$ but only a modest ~ 6 folds selectivity over $\text{hNa}_V1.4$ (Safina et al., 2021). Xenon Pharmaceuticals and Genentech have stopped the development of GDC-0276 and GDC-0310 after Phase I trials for non-disclosed reasons.

We are not aware of any related compounds currently in clinical trials, although improvements in minimizing plasma binding have been observed in a next-generation sulfonamide compound such as AMG8379 (Kornecook et al., 2017). Whether optimizing sulfonamide compounds is still a good strategy for developing pain therapeutics remains to be seen. The activated VSD IV binding site, however, has demonstrated promise for developing subtype selective Na_V channel inhibitors.

Undisclosed Binding Site

It is worth to mention that Vertex has developed a series of pyridone amide-based compounds that are selective against $\text{Na}_V1.8$. The compound VX-150 achieved >400-fold selectivity over other Na_V channels (Hijma et al., 2021). Another Vertex compound VX-548 is currently in phase II clinical trials for acute pain after a bunionectomy. However, binding sites for these small molecules have not been revealed yet.

POTENTIAL USE OF ROSETTA IN STRUCTURAL GUIDED DEVELOPMENT OF PAIN THERAPEUTICS

Designing selective Na_V inhibitors as therapeutics to treat pain has been extremely challenging. Only several highly selective compounds have been identified or created by the massive efforts from the biopharmaceutical industry. However, it is noted that a majority of the approach utilized extensive SAR studies in combination with positional scanning and high-throughput testing, whereas high-resolution structural information remains largely unexplored. Since then, many of Na_V structures have been solved at high resolution, including in complexes with small molecules and peptide toxins, providing a better understanding, both structurally and mechanistically of Na_V inhibitors at their pharmacological binding sites. Advanced computational methods can take advantage of atomic scale

structural information for design and optimization of novel compounds. For example, molecular docking with high-resolution structures led to the identifications of highly selective D_4 dopamine receptor agonists (Wang et al., 2017) and M3R muscarinic acetylcholine receptor antagonists (Liu et al., 2018). Virtual screening of 490 million virtual molecules discovered selective ligands for sigma 2 receptor ($\sigma_2\text{R}$) that showed analgesic effects in animal models for neuropathic pain (Alon et al., 2021). *De novo* protein design functionalities in Rosetta allowed the design of highly potent and selective cytokine mimics (Silva et al., 2019) and picomolar affinity SARS-CoV-2 miniprotein inhibitors (Cao et al., 2020). We expect that high-resolution structures and advanced computational methods will further drive the development of therapeutics targeting Na_V channels to treat pain.

Several tools in the Rosetta computational modeling suite may offer promising strategies for the structure-guided approach, both for small molecule and peptide design and optimization. For example, RosettaLigand has been reliable in docking small molecule ligands and is highly suitable for SAR study (Davis and Baker, 2009). The recent integration of the BCL Cheminformatics package (Brown et al., 2021) will soon allow virtual screening and fragment-based design of small molecules in RosettaLigand. Alternatively, a newly developed application for small molecule docking named Rosetta GALigandDock utilizing a fast genetic algorithm would be suited for the virtual screening of small molecule libraries (Park et al., 2021). Rosetta has demonstrated excellent potential to design novel proteins. A large population of peptide inhibitors of ion channels is from natural toxins such as cysteine knot peptides which can now be designed computationally using Rosetta to access more diverse shapes and sizes (Bhardwaj et al., 2016). Such a library of *de novo* designed peptides can then be incorporated with a binding motif on a Na_V structure—toxin complex using MotifGraft or FunFoldDes (Silva et al., 2016) followed by a sequence design step to achieve novel peptide binders. Additionally, Rosetta also allows the design of peptides with cyclic topologies and with non-canonical amino acids such as macrocycles (Hosseinzadeh et al., 2017). Such approaches would significantly expand peptide compounds' structural and chemical space to increase the chances of achieving potent and selective inhibitors.

CONCLUSION

Human Na_V channel subtypes $\text{Na}_V1.7$, $\text{Na}_V1.8$, and $\text{Na}_V1.9$ have emerged as viable targets for developing novel therapeutics to treat pain. Previous efforts by academia and industry have identified small molecules and peptides targeting $\text{Na}_V1.7$ and $\text{Na}_V1.8$ as potential preclinical leads, with several compounds entering clinical trials. We have categorically grouped these efforts into four pharmacological binding sites; each has distinct characteristics that have attracted developments in the biopharmaceutical industry. We envision computational structural biology approaches, such as Rosetta (Baek et al., 2021), AlphaFold (Tunyasuvunakool et al., 2021) and others will be guiding the future design of highly potent and selective Na_V inhibitors.

Additionally, advanced mathematical modeling and machine learning methods for absorption, distribution, metabolism, and excretion assessment will further guide the development of next-generation investigational compounds for pain therapeutics.

REFERENCES

- Ahern, C. A., Payandeh, J., Bosmans, F., and Chanda, B. (2016). The Hitchhiker's Guide to the Voltage-Gated Sodium Channel Galaxy. *J. Gen. Physiol.* 147, 1–24. doi:10.1085/jgp.201511492
- Ahuja, S., Mukund, S., Deng, L., Khakh, K., Chang, E., Ho, H., et al. (2015). Structural Basis of Nav1.7 Inhibition by an Isoform-Selective Small-Molecule Antagonist. *Science* 350, aac5464. doi:10.1126/science.aac5464
- Alon, A., Lyu, J., Braz, J. M., Tummino, T. A., Craik, V., O'Meara, M. J., et al. (2021). Structures of the $\alpha 2$ Receptor Enable Docking for Bioactive Ligand Discovery. *Nature* 600, 759–764. doi:10.1038/s41586-021-04175-x
- Alsouloum, M., Higerd, G. P., Effraim, P. R., and Waxman, S. G. (2020). Status of Peripheral Sodium Channel Blockers for Non-addictive Pain Treatment. *Nat. Rev. Neurol.* 16, 689–705. doi:10.1038/s41582-020-00415-2
- Baek, M., DiMaio, F., Anishchenko, I., Dauparas, J., Ovchinnikov, S., Lee, G. R., et al. (2021). Accurate Prediction of Protein Structures and Interactions Using a Three-Track Neural Network. *Science* 373, 871–876. doi:10.1126/science.abj8754
- Bagal, S. K., Chapman, M. L., Marron, B. E., Prime, R., Storer, R. I., and Swain, N. A. (2014). Recent Progress in Sodium Channel Modulators for Pain. *Bioorg. Med. Chem. Lett.* 24, 3690–3699. doi:10.1016/j.bmcl.2014.06.038
- Beckley, J. T., Pajouhesh, H., Luu, G., Klas, S., Delwig, A., Monteleone, D., et al. (2021). Antinociceptive Properties of an Isoform-Selective Inhibitor of Nav1.7 Derived from Saxitoxin in Mouse Models of Pain. *Pain* 162, 1250–1261. doi:10.1097/j.pain.0000000000002112
- Bhardwaj, G., Mulligan, V. K., Bahl, C. D., Gilmore, J. M., Harvey, P. J., Cheneval, O., et al. (2016). Accurate De Novo Design of Hyperstable Constrained Peptides. *Nature* 538, 329–335. doi:10.1038/nature19791
- Bladen, C., and Zamponi, G. W. (2012). Common Mechanisms of Drug Interactions with Sodium and T-type Calcium Channels. *Mol. Pharmacol.* 82, 481–487. doi:10.1124/mol.112.079715
- Brown, B. P., Mendenhall, J., Geanes, A. R., and Meiler, J. (2021). General Purpose Structure-Based Drug Discovery Neural Network Score Functions with Human-Interpretable Pharmacophore Maps. *J. Chem. Inf. Model.* 61, 603–620. doi:10.1021/acs.jcim.0c01001
- Cao, L., Goreschnik, I., Coventry, B., Case, J. B., Miller, L., Kozodoy, L., et al. (2020). De Novo design of Picomolar SARS-CoV-2 Miniprotein Inhibitors. *bioRxiv* 370, 426–431. doi:10.1101/2020.08.03.234914
- Cardoso, F. C., Dekan, Z., Rosengren, K. J., Erickson, A., Vetter, I., Deuis, J. R., et al. (2015). Identification and Characterization of ProTx-III [μ -TRTX-Tp1a], a New Voltage-Gated Sodium Channel Inhibitor from Venom of the Tarantula *Thrixopelma Pruriens*. *Mol. Pharmacol.* 88, 291–303. doi:10.1124/mol.115.098178
- Catterall, W. A., Goldin, A. L., and Waxman, S. G. (2005). International Union of Pharmacology. XLVII. Nomenclature and Structure-Function Relationships of Voltage-Gated Sodium Channels. *Pharmacol. Rev.* 57, 397–409. doi:10.1124/pr.57.4.4
- Cheng, X., Choi, J. S., Waxman, S. G., and Dib-Hajj, S. D. (2021). Mini-review - Sodium Channels and beyond in Peripheral Nerve Disease: Modulation by Cytokines and Their Effector Protein Kinases. *Neurosci. Lett.* 741, 135446. doi:10.1016/j.neulet.2020.135446
- Craig, R. A., Garrison, C. E., Nguyen, P. T., Yarov-Yarovoy, V., and Du Bois, J. (2020). Veratridine: A Janus-Faced Modulator of Voltage-Gated Sodium Ion Channels. *ACS Chem. Neurosci.* 11, 418–426. doi:10.1021/acchemneuro.9b00621
- Crucchi, G., Gronseth, G., Alksne, J., Argoff, C., Brainin, M., Burchiel, K., et al. (2008). AAN-EFNS Guidelines on Trigeminal Neuralgia Management. *Eur. J. Neurol.* 15, 1013–1028. doi:10.1111/j.1468-1331.2008.02185.x
- Davis, I. W., and Baker, D. (2009). RosettaLigand Docking with Full Ligand and Receptor Flexibility. *J. Mol. Biol.* 385, 381–392. doi:10.1016/j.jmb.2008.11.010

AUTHOR CONTRIBUTIONS

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- Deuis, J. R., Dekan, Z., Wingerd, J. S., Smith, J. J., Munasinghe, N. R., Bhola, R. F., et al. (2017). Pharmacological Characterisation of the Highly Nav1.7 Selective Spider Venom Peptide Pn3a. *Sci. Rep.* 7, 40883. doi:10.1038/srep40883
- Flinspach, M., Xu, Q., Piekarz, A. D., Fellows, R., Hagan, R., Gibbs, A., et al. (2017). Insensitivity to Pain Induced by a Potent Selective Closed-State Nav1.7 Inhibitor. *Sci. Rep.* 7, 39662. doi:10.1038/srep39662
- Gingras, J., Smith, S., Matson, D. J., Johnson, D., Nye, K., Couture, L., et al. (2014). Global Nav1.7 Knockout Mice Recapitulate the Phenotype of Human Congenital Indifference to Pain. *PLoS One* 9, e105895. doi:10.1371/journal.pone.0105895
- Hijma, H. J., Siebenga, P. S., de Kam, M. L., and Groeneveld, G. J. (2021). A Phase 1, Randomized, Double-Blind, Placebo-Controlled, Crossover Study to Evaluate the Pharmacodynamic Effects of VX-150, a Highly Selective Nav1.8 Inhibitor, in Healthy Male Adults. *Pain Med.* 22, 1814–1826. doi:10.1093/pm/pnab032
- Hille, B. (1977). Local Anesthetics: Hydrophilic and Hydrophobic Pathways for the Drug-Receptor Reaction. *J. Gen. Physiol.* 69, 497–515. doi:10.1085/jgp.69.4.497
- Hinckley, C. A., Kuryshv, Y., Sers, A., Barre, A., Buisson, B., Naik, H., et al. (2021). Characterization of Vixotrigine, a Broad-Spectrum Voltage-Gated Sodium Channel Blocker. *Mol. Pharmacol.* 99, 49–59. doi:10.1124/molpharm.120.000079
- Hosseinizadeh, P., Bhardwaj, G., Mulligan, V. K., Shortridge, M. D., Craven, T. W., Pardo-Avila, F., et al. (2017). Comprehensive Computational Design of Ordered Peptide Macrocycles. *Science* 358, 1461–1466. doi:10.1126/science.aap7577
- Jiang, D., Shi, H., Tonggu, L., Gamal El-Din, T. M., Lenaus, M. J., Zhao, Y., et al. (2020). Structure of the Cardiac Sodium Channel. *Cell* 180, 122–e10. doi:10.1016/j.cell.2019.11.041
- Kornecook, T. J., Yin, R., Altmann, S., Be, X., Berry, V., Ilch, C. P., et al. (2017). Pharmacologic Characterization of AMG8379, a Potent and Selective Small Molecule Sulfonamide Antagonist of the Voltage-Gated Sodium Channel Nav1.7. *J. Pharmacol. Exp. Ther.* 362, 146–160. doi:10.1124/jpet.116.239590
- Lattanzi, S., Cagnetti, C., Foschi, N., Provinciali, L., and Silvestrini, M. (2015). Lacosamide Monotherapy for Partial Onset Seizures. *Seizure* 27, 71–74. doi:10.1016/j.seizure.2015.03.003
- Lee, S., Jo, S., Talbot, S., Zhang, H. B., Kotoda, M., Andrews, N. A., et al. (2019). Novel Charged Sodium and Calcium Channel Inhibitor Active against Neurogenic Inflammation. *Elife* 8, e48118. doi:10.7554/eLife.48118
- Liu, H., Hofmann, J., Fish, I., Schaake, B., Eitel, K., Bartuschat, A., et al. (2018). Structure-guided Development of Selective M3 Muscarinic Acetylcholine Receptor Antagonists. *Proc. Natl. Acad. Sci. U S A.* 115, 12046–12050. doi:10.1073/pnas.1813988115
- Liu, Y., Wu, Z., Tang, D., Xun, X., Liu, L., Li, X., et al. (2014). Analgesic Effects of Huwentoxin-IV on Animal Models of Inflammatory and Neuropathic Pain. *Protein Pept. Lett.* 21, 153–158. doi:10.2174/09298665113206660119
- McCormack, K., Santos, S., Chapman, M. L., Krafte, D. S., Marron, B. E., West, C. W., et al. (2013). Voltage Sensor Interaction Site for Selective Small Molecule Inhibitors of Voltage-Gated Sodium Channels. *Proc. Natl. Acad. Sci. U S A.* 110, E2724–E2732. doi:10.1073/pnas.1220844110
- McDonnell, A., Collins, S., Ali, Z., Iavarone, L., Surujbally, R., Kirby, S., et al. (2018). Efficacy of the Nav1.7 Blocker PF-05089771 in a Randomised, Placebo-Controlled, Double-Blind Clinical Study in Subjects with Painful Diabetic Peripheral Neuropathy. *Pain* 159, 1465–1476. doi:10.1097/j.pain.0000000000001227
- Moyer, B. D., Murray, J. K., Ligutti, J., Andrews, K., Favreau, P., Jordan, J. B., et al. (2018). Pharmacological Characterization of Potent and Selective Nav1.7 Inhibitors Engineered from Chilobrachys Jingzhao Tarantula Venom Peptide JzTx-V. *PLoS One* 13, e0196791. doi:10.1371/journal.pone.0196791
- Mulcahy, J., Pajouhesh, H., Delwig, A., Beckley, J., Shibuya, G. M., Du Bois, J., et al. (2018). 11,13-modified Saxitoxins for the Treatment of Pain. Patent WO2018183781. Available at: <https://patents.google.com/patent/WO2018183781A1/>.

- Mulcahy, J. V., Pajouhesh, H., Beckley, J. T., Delwig, A., Du Bois, J., and Hunter, J. C. (2019). Challenges and Opportunities for Therapeutics Targeting the Voltage-Gated Sodium Channel Isoform Nav1.7. *J. Med. Chem.* 62, 8695–8710. doi:10.1021/acs.jmedchem.8b01906
- Murray, J. K., Ligutti, J., Liu, D., Zou, A., Poppe, L., Li, H., et al. (2015). Engineering Potent and Selective Analogues of GpTx-1, a Tarantula Venom Peptide Antagonist of the Na(V)1.7 Sodium Channel. *J. Med. Chem.* 58, 2299–2314. doi:10.1021/jm501765v
- Murray, J. K., Long, J., Zou, A., Ligutti, J., Andrews, K. L., Poppe, L., et al. (2016). Single Residue Substitutions that Confer Voltage-Gated Sodium Ion Channel Subtype Selectivity in the Nav1.7 Inhibitory Peptide GpTx-1. *J. Med. Chem.* 59, 2704–2717. doi:10.1021/acs.jmedchem.5b01947
- Nguyen, P. T., DeMarco, K. R., Vorobyov, I., Clancy, C. E., and Yarov-Yarovoy, V. (2019). Structural Basis for Antiarrhythmic Drug Interactions with the Human Cardiac Sodium Channel. *Proc. Natl. Acad. Sci. U S A.* 116, 2945–2954. doi:10.1073/pnas.1817446116
- Nolan, S. J., Tudur Smith, C., Weston, J., and Marson, A. G. (2016). Lamotrigine versus Carbamazepine Monotherapy for Epilepsy: an Individual Participant Data Review. *Cochrane Database Syst. Rev.* 11, CD001031. doi:10.1002/14651858.CD001031.pub3
- Pajouhesh, H., Beckley, J. T., Delwig, A., Hajare, H. S., Luu, G., Monteleone, D., et al. (2020). Discovery of a Selective, State-independent Inhibitor of Nav1.7 by Modification of Guanidinium Toxins. *Sci. Rep.* 10, 14791. doi:10.1038/s41598-020-71135-2
- Pan, X., Li, Z., Huang, X., Huang, G., Gao, S., Shen, H., et al. (2019). Molecular Basis for Pore Blockade of Human Na⁺ Channel Nav1.2 by the μ -conotoxin KIIIA. *Science* 363, 1309–1313. doi:10.1126/science.aaw2999
- Park, H., Zhou, G., Baek, M., Baker, D., and DiMaio, F. (2021). Force Field Optimization Guided by Small Molecule Crystal Lattice Data Enables Consistent Sub-angstrom Protein-Ligand Docking. *J. Chem. Theor. Comput.* 17, 2000–2010. doi:10.1021/acs.jctc.0c01184
- Payne, C. E., Brown, A. R., Theile, J. W., Loucif, A. J., Alexandrou, A. J., Fuller, M. D., et al. (2015). A Novel Selective and Orally Bioavailable Nav 1.8 Channel Blocker, PF-01247324, Attenuates Nociception and Sensory Neuron Excitability. *Br. J. Pharmacol.* 172, 2654–2670. doi:10.1111/bph.13092
- Pero, J. E., Rossi, M. A., Lehman, H. D. G. F., Kelly, M. J., Mulhearn, J. J., Wolkenberg, S. E., et al. (2017). Benzoxazolinone Aryl Sulfonamides as Potent, Selective Nav1.7 Inhibitors with *In Vivo* Efficacy in a Preclinical Pain Model. *Bioorg. Med. Chem. Lett.* 27, 2683–2688. doi:10.1016/j.bmcl.2017.04.040
- Safina, B. S., McKerrall, S. J., Sun, S., Chen, C. A., Chowdhury, S., Jia, Q., et al. (2021). Discovery of Acyl-Sulfonamide Nav1.7 Inhibitors GDC-0276 and GDC-0310. *J. Med. Chem.* 64, 2953–2966. doi:10.1021/acs.jmedchem.1c00049
- Schmalhofer, W. A., Calhoun, J., Burrows, R., Bailey, T., Kohler, M. G., Weinglass, A. B., et al. (2008). ProTx-II, a Selective Inhibitor of Nav1.7 Sodium Channels, Blocks Action Potential Propagation in Nociceptors. *Mol. Pharmacol.* 74, 1476–1484. doi:10.1124/mol.108.047670
- Shen, H., Liu, D., Wu, K., Lei, J., and Yan, N. (2019). Structures of Human Nav1.7 Channel in Complex with Auxiliary Subunits and Animal Toxins. *Science* 363, 1303–1308. doi:10.1126/science.aaw2493
- Silva, D. A., Correia, B. E., and Procko, E. (2016). Motif-Driven Design of Protein-Protein Interfaces. *Methods Mol. Biol.* 1414, 285–304. doi:10.1007/978-1-4939-3569-7_17
- Silva, D. A., Yu, S., Ulge, U. Y., Spangler, J. B., Jude, K. M., Labão-Almeida, C., et al. (2019). De Novo design of Potent and Selective Mimics of IL-2 and IL-15. *Nature* 565, 186–191. doi:10.1038/s41586-018-0830-7
- Stevens, M., Peigneur, S., and Tytgat, J. (2011). Neurotoxins and Their Binding Areas on Voltage-Gated Sodium Channels. *Front. Pharmacol.* 2, 71. doi:10.3389/fphar.2011.00071
- Storer, R. I., Pike, A., Swain, N. A., Alexandrou, A. J., Bechle, B. M., Blakemore, D. C., et al. (2017). Highly Potent and Selective Nav1.7 Inhibitors for Use as Intravenous Agents and Chemical Probes. *Bioorg. Med. Chem. Lett.* 27, 4805–4811. doi:10.1016/j.bmcl.2017.09.056
- Sun, S., Jia, Q., Zenova, A. Y., Wilson, M. S., Chowdhury, S., Focken, T., et al. (2019). Identification of Selective Acyl Sulfonamide-Cycloalkylether Inhibitors of the Voltage-Gated Sodium Channel (Nav) 1.7 with Potent Analgesic Activity. *J. Med. Chem.* 62, 908–927. doi:10.1021/acs.jmedchem.8b01621
- Swain, N. A., Batchelor, D., Beaudoin, S., Bechle, B. M., Bradley, P. A., Brown, A. D., et al. (2017). Discovery of Clinical Candidate 4-[2-(5-Amino-1h-Pyrazol-4-Yl)-4-Chlorophenoxy]-5-Chloro-2-Fluoro-N-1,3-Thiazol-4-Ylbenzenesulfonamide (PF-05089771): Design and Optimization of Diaryl Ether Aryl Sulfonamides as Selective Inhibitors of Nav1.7. *J. Med. Chem.* 60, 7029–7042. doi:10.1021/acs.jmedchem.7b00598
- Tunyasuvunakool, K., Adler, J., Wu, Z., Green, T., Zielinski, M., Židek, A., et al. (2021). Highly Accurate Protein Structure Prediction for the Human Proteome. *Nature* 596, 590–596. doi:10.1038/s41586-021-03828-1
- US National Library of Medicine. 2021 US National Library of Medicine. ClinicalTrials.gov. Available at: <https://clinicaltrials.gov/ct2/show/NCT01529346>.
- Walker, J. R., Novick, P. A., Parsons, W. H., McGregor, M., Zablocki, J., Pande, V. S., et al. (2012). Marked Difference in Saxitoxin and Tetrodotoxin Affinity for the Human Nociceptive Voltage-Gated Sodium Channel (Nav1.7) [corrected]. *Proc. Natl. Acad. Sci. U S A.* 109, 18102–18107. doi:10.1073/pnas.1206952109
- Wang, S., Wacker, D., Levit, A., Che, T., Betz, R. M., McCorvy, J. D., et al. (2017). D4 Dopamine Receptor High-Resolution Structures Enable the Discovery of Selective Agonists. *Science* 358, 381–386. doi:10.1126/science.aan5468
- Wang, S. Y., Tikhonov, D. B., Mitchell, J., Zhorov, B. S., and Wang, G. K. (2007). Irreversible Block of Cardiac Mutant Na⁺ Channels by Batrachotoxin. *Channels (Austin)* 1, 179–188. doi:10.4161/chan.4437
- Wu, B., Murray, J. K., Andrews, K. L., Sham, K., Long, J., Aral, J., et al. (2018). Discovery of Tarantula Venom-Derived Nav1.7-Inhibitory JzTx-V Peptide 5-Br-Trp24 Analogue AM-6120 with Systemic Block of Histamine-Induced Pruritis. *J. Med. Chem.* 61, 9500–9512. doi:10.1021/acs.jmedchem.8b00736
- Xiao, Y., Bingham, J. P., Zhu, W., Moczydlowski, E., Liang, S., and Cummins, T. R. (2008). Tarantula Huwentoxin-IV Inhibits Neuronal Sodium Channels by Binding to Receptor Site 4 and Trapping the Domain II Voltage Sensor in the Closed Configuration. *J. Biol. Chem.* 283, 27300–27313. doi:10.1074/jbc.M708447200
- Xu, H., Li, T., Rohou, A., Arthur, C. P., Tzakoniati, F., Wong, E., et al. (2019). Structural Basis of Nav1.7 Inhibition by a Gating-Modifier Spider Toxin. *Cell* 176, 702–e14. doi:10.1016/j.cell.2018.12.018

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