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RESEARCH ARTICLE

The novel persistent sodium current inhibitor PRAX-562 has potent anticonvulsant activity with improved protective index relative to standard of care sodium channel blockers

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Abstract

Objective: This study investigates the effects of PRAX-562 on sodium current (I_{Na}), intrinsic neuronal excitability, and protection from evoked seizures to determine whether a preferential persistent I_{Na} inhibitor would exhibit improved preclinical efficacy and tolerability compared to two standard voltage-gated sodium channel (Na_V) blockers.

Methods: Inhibition of I_{Na} was characterized using patch clamp analysis. The effect on intrinsic excitability was measured using evoked action potentials recorded from hippocampal CA1 pyramidal neurons in mouse brain slices. Anticonvulsant activity was evaluated using the maximal electroshock seizure (MES) model, and tolerability was assessed by measuring spontaneous locomotor activity (sLMA).

Results: PRAX-562 potently and preferentially inhibited persistent I_{Na} induced by ATX-II or the *SCN8A* mutation N1768D (half-maximal inhibitory concentration [IC_{50}] = 141 and 75 nmol·L^{-1}, respectively) relative to peak I_{Na} tonic/resting block (60× preference). PRAX-562 also exhibited potent use-dependent block (31× preference to tonic block). This profile is considerably different from standard Na_V blockers, including carbamazepine (CBZ; persistent I_{Na} IC_{50} = 77 500 nmol·L^{-1}, preference ratios of 30× [tonic block], less use-dependent block observed at various frequencies). In contrast to CBZ, PRAX-562 reduced neuronal intrinsic excitability with only a minor reduction in action potential amplitude. PRAX-562 (10 mg/kg po) completely prevented evoked seizures without affecting sLMA (MES unbound brain half-maximal efficacious concentration = 4.3 nmol·L^{-1}, sLMA half-maximal tolerated concentration = 69.7 nmol·L^{-1}, protective index [PI] = 16×). In contrast, CBZ and lamotrigine (LTG) had PIs of approximately 5.5×, with significant overlap between doses that were anticonvulsant and that reduced locomotor activity.

Significance: PRAX-562 demonstrated robust preclinical anticonvulsant activity similar to CBZ but improved compared to LTG. PRAX-562 exhibited significantly improved preclinical tolerability compared with standard Na_V blockers (CBZ and

LTG), potentially due to the preference for persistent I_{Na}. Preferential targeting of persistent I_{Na} may represent a differentiated therapeutic option for diseases of hyperexcitability, where standard Na_V blockers have demonstrated efficacy but poor tolerability.

KEYWORDS

antiepileptic drugs, persistent sodium current, sodium channel blocker, tolerability

1 | INTRODUCTION

Epilepsy is the fourth most common neurological disorder, affecting 3.4 million people in the United States, including 470 000 children.[1,2] Although the introduction of second and third generation antiepileptic drugs (AEDs) has reduced drug–drug interactions and teratogenic risk, these newer agents have not addressed the 30% of patients unable to achieve seizure freedom.[3] Therefore, additional therapeutic options with improved efficacy and tolerability are desperately needed.

Epilepsy is a group of heterogeneous disorders classified into distinct syndromes by etiology, seizure type(s), and comorbidities.[4] The most common cause of genetic epilepsy is mutations within voltage-gated sodium channel (Na_V) genes leading to gain-of-function and/or loss-of-function changes in channel activity.[5] Affected patients typically present as children or neonates and have prognoses ranging from benign seizures that spontaneously remit to devastating developmental epileptic encephalopathies (DEEs).[6]

Na_V channels are an important therapeutic target for AEDs.[7] Their blockade, and consequent inhibition of neuronal sodium current (I_{Na}), is ideally positioned to reduce excitability, as peak I_{Na} in the axonal initial segment and node of Ranvier is responsible for the initiation and propagation of action potentials (APs), respectively.[8,9] However, the clinical utility of standard Na_V-targeting AEDs is limited because current agents, including carbamazepine (CBZ), oxcarbazepine, and phenytoin, can show severe toxicity at therapeutic doses. This toxicity includes ataxia, lethargy, vomiting, and seizures and reflects compromised physiologic neuronal function as a result of excessive peak I_{Na} inhibition or off-target (non-Na_V-mediated) activities.[10–13] Identification of novel I_{Na} inhibitors with improved tolerability would thus represent a clinically meaningful alternative treatment option.

Physiological persistent I_{Na} is a small, subthreshold current that contributes to the amplification of synaptic responses and the enhancement of repetitive firing.[14,15] Functional studies of *SCN2A* (encoding $Na_V1.2$) and *SCN8A* (encoding $Na_V1.6$) DEE variants have demonstrated small increases in persistent I_{Na} that can cause hyperexcitability, seizures, and developmental comorbidities.[6,15–18] Current Na_V-targeting AEDs are predicted to inhibit both peak I_{Na} and persistent I_{Na} at or near therapeutic concentrations (high $\mu mol \cdot L^{-1}$ range[10,11]), with excessive peak I_{Na} inhibition compromising physiological neuronal activity. Therefore, improved selectivity for Na_V activity and preference in the targeting of persistent I_{Na} could meaningfully improve tolerability.

Key Points

- PRAX-562 exhibits increased potency for I_{Na}, improved preference for persistent I_{Na}, and enhanced use-dependent block relative to standard Na_V-targeting AEDs
- PRAX-562 reduces neuronal AP firing with only minor effects on AP amplitude, suggesting limited inhibition of peak I_{Na} compared to other Na_V inhibitors; this profile may reduce hyperexcitability in disease states such as seizures, without impacting physiologically relevant activity
- PRAX-562 protects mice from electrically induced seizures and has a larger acute PI compared with standard Na_V-targeting AEDs
- The profile of PRAX-562 may translate into a clinically effective therapy that is well tolerated in epilepsy as well as other indications caused by neuronal hyperexcitability

The therapeutic potential of preferential persistent I_{Na} inhibitors in epilepsy is supported by previous work with PRAX-330 (GS967).[19–23] In animal models of Na_V DEE caused by pathologically enhanced persistent I_{Na}, PRAX-330 protected mice from seizures and premature death caused by either Scn8a ($Na_V1.6$-N1768D/+,[20] $Na_V1.6$-R1872W/+[21]) or Scn2a ($Na_V1.2$-Q54[22]) gain-of-function variants.

PRAX-562 was discovered following efforts aimed at developing a highly differentiated, potent, and preferential inhibitor of persistent I_{Na} that could overcome the tolerability limitations of standard Na_V-targeting AEDs.

The structure of PRAX-562 (3-[ethoxydifluoromethyl]-6-[5-fluoro-6-(2,2,2-trifluoroethoxy)pyridin-3-yl]-[1,2,4]triazolo[4,3-*a*]pyrazine) is depicted in Figure 1A. PRAX-562 potently and preferentially inhibits physiologic persistent I_{Na} to produce preclinical anticonvulsant activity with a significantly improved protective index (PI) compared to CBZ and lamotrigine (LTG). Brain slice studies suggest the improvement in PI correlates with a lower effect on intrinsic excitability than observed with CBZ, which demonstrates lower potency and preference for persistent I_{Na} inhibition. These data suggest PRAX-562 holds promise as an improved Na_V-targeting AED, particularly where the low tolerability of Na_V blockers limits the ability to dose high enough to realize full antiepileptic potential.

2 | MATERIALS AND METHODS

Additional details can be found in Appendix S1.

2.1 | Electrophysiology using HEK-293 cells

Whole-cell patch clamp analysis was performed using a PatchXpress automated electrophysiology platform (Molecular Devices). HEK-293 cell lines stably expressing either human (h), rat (r), canine (d), or mouse (m) orthologs were used as follows: $hNa_V1.1$ (NP_008851.3), $hNa_V1.2$ (NP_066287.2), $rNa_V1.2$ (NP_036779.1), $dNa_V1.2$ (XP_013966299.1), $hNa_V1.5$ (NP_000326.2), $hNa_V1.6$ (NP_055006), $mNa_V1.6$ (NP_035453.2), or $rNa_V1.6$ (NP_062139). Data were processed using DataXpress 2.0. Persistent I_{Na} (200 nmol·L^{-1} ATX-II or *SCN8A* DEE variant N1768D) or peak I_{Na} was measured using voltage protocols included as figure insets, with effect of compound measured at the depicted blue arrowheads (Figures 1 and 2); average persistent I_{Na} was measured during the last 20 ms, and peak I_{Na} was measured at the beginning of the step.

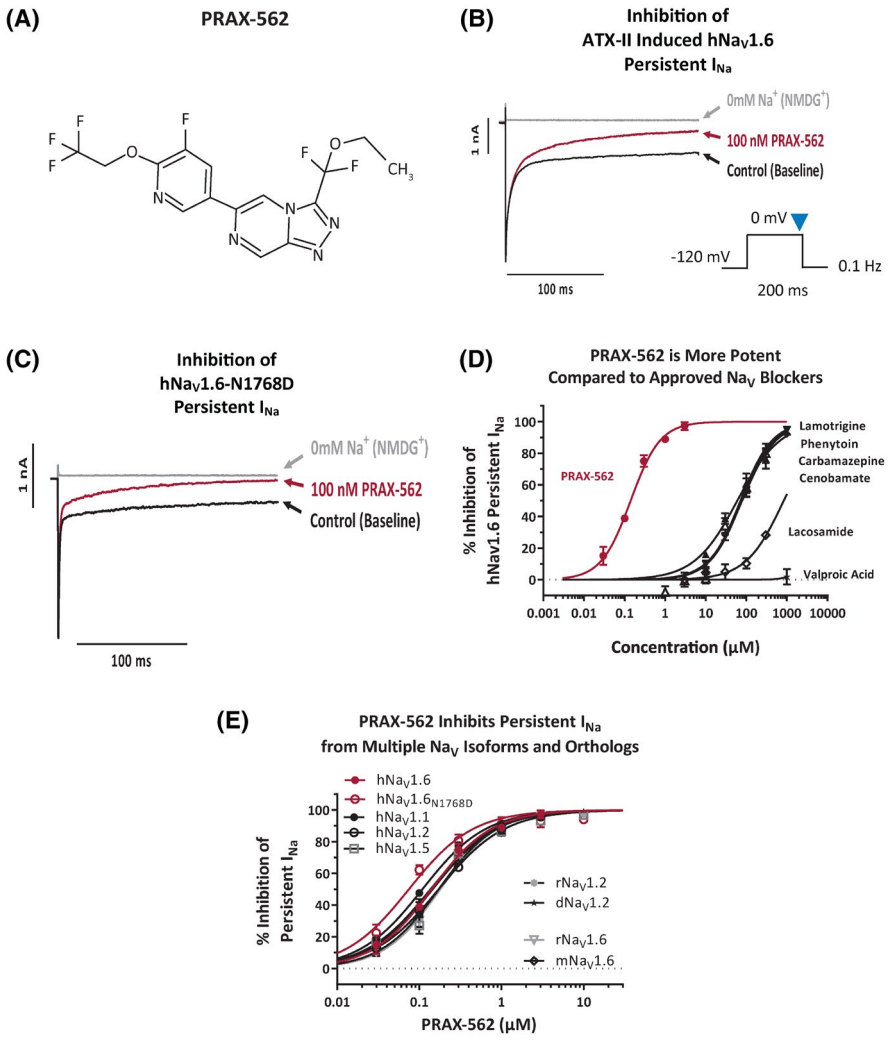

FIGURE 1 PRAX-562 exhibits potent inhibition of $Na_V1.6$ persistent sodium current (I_{Na}). (A) Structure of PRAX-562. PRAX-562 reduced (B) ATX-II-evoked $hNa_V1.6$ persistent I_{Na} and (C) $hNa_V1.6$-N1768D (developmental epileptic encephalopathy variant)-expressed persistent I_{Na}. (D) PRAX-562 demonstrated increased potency for persistent I_{Na} relative to standard Na_V-targeting antiepileptic drugs. (E) PRAX-562 inhibited ATX-II- or N1768D-induced persistent I_{Na} expressed by multiple Na_V isoforms and orthologs. Voltage protocols are included as panel insets, pharmacology was measured at blue arrowhead, and points represent mean ± SEM. NMDG, N-methyl-D-glucamine

Plotting and fitting were performed using Prism (GraphPad Software). Percent inhibition was calculated, expressed as mean ± SEM, and plotted versus the tested concentration. Data were fitted using a Hill equation (Max_Effect / [1 + (IC_{50} / x) ^ Hill_Slope]) to estimate the concentration of compound producing half-maximal inhibition (IC_{50}) and hill slope. Max_Effect was allowed to vary for the use-dependent block assay but was fixed at 100 for all other assays.

2.2 | Animal use statement

All experiments using mice were conducted in accordance with relevant institutional and national guidelines for ethical use of animals in research. Brain slice experiments were conducted in accordance with the Prevention of Cruelty to Animals Act 1986, under the guidelines of the National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia, and were approved by the Florey Neuroscience Institute Animal Ethics Committee (AEC 18-126 FINMH). Behavioral studies performed at ChemPartner, Shanghai, China, were conducted in accordance with guidelines for animal welfare of the Animal Care and Use Committee of the Organization which has been accredited by Association for Assessment and Accreditation of Laboratory Animal Care International since 2010.

2.3 | Electrophysiology using brain slices

Mice (postnatal day 17–21) were anesthetized using 2% isoflurane and decapitated. Coronal hippocampal slices (300 μm) were prepared and transferred to a submerged recording chamber on an upright microscope (Slicescope Pro 1000; Scientifica) and perfused (2 ml/min) with extracellular recording solution at 32°C. Patch clamp recordings were made from hippocampal CA1 pyramidal neurons.

Once whole-cell configuration was obtained for 2 min, a holding current was injected to maintain a membrane potential of approximately −70 mV. Current steps (injected current of between −60 and 340 pA in 20-pA steps, 400-ms duration) were applied in current clamp mode. The amplitude of current injections was relative to the holding current. The intersweep interval was 5 s (.2 Hz). Acceptance criteria were access resistance < 20 MΩ and holding current < −200 pA. Once baseline AP firing was determined in the extracellular recording solution (artificial cerebral spinal fluid), the compound was washed onto the slice for 5 min and the AP-generating protocol was repeated.

Data were analyzed using AXOGRAPH X software. Individual APs were identified and counted using a +50-mV amplitude threshold relative to pre-event baseline. The average number of APs evoked for each current injection was calculated. AP amplitudes were determined relative to the pre-event baseline at the +200-pA current injection step for each cell. The average AP amplitude for each evoked AP in the train was calculated. Once an AP count had less than three cells contributing to the averaged amplitude, it was excluded from analysis. For statistical comparisons between groups, the total number of APs fired and the summed AP amplitude for all APs at the +200-pA step were calculated per cell. Statistical analysis was performed using Prism software. Paired two-tailed Student *t*-tests were used to test the effect of compound on AP firing compared to baseline. For all analyses, significance was set at an alpha value of .05.

2.4 | Mouse maximal electroshock seizure model

The effects of PRAX-562 on latency to seizure in the maximal electroshock seizure (MES) assay were evaluated in two separate experiments in male CD-1 mice (~35 g). In the first, mice were administered PRAX-562 (.3, 1, or 3 mg/kg po); in the second, a higher dose range was evaluated (1, 3, or 10 mg/kg po). In both studies, PRAX-562 or vehicle (35% 2-hydroxypropyl-β-cyclodextrin [HPBCD], 10 ml/kg po; $n = 12$/group) was administered 30 min before the MES test. Each experiment included groups of mice dosed with vehicle (.9% saline; 10 ml/kg ip) or valproic acid (VPA; 400 mg/kg ip) to serve as the positive control. Separate studies tested the anticonvulsant efficacy of CBZ and LTG. Mice were dosed with CBZ (HY-B0246, MedChemExpress; 3–10 mg/kg ip) or vehicle (35% HPBCD, 10 ml/kg ip) 30 min prior to MES, or were dosed with LTG (HY-B0495, MedChemExpress; 1–10 mg/kg ip) or vehicle (saline, 10 ml/kg ip) 60 min prior to MES. All mice were evaluated by blinded observation before the MES test to determine whether compound administration affected baseline behavior or caused sedation.

Tonic hindlimb extension seizures were induced via an electroshock apparatus set to deliver a 50-mA square-wave stimulus, with a .8-s duration, a pulse width of 10 ms, and a frequency of 50 Hz. Custom stainless-steel ear-clip electrodes were soaked in .2% Agar and used to apply bilateral transauricular stimulation in manually restrained mice. Immediately after the stimulation, mice were placed into a clean cage and observed continuously for a period of 1 min by individuals blinded to treatment conditions. The latency to seizure and number of mice developing seizures were recorded. At the end of the observation period, mice were euthanized with CO_2, and terminal plasma and brain

tissue samples were collected and stored at −80°C for subsequent analysis of drug concentration.

Statistical analyses were conducted using Prism 7.0. Mann–Whitney *U* test was used to detect differences in latency and number of seizures between saline and VPA groups. One-way analysis of variance (ANOVA) on ranks (Kruskal–Wallis test) followed by Dunn test was used to detect differences in latency and number of seizures between vehicle- and test drug-treated mice. Dose–response and concentration–response curves for plasma and brain were fitted for each test drug to calculate the half-maximal efficacious dose (ED_{50}) and half-maximal efficacious concentration (EC_{50}) for increasing latency to tonic seizure.

2.5 | Mouse spontaneous locomotor activity assay

The effect of PRAX-562 on locomotor activity was assessed with the spontaneous locomotor activity (sLMA) test in two separate experiments. In both studies, male CD-1 mice (~35 g) were acclimated to the test room at least 30 min before the start of the experiment. Mice ($n = 10$/group) were orally administered PRAX-562 (10–40 mg/kg) or 35% HPBCD (vehicle, 10 ml/kg) 30 min prior to the sLMA test. Separate studies tested the effects of CBZ and LTG on sLMA. Mice were dosed with CBZ (HY-B0246, MedChemExpress; 30, 54, and 96 mg/kg ip) or vehicle (35% HPBCD, 10 ml/kg ip) 30 min prior to testing, or were dosed with LTG (HY-B0495, MedChemExpress; 20, 35.6, and 63.4 mg/kg ip) or vehicle (saline, 10 ml/kg ip) 60 min prior to testing.

Each mouse was placed at the center of the test chamber (40 × 40 × 30 cm, 45 ± 5 lux on the floor) for the 30-min sLMA video recording. Each mouse was automatically tracked with overhead cameras using a 1-min sampling window. Spontaneous locomotion was analyzed offline. Locomotor status was defined as >2 mm of movement every 200 ms (four frames recorded at 20 frames/s). The accumulated shift of tracking spots within the 200 ms was identified as a locomotor epoch. Distance traveled was calculated from all the locomotion epochs and analyzed. Following the test, all mice were euthanized with CO_2. Terminal plasma and brain tissue samples were collected as described above and stored at −80°C for subsequent analysis of drug concentration.

Statistical analyses were conducted using Prism 7.0. ANOVA followed by Dunnett test was used to detect differences in total traveling distance over 30 min between vehicle and treatment groups. Dose–response and concentration–response curves for plasma and brain were fitted for each test drug to calculate half-maximal tolerated dose (TD_{50}) and half-maximal tolerated concentration (TC_{50}).

TABLE 1 PRAX-562 demonstrates greater potency and preference for $hNa_V1.6$ persistent I_{Na} compared with standard Na_V-targeting antiepileptic drugs

	Persistent I_{Na}, IC_{50}, nmol·L^{-1} (slope)	Peak I_{Na} TB, IC_{50}, nmol·L^{-1} (slope)	Ratio to persistent I_{Na}	Peak I_{Na} UDB, 10 Hz, IC_{50}, nmol·L^{-1} (slope)	Ratio to persistent I_{Na}	Ratio to peak I_{Na} TB	Peak I_{Na} VDB, IC_{50}, nmol·L^{-1} (slope)	Ratio to persistent I_{Na}
PRAX-562	141 (1.2)	8472 (1.0)	60	271 (1.3) MAX = 75%	2	31	317 (1.0)	2.2
Cenobamate	71 690 (1.1)	1 719 000 (1.1)	24	749 300 (.7)	11	2.3	66 710 (.9)	.9
Phenytoin	59 820 (.8)	n/a[a]	—	876 600 (.6)	15	—	47 780 (1.0)	.8
Carbamazepine	77 490 (1.1)	2 307 000 (1.0)	30	1 418 000 (.9)	18	1.6	44 370 (.9)	.6
Oxcarbazepine	123 700 (1.0)	1 035 000 (1.7)	8	n.d.	—	—	42 000 (1.1)	.3
Lamotrigine	78 480 (1.0)	1 249 000 (.8)	16	515 800 (1.0)	6.6	2.4	39 090 (.9)	.5
Lacosamide	832 700 (.9)	n/a[a]	—	682 200 (1.3)	.8	—	269 300 (1.2)	.3
Valproic acid	2% @ 1 mmol·L^{-1}	11% @ 1 mmol·L^{-1}	—	8% @ 1 mmol·L^{-1}	—	—	18% @ 1 mmol·L^{-1}	—

Abbreviations: IC_{50}, half-maximal inhibitory concentration; I_{Na}, sodium current; MAX, maximum; n.d., not determined; n/a, not available; Na_V, voltage-gated sodium channel; TB, tonic block; UDB, use-dependent block; VDB, voltage-dependent block.

[a]Could not be determined due to compound solubility limit.

3 | RESULTS

3.1 | PRAX-562 potently inhibits persistent I_{Na}

PRAX-562 potently inhibits ATX-II-induced persistent I_{Na} expressed by wild-type $hNa_V1.6$ (Figure 1B; IC_{50} = 141 nmol·L^{-1}) and persistent I_{Na} expressed by the DEE mutant $hNa_V1.6$-N1768D (Figure 1C; IC_{50} = 75 nmol·L^{-1}). Persistent I_{Na} (control, black trace; PRAX-562, red trace) was activated from resting/closed channel conformations by maintaining a hyperpolarized holding potential (−120 mV). Removal of extracellular sodium ($NMDG^+$, gray trace) completely inhibited sodium-dependent conductance. The potency of PRAX-562 for ATX-II or N1768D persistent I_{Na} was at least 550-fold greater than that of standard Na_V-targeting AEDs (Figure 1D, Table 1).

PRAX-562 displayed similar potency for the inhibition of persistent I_{Na} expressed by other human Na_V isoforms ($hNa_V1.1$, $hNa_V1.2$, $hNa_V1.5$) as well as rat, dog, and mouse orthologs ($rNa_V1.2$, $dNa_V1.2$, $mNa_V1.6$, $rNa_V1.6$), with IC_{50} values ranging 109–180 nmol·L^{-1} (Figure 1E, Tables S1 and S2).

3.2 | PRAX-562 exhibits enhanced preference for the inhibition of persistent I_{Na} over peak I_{Na}

The inhibition of peak I_{Na} was investigated using three assays with increasing levels of $hNa_V1.6$ activation. Tonic block of physiologic peak I_{Na} is measured at a low stimulation frequency (.1 Hz) from resting/closed channel conformations (Figure 2A). PRAX-562 exhibited tonic block with lower potency (IC_{50} = 8470 nmol·L^{-1}), demonstrating 60-fold preference for persistent I_{Na} (Table 1). PRAX-562 also exhibited preference for persistent I_{Na} over peak I_{Na} tonic block for other human Na_V isoforms: $hNa_V1.1$ (173-fold, 109 nmol·L^{-1} vs. 18 870 nmol·L^{-1}), $hNa_V1.2$ (80-fold, 172 nmol·L^{-1} vs. 13 690 nmol·L^{-1}), and $hNa_V1.5$ (>174-fold, 172 nmol·L^{-1} vs. 12% inhibition at 30 000 nmol·L^{-1}; Table S2).

The use- (activity-) dependent block of $hNa_V1.6$ by PRAX-562 was measured using a train of short voltage steps at a frequency of 10 Hz to represent periods of elevated neuronal firing (e.g., during a seizure) where use-dependent block of peak I_{Na} may have a therapeutic benefit. PRAX-562 exhibited use-dependent block of

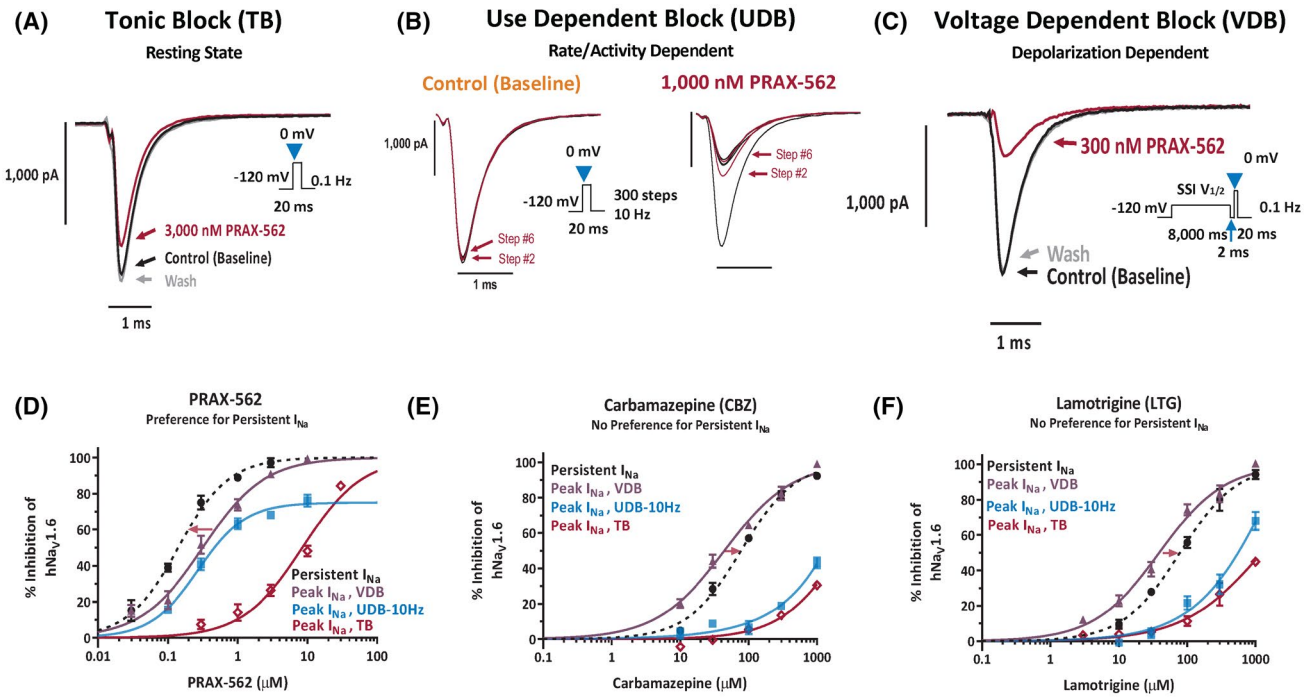

FIGURE 2 PRAX-562 demonstrates increased preference for $hNa_V1.6$ persistent sodium current (I_{Na}) over peak I_{Na} relative to the standard Na_V-targeting antiepileptic drugs CBZ and LTG. Inhibition of peak I_{Na} assessed using assays for (A) tonic block, (B) use-dependent block, or (C) voltage-dependent block. (D) PRAX-562 demonstrates preference for persistent I_{Na} relative to peak I_{Na} for all assay conditions (red arrow). (E) CBZ and (F) LTG exhibited lower potency and preference for persistent I_{Na} (red arrows). Voltage protocols are included as panel insets, pharmacology was measured at blue arrowheads, and points represent mean ± SEM

hNa$_V$1.6 peak I_{Na} with an IC_{50} of 271 nmol·L^{-1} and maximum inhibition of 75% (blue trace, Figure 2B; Table 1). Notably, use-dependent block was not observed for either CBZ or LTG at a stimulation frequency of 10 Hz (blue trace, Figure 2E,F, respectively). Use-dependent block could be observed for CBZ by increasing the frequency of depolarization from 10 Hz to either 30 Hz or 50 Hz (Figure S1). The degree of use-dependent block observed for PRAX-562 was significantly greater compared with CBZ at all frequencies, suggesting an increased ability of PRAX-562 to respond to acute changes in neuronal activity (acute hyperexcitability).

The peak I_{Na} voltage-dependent block assay employs a sustained, nonphysiological inactivating voltage step to midpoint ($V_{1/2}$) of the steady-state inactivation (determined in real time for each cell) to place half the channels into the inactivated state. This approach effectively explores isoform selectivity, as the differences in voltage-sensing that regulate binding site access are minimized, and the extended time allows for most inhibitors to reach binding equilibrium. PRAX-562 exhibited a voltage-dependent block IC_{50} of 317 nmol·L^{-1} (Figure 2C, Table 1). Importantly, these data demonstrate a 2.2-fold preference for persistent I_{Na} is retained as channels are inactivated (red arrow, Figure 2D; Table 1). PRAX-562 exhibited a similar preference for persistent I_{Na} over peak I_{Na} voltage-dependent block for hNa$_V$1.1 (6.3-fold), hNa$_V$1.2 (8.2-fold), and hNa$_V$1.5 (5.8-fold; Figure S2, Table S2). PRAX-562 also induced a concentration-dependent stabilization of hNa$_V$1.6 inactivation, as evidenced by a significant left shift in the $V_{1/2}$ of the steady-state inactivation curve: shifts of −2.6 mV for dimethylsulfoxide (DMSO)/control, −6.2 mV for .3 μmol·L^{-1} PRAX-562, and −11.7 mV for 1 μmol·L^{-1} PRAX-562 (Figure S3, Table S3). Only minor shifts in the $V_{1/2}$ of the activation curve were observed: shifts of −1.3 mV for DMSO/control, −2.3 mV for .3 μmol·L^{-1} PRAX-562, and −2.7 mV for 1 μmol·L^{-1} PRAX-562 (Figure S3, Table S3). These data suggest PRAX-562 enhances fast inactivation with minimal effects on activation gating.

A panel of standard Na$_V$-targeting AEDs was tested in the same persistent I_{Na} and peak I_{Na} assays. Compared with PRAX-562, all tested inhibitors were less potent in all assays (Table 1). More importantly, the moderate persistent I_{Na} preference observed for other I_{Na} inhibitors in the tonic block assay (eightfold to 30-fold) was lost as the channels transitioned to more activated/inactivated states, as in the voltage-dependent block assay (.3-fold to .9-fold preference). Notably, both CBZ and LTG were more potent for voltage-dependent block peak I_{Na} compared to persistent I_{Na} (.6-fold and .5-fold, respectively), demonstrating a preference for peak I_{Na} under these conditions.

3.3 | PRAX-562 reduces intrinsic excitability of wild-type CA1 pyramidal neurons

The preferential inhibition of persistent I_{Na} is predicted to reduce neuronal hyperexcitability without excessive disruption of AP morphology, including AP amplitude, as this feature depends on the expression of peak I_{Na}. The effects of PRAX-562 and CBZ on neuronal intrinsic excitability were measured using evoked AP firing (input–output curves) at the equivalent effective concentrations of the peak I_{Na} voltage-dependent block IC_{50} (Table 1). At .3 μmol·L^{-1}, PRAX-562 significantly reduced the intrinsic excitability as measured by the number of evoked APs (Figure 3A). In contrast, 45 μmol·L^{-1} CBZ produced a more robust reduction in neuronal excitability (Figure 3D). Importantly, CBZ caused a more pronounced reduction in AP amplitude compared to PRAX-562, suggesting greater inhibition of peak I_{Na} (Figure 3F). These data demonstrate that although both agents generate a reduction in the excitability of wild-type CA1 neurons, PRAX-562 reduces excitability in a manner that likely maintains physiological activity over a broader range of concentrations than CBZ by leaving a greater proportion of peak I_{Na} intact.

3.4 | PRAX-562 achieves full anticonvulsant efficacy without affecting locomotor activity

To assess whether a persistent I_{Na} inhibitor with the in vitro profile of PRAX-562 can prevent seizures, anticonvulsant activity in the mouse MES model was investigated. This model has predictive validity for clinical anticonvulsant activity.[24] We compared PRAX-562 to the standard Na$_V$-targeting AEDs, CBZ and LTG. PRAX-562 produced dose-dependent protection (increase in latency) of mice against MES-induced tonic hindlimb seizures (Figure 4A). Near complete protection was achieved at 10 mg/kg, where 11 of 12 mice did not exhibit tonic seizures (Figure 4B). This effect was comparable to that observed with the positive control, VPA. The calculated ED_{50} value for increasing latency to tonic extension seizures was 2 mg/kg, with calculated EC_{50} values of 90.1 ng/ml (17.9 nmol·L^{-1} free) and 116 ng/g (4.3 nmol·L^{-1} free) in plasma and brain, respectively (Table 2).

CBZ and LTG also provided dose-dependent protection of mice against MES-induced tonic hindlimb seizures, with CBZ (30 mg/kg) protecting all mice (Figure S2A,B) and LTG protecting eight of 12 mice at the highest dose tested (10 mg/kg; Figure S2D,E). The calculated ED_{50} values for increased latency to tonic extension

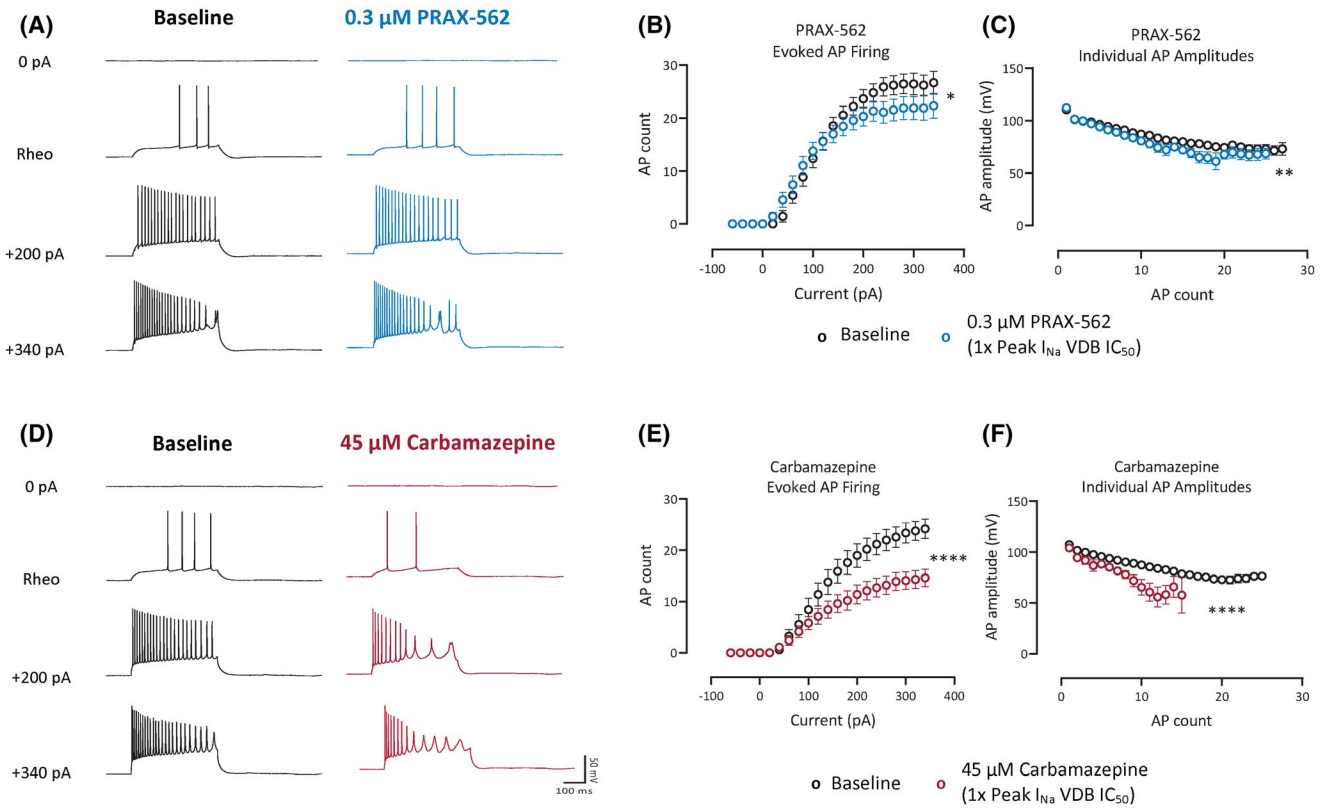

FIGURE 3 PRAX-562 reduces intrinsic excitability of hippocampal CA1 pyramidal neurons without compromising action potential (AP) amplitude. The effect is shown of PRAX-562 (blue) and carbamazepine (CBZ; red) at equivalent effective concentrations (half-maximal inhibitory concentration [IC_{50}] of peak sodium current [I_{Na}] voltage-dependent block [VDB]) on AP firing recorded from CA1 pyramidal neurons from wild-type mice. Representative AP traces show the predrug (black, baseline) and after-drug records for (A) .3 $\mu mol \cdot L^{-1}$ PRAX-562 (blue) or (D) 45 $\mu mol \cdot L^{-1}$ CBZ (red). (B, E) Input–output relationships and (C, F) AP amplitude adaptation for PRAX-562 and CBZ at a current injection of +200 pA. Data are presented as mean ± SEM. *$p < .05$, **$p < .01$, ****$p < .0001$

seizures were 5 and 3.4 mg/kg for CBZ and LTG, respectively (Table 2).

To determine the tolerability of PRAX-562, the effects on sLMA were measured. PRAX-562 produced reductions in the distance moved at 20 and 40 mg/kg (Figure 4C). The dose of PRAX-562 (10 mg/kg) that resulted in seizure prevention in 11 of 12 mice had no effect on locomotor function. The dose of PRAX-562 required to reduce sLMA by 50% (TD_{50}) was calculated to be 44 mg/kg. The PRAX-562 concentrations associated with the 50% effect (TC_{50}) were calculated to be 1553 ng/ml (308.9 $nmol \cdot L^{-1}$ free) and 1899 ng/g (69.7 $nmol \cdot L^{-1}$ free) in plasma and brain, respectively (Table 2).

CBZ and LTG also produced dose-dependent reductions in sLMA, with ED_{50} values of 37.6 and 26.5 mg/kg, respectively (Table 2, Figure S2C,F). Notably, CBZ produced a significant reduction in sLMA at the dose required for complete seizure prevention.

The ratio of tolerability to efficacy (PI) was calculated for each molecule by dividing the brain or plasma TC_{50} for reduction in sLMA by the brain or plasma EC_{50} for increasing latency to seizures (Figure 4D). PRAX-562 had a significantly improved PI of approximately 16-fold (based on calculated free brain concentrations) and 17-fold (based on free plasma concentrations). This represents an improvement in PI compared with both CBZ (brain, 5.9×; plasma, 3.4×) and LTG (brain, 4.7×; plasma, 6.4×; Figure 4E). Preclinical models are inherently imperfect, and thus the clinical therapeutic margin of PRAX-562 dosed acutely as well as chronically will need to be defined in patients.

4 | DISCUSSION

Na_V blockers have been a critical component of the pharmacological management of epilepsy for decades.[8,9] However, the efficacy of currently approved standard Na_V blockers is constrained by their narrow therapeutic window, limiting the ability to dose high enough to realize their full antiepileptic potential. The narrow therapeutic window may in part be a consequence

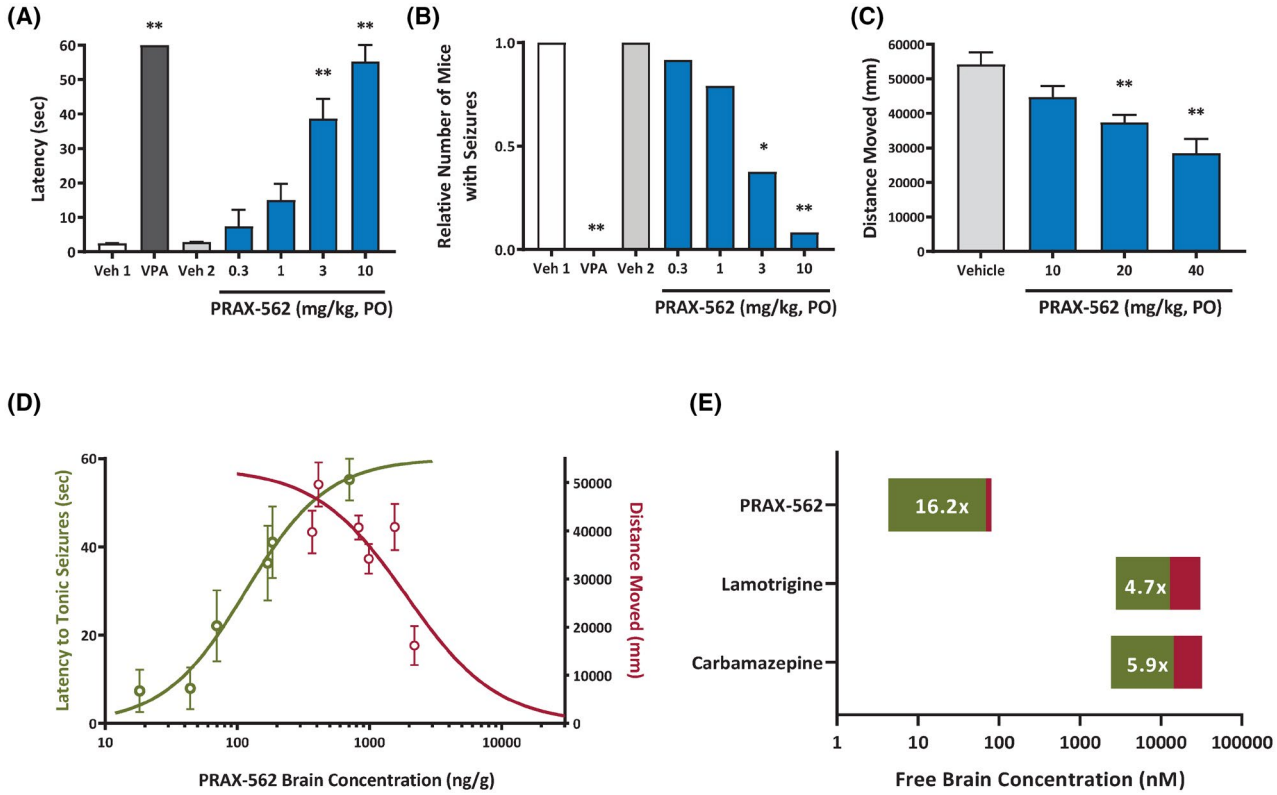

FIGURE 4 PRAX-562 has an improved preclinical protective index (PI) compared to carbamazepine (CBZ) or lamotrigine (LTG). PRAX-562 (.3–10 mg/kg po) produced dose-dependent increases in (A) latency to tonic extension seizures and (B) decreases in the relative number of mice developing seizures in the maximal electroshock seizure (MES) model. Maximal effects were equivalent to the positive control valproic acid (VPA; 400 mg/kg ip). (C) PRAX-562 (10–40 mg/kg po) produced dose-dependent reductions in distance moved in the spontaneous locomotor activity (sLMA) assay. (D) Total brain concentrations of PRAX-562 associated with anticonvulsant efficacy (green symbols, left y-axis) were separated from those associated with decreases in total distance moved (red symbols, right y-axis). (E) The range of calculated free brain concentrations of PRAX-562, CBZ, and LTG associated with anticonvulsant effects (green bars) and reductions in locomotor activity (red bars) are shown. PIs for each molecule are shown. Data presented as mean ± SEM. MES: n = 12–24/group, analysis of variance (ANOVA)/Dunn test; sLMA: n = 20/group, ANOVA/Dunnett test. *$p < .05$ versus vehicle (Veh), **$p < .01$ versus Veh

TABLE 2 PRAX-562 has improved preclinical PI compared to CBZ and LTG

	MES		sLMA		
	ED_{50}, mg/kg	EC_{50}, free brain, nmol·L^{-1}	TD_{50}, mg/kg	TC_{50}, free brain, nmol·L^{-1}	PI
PRAX-562	2.0	4.3	44	69.7	16.2
LTG	5.0	2754	37.6	12 853	4.7
CBZ	3.4	2410	26.5	14 350	5.9

Note: Mean drug concentrations associated with MES ED_{50}/EC_{50} and sLMA TD_{50}/TC_{50} are shown. PI was calculated as brain TC_{50}/brain EC_{50}.

Abbreviations: CBZ, carbamazepine; EC_{50}, half-maximal efficacious concentration; ED_{50}, half-maximal efficacious dose; LTG, lamotrigine; MES, maximal electroshock seizure; PI, protective index; sLMA, spontaneous locomotor activity; TC_{50}, half-maximal tolerated concentration; TD_{50}, half-maximal tolerated dose.

of their mechanism of Na_V block and/or could reflect off-target (non-Na_V-mediated) activities.[10–13] Enhanced persistent I_{Na} is commonly observed in various excitability disorders.[14–18] Notably, standard Na_V blockers are not selective for persistent I_{Na}, and some even show preference for peak I_{Na} under relevant physiologic conditions where the concomitant inhibition of persistent I_{Na} and peak I_{Na} may contribute to the observed narrow therapeutic window. Here, we test the hypothesis that preferential targeting of persistent I_{Na} would widen the

preclinical PI as a consequence of normalizing pathologic hyperexcitability while sparing normal neuronal activity.

During periods of elevated neuronal firing, such as during a seizure, some degree of use- (activity-) dependent block of peak I_{Na} is likely to drive therapeutic benefit. Use-dependent block has been proposed to represent a form of functional selectivity in which peak I_{Na} is inhibited to a greater degree at higher AP frequencies (seizure activity) and inhibited to a lesser degree, or not at all, at lower AP frequencies (physiological brain function).[25] Standard Na_V-targeting AEDs exhibit varying degrees of use-dependent block. However, as the concentration of standard Na_V-targeting AEDs increases, inhibition of peak I_{Na} transitions from use-dependent block to a tonic block (activity independent) dramatically reducing AP firing. Small molecules that display use-dependent block with specificity for persistent I_{Na} over peak I_{Na} should have a wider therapeutic window, as they simultaneously reduce excitability by limiting firing rate via both use-dependent block and reduction in excitability, enhancing persistent I_{Na}.[26]

PRAX-562 is a novel, orally active, small molecule that exhibits not only a 60-fold preference for persistent I_{Na} over peak I_{Na} but also a remarkable and unexpected 30-fold preference for use-dependent block over tonic block. This profile clearly differentiates it from standard Na_V-targeting AEDs examined in this study. Notably, the set of approved AEDs examined included the newer agent lacosamide, which has been proposed to interact with sodium channels in a nonstandard manner. Lacosamide is thought to preferentially bind to slow inactivated states instead of fast inactivated states.[26] The voltage protocols used in this study do not fully invoke slow inactivation, which limits the interpretation of lacosamide activity related to persistent I_{Na}. However, lacosamide did exhibit less use-dependent block relative to PRAX-562. Additional studies will be needed to determine whether PRAX-562 affects Na_V slow inactivation in a manner similar to that of lacosamide. Compared to standard Na_V-targeting AEDs, the unique profile of PRAX-562 may translate to a more efficacious inhibition of hyperexcitability and contribute to a wider therapeutic window.

Both PRAX-562 and CBZ were able to reduce hippocampal CA1 neuron AP firing consistent with a role of I_{Na} in neuronal intrinsic excitability. However, the potency and profile of each was distinct. PRAX-562 reduced the number of evoked APs (intrinsic excitability) with minimal effects on AP amplitude, which reflects minimal inhibition of peak I_{Na}. At an equivalent effective concentration (IC_{50} of peak I_{Na} voltage-dependent block), CBZ produced a larger reduction in both intrinsic excitability and AP amplitude. These data demonstrate that CBZ inhibited both persistent I_{Na} and peak I_{Na}, which would be predicted to more readily impair the ability of neurons to respond to physiological stimuli and incur toxicity at lower relative concentrations.

We investigated the preclinical activity of PRAX-562 in the MES model, which has good predictive validity for clinical anticonvulsant activity,[24] and compared it with the effects of standard Na_V blockers CBZ and LTG. In this model, seizures arise due to induced network synchronization in an otherwise normal brain. The maximally efficacious dose of PRAX-562 (10 mg/kg, conveying protection in 11 of 12 mice) prevented seizures but did not impair locomotor function. In contrast, CBZ and LTG only achieved full seizure prevention in this model at doses that also show impaired locomotion. These acute observations on efficacy and tolerability demonstrate a wider preclinical PI for PRAX-562, suggesting the potential for a corresponding wider therapeutic window in patients.

The narrow therapeutic window of standard Na_V blockers arises from both Na_V- and non-Na_V-mediated activities. Toxic side effects (including ataxia, double vision, vertigo) appear to be a class attribute (i.e., Na_V mediated), with potential tolerance following chronic treatment. In addition, the lower Na_V potency of approved agents requires higher clinical exposures that can lead to off-target non-Na_V-mediated activity, which may underlie drug-specific toxicities (such as the incidence of somnolence).[10–13] PRAX-562 is approximately 550-fold more potent for persistent I_{Na} compared to standard Na_V-targeting AEDs, thus reducing the potential for non-Na_V-mediated activity at clinical exposures. PRAX-562 displayed a 3× wider PI compared to CBZ and LTG when acute toxicity was assessed using MES and sLMA preclinical models. Additional studies are warranted to investigate the preclinical PI of PRAX-562 following chronic treatment, which would better reflect the anticipated clinical use of PRAX-562.

Recent preclinical studies have shown that GS967/PRAX-330, a molecule that also shows preference for persistent I_{Na}, can reduce seizures and prevent premature death in several transgenic mouse models of Na_V DEE, including the Scn8A-N1768D, Scn8a-R1782W, Scn2a-Q54, and $Scn1a^{+/-}$ mouse models.[19–23] We demonstrate here that PRAX-562 potently inhibits the persistent I_{Na} expressed by $hNa_V1.6$-N1768D in a heterologous expression system (Figure 1C). These data suggest that PRAX-562 may also exhibit activity in *SCN8A* and *SCN2A* DEE via preferential normalization of pathologically enhanced persistent I_{Na}. Additional studies will be required to explore the activity of PRAX-562 in these models.

PRAX-562 preferentially inhibits persistent I_{Na} in the Na_V channel isoforms $hNa_V1.1$, $hNa_V1.2$, and $hNa_V1.5$, which have all been associated with diseases of hyperexcitability.[27] This activity exhibits similar potencies and greater preference over peak I_{Na} compared to $hNa_V1.6$,

suggesting a profile of enhanced efficacy and improved tolerability compared with the currently available pan-Na_V blockers. Moreover, activity at several Na_V isoforms may broaden its potential clinical utility compared with isoform-specific molecules currently in development.[28] Additional studies will be required to determine whether PRAX-562 has efficacy and/or disease-modifying activity in models of diseases where increased persistent I_{Na} in these isoforms has been implicated, including migraine,[29] genetically defined seizure disorders,[30] pain,[31] and long-QT syndrome.[32]

In summary, PRAX-562 demonstrated robust anticonvulsant activity in vivo, with significantly improved preclinical tolerability compared with other Na_V blockers, suggesting a potential for an improved clinical therapeutic window. Given the role of persistent I_{Na} in modulating excitability, PRAX-562 has the potential to be a broadly efficacious and well-tolerated AED for both genetic and nongenetic epilepsies. The profile of PRAX-562 may also prove to be more broadly useful in diseases of hyperexcitability where Na_V blocker use is associated with limited efficacy due to poor tolerability.

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CONFLICT OF INTEREST

K.M.K. and L.S. are employees and shareholders of Praxis Precision Medicines; R.J.H. serves as a paid consultant to Praxis Precision Medicines and is not a shareholder; A.G. serves as a paid consultant to Praxis Precision Medicines and is a shareholder; Z.A.H. was an employee of Praxis Precision Medicines during study conduct and manuscript preparation, who has served as a paid consultant to Praxis Precision Medicines and is a shareholder; G.M.B. and M.W. were employees of Praxis Precision Medicines during study conduct and manuscript preparation, who serve as paid consultants to Praxis Precision Medicines and are shareholders. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

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