Discovery of a Novel Class of State-Dependent Na$_v$1.7 Inhibitors for the Treatment of Neuropathic Pain

Kyosuke Tanaka, a Hiroyuki Kobayashi, a Sayaka Suzuki, a Satoshi Shibuya, a Hiroko Kimoto, a,b Yuki Domon, a Kazufumi Kubota, a Yutaka Kitano, a Tomihisa Yokoyama, a Akiko Shimizugawa, a Ryuta Koishi, a,b Chie Fujiwara, a Daigo Asano, a and Tsuyoshi Shinozuka*, a

a R&D Division, Daitichi Sankyo Co., Ltd.; 1–2–58 Hiromachi, Shinagawa-ku, Tokyo 140–8710, Japan: and b Daiichi Sankyo RD Novare Co., Ltd.; 1–16–13 Kitakasai, Edogawa-ku, Tokyo 134–8630, Japan.

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The discovery of a novel class of state-dependent voltage-gated sodium channel (Na$_v$)1.7 inhibitors is described. By the modification of amide or urethane bond in Na$_v$1.7 blocker III, structure–activity relationship studies that led to the identification of novel Na$_v$1.7 inhibitor 2i (DS01171986) were performed. Compound 2i exhibited state-dependent inhibition of Na$_v$1.7 without Na$_v$1.1, Na$_v$1.5 or human ether-a-go-go related gene (hERG) liabilities at concentrations up to 100 μM. Further biological profiling successfully revealed that 2i possessed potent analgesic properties in a murine model of neuropathic pain (ED$_{50}$: 3.4 mg/kg) with an excellent central nervous system (CNS) safety margin (>600 fold).

Key words voltage-gated sodium channel; pain; central nervous system (CNS) side effect; quinolone; urethane

Introduction

Neuropathic pain (NP) is a debilitating disease that affects patients spontaneously and has a significant impact on their QOL. Diabetic peripheral neuropathy (DPN) is one of the most common types of NP. It is estimated that around half of diabetic patients suffer from DPN. The first-line treatments for DPN include calcium channel α₂δ ligands and tricyclic antidepressants. Although these drugs are efficacious and tolerable, central nervous system (CNS) adverse effects, such as drowsiness, lightheadedness, dizziness, and sedation, limit their use. Consequently, novel potent analgesic drugs with less CNS adverse effects have a high demand.

Voltage-gated sodium channels (Na$_v$) are responsible for transmitting neuronal signals not only in CNS but also in the peripheral nervous system (PNS). In humans, nine Na$_v$ subtypes (Na$_v$1.1–Na$_v$1.9) have been identified, and Na$_v$1.7 has been shown to play a crucial role in pain signaling. Several Na$_v$ blockers, such as lidocaine (I) and mexiletine (II) (Fig. 1), have been used to treat chronic pain in a clinical setting. Furthermore, a high safety index over CNS adverse effects is expected by selective Na$_v$1.7 inhibition, because the Na$_v$1.7 isoform is predominantly expressed in the peripheral nervous system (PNS). In the discovery of novel Na$_v$1.7 inhibitors, a high subtype selectivity is crucial, because multiple Na$_v$ subtypes are differentially expressed in various organs; non-selective inhibitors could cause adverse effects. For example, Na$_v$1.1 is expressed in brain, and Na$_v$1.1 inhibition is known to cause CNS adverse effects, whereas the inhibition of Na$_v$1.5 leads to cardiac arrhythmias. In fact, Na$_v$ blockers used in clinics are non-selective, and the lack of selectivity limits the usage of such drugs due to safety concerns. Therefore, the selective inhibition of Na$_v$1.7 is a promising analgesic target.

During the course of a project directed at acquiring potent selective Na$_v$1.7 inhibitors, we focused on benzazepinone derivative III (Fig. 1), reported by the Merck group, owing to its high state dependency. As two amide and one urethane bonds are found in this molecule, our derivatization strategy consists of the modifications of such bonds. Herein, we describe the conversion of these moieties to identify a novel class of state-dependent Na$_v$1.7 inhibitors with potent analgesic efficacy in a model of NP as well as an excellent CNS safety margin.

Results and Discussion

Human in Vitro Profile The in vitro evaluation of the compounds was performed utilizing the IonWorks Quattro automated electrophysiology platform. The inhibitory activity of hNa$_v$1.7 was evaluated at a half-inactivated state ($V_{\text{hold}}$ = −59 mV). In addition, the inhibitory potency of hNa$_v$1.7 was evaluated at an elevated membrane voltage ($V_{\text{hold}}$ = −30 mV), because an elevated Na$_v$1.7 membrane voltage is reported in damaged dorsal root ganglion (DRG) neurons. This screening process enabled us to provide an analgesic agent with high safety index owing to the ability to inhibit only abnormal hyperactive neurons with frequent action potentials without affecting normal firing activity. In this assay condition, we confirmed the inhibitory activity of the antiepileptic drug lacosamide, which is known to enhance slow inactivation of voltage-gated sodium channels selectively. In the in vitro

![Fig. 1. Structures of Lidocaine (I), Mexiletine (II), and Compound III, Reported by the Merck Group](image)

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screening, hNaV1.1 and hNaV1.5 inhibitory activities were acquired to monitor adverse effects.

We initially focused on the conversion of the benzazepinone moiety, as shown in Table 1. Compound III showed good inhibitory potency against hNaV1.7 at $V_{\text{hold}} - 30$ mV ($IC_{50} = 2.5 \mu M$) without hNaV1.1 or hNaV1.5 liability. Compound III was found to inhibit hNaV1.7 in a high state-dependent manner, because it did not inhibit hNaV1.7 at a half-inactivated state. As the removal of the trifluoromethyl group or the reduction of ring size (benzazepinone to dihydroquinolinone) did not affect hNaV1.7 potency (data not shown), tetrahydroquinoline 1a was synthesized, which resulted in the retention of hNaV1.7 potency. Compound 1a was prepared as a diastereomeric mixture (1:1). These results encouraged us to synthesize quinoline and isoquinoline derivatives. As expected, isoquinoline 1b and quinoline 1c exhibited potent hNaV1.7 activity. Compounds 1b and 1c also showed no hNaV1.1 or hNaV1.5 liabilities. As other regioisomers of 1b and 1c did not show potent inhibitory activity (data not shown), alternative heteroaromatic rings were investigated. 2-Benzazoles such as 1d, 1e and 1f retained hNaV1.7 activity. In particular, 2-benzimidazole 1f exhibited the best inhibitory potency among this series of compounds with increased hNaV1.1 or hNaV1.5 potency. In contrast to quinolone derivatives, the regioisomer of 1d, 5-benzo thiazole 1g retained hNaV1.7 potency without hNaV1.1 or hNaV1.5 liabilities. Although the enhancement of hNaV1.7 activity was expected by the introduction of a nitrogen in 1g, thiazolopyridine 1h showed attenuated hNaV1.7 potency.

The replacement of acid labile Boc group of 1c was then investigated (Table 2). hNaV1.7 activity was found to be correlated to the lipophilicity of molecules in the modification of t-butyl group. The inhibitory activity of isopropyl 2a and ethyl derivative 2b is 2.3 and 10 $\mu M$, respectively, whereas methyl analog 2c almost lost activity. Consequently, t-butyl group was optimal in this position. The introduction of methyl group in the urethane moiety led to the loss of potency in vitro, suggesting that a hydrogen bond may exist between this moiety and the ion channel (compound 2d). The importance of this hydrogen bond network was confirmed by amide derivatives, because $\beta$-alanine 2e and neopentyl amide 2f maintained the activity. As the hydrogen bond donor exists in this position,

| Compound | R | hNaV1.1 | hNaV1.5 | hNaV1.7 | hNaV1.7 |
|----------|---|---------|---------|---------|---------|
|          |   | $IC_{50}$ (µM) | $IC_{50}$ (µM) | $IC_{50}$ (µM) | $IC_{50}$ (µM) |
| III      |   | >100     | >100    | >100    | 2.5 ± 0.45 |
| 1a       |   | 32       | 45      | 72      | 6.9      |
| 1b       |   | 47       | 73      | >100    | 3.0      |
| 1c       |   | >100     | 33 ± 7.7| >100    | 1.6 ± 0.13 |
| 1d       |   | >100     | >100    | >100    | 9.1      |
| 1e       |   | >100     | 36      | >100    | 4.8      |
| 1f       |   | 17       | 32      | >100    | 1.4      |
| 1g       |   | >100     | >100    | >100    | 5.2 ± 0.33 |
| 1h       |   | 51       | 16      | 68      | 8.1      |

- Values of at least two independent experiments run in quadruplicate, unless otherwise noted. Each value represents the mean ± standard error of the mean (S.E.M.)
- Values at a half-inactivated state.
- Values at $V_{\text{hold}} - 30$ mV.
- Diastereomeric mixture (1:1).
- Values of a single experiment run in quadruplicate.

Table 1. *In Vitro* Profile of NaV1.7 Inhibitors 1-10
urea 2h maintained the activity. Compounds 2e and 2g were prepared as a racemic mixture. Reverse urethane 2i displayed decent hNaV1.7 potency (IC$_{50}$ = 5.2 µM), and 2i did not exhibit hNaV1.1 or hNaV1.5 liabilities at concentrations up to 100 µM.

Mice in Vitro Profile, in Vitro ADME Properties, and Pharmacokinetics (PK) Parameters in Mice Potent compounds without hNaV1.1 or hNaV1.5 liabilities were evaluated for mouse NaV activities, in vitro ADME properties and PK parameters in mice as shown in Table 3. To evaluate the state dependency, mNaV1.7 activity at V$_{hold}$ − 30 mV was measured, and all selected compounds exhibited comparable mouse in vitro profiles to that of human. Compounds 1c, 1g, and 2i exerted high membrane permeability in Parallel Artificial Membrane Permeation Assay (PAMPA) and were expected to exhibit high plasma exposure in the PK study. Compound 1b lacked adequate aqueous solubility, whereas better solubility was observed in other compounds. Additionally, all evaluated compounds exhibited acceptable plasma protein binding (PB) ability (free fraction >1.0%). PK parameters were acquired by orally administering the compounds to mice at 30 mg/kg. Compounds 1b and 1c showed poor plasma exposure of the test compound, whereas a slight improvement of the PK profile was observed in reverse urethane 2i. Further improvement of the plasma exposure was observed in benzoazole 1g. Considering the mouse in vitro activity, plasma PB ability, and PK parameters, 1g was expected to exhibit potent efficacy. In the PK evaluation, all evaluated compounds exhibited T$_{max}$ less than 1 h, which would be expected immediate onset of pharmacological efficacy in vivo.

To unveil the brain penetration of the compound, the Kp brain value of 1c and 2i was assessed. After 0.5 h of the compound administration (30 mg/kg, p.o.), the brain concentration of the compounds was determined (n = 2). Compound 2i indicated less exposure in the brain than in the plasma, while higher brain concentration of 1c was observed.

In Vivo Efficacy The assessment of in vivo efficacy was conducted on thermal hyperalgesia in mice evoked by partial sciatic nerve ligation (PSL, Seltzer), a model of NP 21) (Fig. 2). The model was prepared by partial ligation of 1/2 to 1/3 of the left sciatic nerve with silk suture. The paw withdrawal latency (PWL, second) was assessed after 30, 60, 120, and 180 min of oral administration of the test compound (Plantar...
Compared with the normal group, the pain threshold was significantly lowered in the operated group, indicating the development of hyperalgesia. The time course of the efficacy of compound 2i administered to PSL mice is shown in Fig. 2a. The administration of 2i at 3 mg/kg p.o. (red curve) reversed the thermal PWL from 30 to 120 min. This reversal of hyperalgesia was maximized significantly from 30 to 60 min (p < 0.01 at 30 min, and p < 0.05 at 60 min). The peak efficacy improved PWL to almost normal levels. The therapeutic efficacy of 2i at 3 mg/kg was maximized from 30 to 60 min, whereas the plasma concentration of 2i at 30 mg/kg peaked at 30 min in the PK study. The thermal hyperalgesia was suppressed in a dose dependent manner (3, 10, and 30 mg/kg), and the administration of 2i at 30 mg/kg significantly reversed hyperalgesia at all timepoints evaluated (purple curve, p < 0.01 from 30 to 120 min, and p < 0.05 at 180 min). Potent analgesic efficacy lasted until 180 min when 10 or 30 mg/kg of 2i was administered. The retention of potent efficacy at 30 mg/kg administered group correlates with the PK parameters, because T1/2 of 2i at 30 mg/kg was 6.8 h (Table 3). AUC0–3h (area under curve for 0–3 h) for each curve was calculated to analyze the total anti-hyperalgesic effect (Fig. 2b). The anti-hyperalgesic

Table 3. Mouse NaV IC50 Values, in Vitro ADME Properties and PK Parameters

| Compound | 1b | 1c | 1g | 2i |
|----------|----|----|----|----|
| mNaV1.1 IC50 (µM) | 18b | 29 ± 8.4 | >100b | >100b |
| mNaV1.5 IC50 (µM) | 43b | 43 ± 12 | >100b | >100b |
| mNaV1.7 IC50 (µM) | >100b | >100b | >100b | >100b |
| PAMPA P_app (10−6 cm/s) | NTi | 2.0 ± 0.24 | 4.8 ± 0.70 | 11.8 ± 2.2 |
| Solubility (µg/mL) | 1.4 | 23 | 28 | 17 |
| PK parameters in ddY mice at 30 mg/kg | Cmax (µg/mL) | 0.010 ± 0.0036 | 0.032 ± 0.0077 | 1.45 ± 0.090 | 0.10 ± 0.019 |
| T max (h) | 0.67 ± 0.29 | 0.67 ± 0.29 | 0.50 ± 0.00 | 0.50 ± 0.00 |
| AUC 0–24h (µg/mL) | 0.031 ± 0.020 | 0.051 ± 0.011 | 2.84 ± 0.24 | 0.20 ± 0.047 |
| T1/2 (h) | 1.4 ± 0.47 | 1.1 ± 0.53 | 6.0 ± 2.4 | 6.8 ± 3.3 |
| Kp Brain | NTi | 1.65 | NTi | 0.69 |

a) Values of at least two independent experiments run in quadruplicate unless otherwise noted. Each value represents the mean ± S.E.M.
b) Values at a half-inactivated state.
c) Values at Vhold = −30 mV.
d) PAMPA was performed at pH 7.4. Values of a single experiment run in duplicate.
e) Aqueous thermodynamic solubility at pH 6.8. Values of a single experiment run in duplicate.
f) Unbound fractions (%) in mouse plasma.
g) Average of three male ddY mice dosed at 30 mg/kg p.o. (p.o.) in N,N-dimethylacetamide/Tween80/saline: 10/10/80.
h) Values of a single experiment run in quadruplicate.
i) Not tested.
effect occurred in a clear dose dependent manner, and ED\textsubscript{50} of 2i was estimated to be 3.4 mg/kg. Compound 2i at 30 mg/kg significantly improved the total hyperalgesic effect to the normal levels (p < 0.01).

Isoquinoline 1b failed to show potent efficacy on thermal hyperalgesia at 30 mg/kg (data not shown), owing to less plasma exposure of 1b, as suggested by the PK study. The total anti-hyperalgesic effect of 1c and 1g is shown in Figs. 2c and 2d. Compound 1c suppressed thermal hyperalgesia in a dose-dependent manner (3, 10, 30 mg/kg), and ED\textsubscript{50} was estimated to be 23 mg/kg. Although a remarkable improvement in PK parameters was observed in 5-benzothiazole 1g, the efficacy of 1g in PSL mice was comparable to that of 1c, with ED\textsubscript{50} = 16 mg/kg. There was a difference observed in AUC\textsubscript{0–3 h} of control groups (vehicle-treated and normal groups) in Figs. 2b, 2c, and 2d. AUC\textsubscript{0–3 h} of control groups in Fig. 2d is higher than those in Figs. 2b and 2c. Consequently, the pain threshold of the study with 1g is higher than those with 2i and 1c.

As ED\textsubscript{50} value is calculated defined PWL of vehicle-treated group as 0%, and PWL of normal mice as 100%, the inter-assay variation in pain threshold has no significant influence on ED\textsubscript{50}.

**Toxicological Evaluation** Compounds with potent analgesic efficacy (1c, 1g, and 2i) were selected for CNS toxicological evaluation as indicated in Fig. 3. The in vivo CNS side effects were assessed using a mice rotarod test, a model of motor coordination at 30, 100, and 300 mg/kg (n = 10). TK parameters were acquired at 100 or 300 mg/kg from a satellite group (Table 4, n = 1–3). As the administration of compound 1g at 300 mg/kg was lethal (two mice died after 120 min), the results of rotarod test are shown in 30 and 100 mg/kg administered group (Fig. 2b). The administration of 1g at 30 or 100 mg/kg did not effect on rotarod activity in mice, and ID\textsubscript{50} was calculated to be >100 mg/kg. Compared to anti-thermal hyperalgesia efficacy, plasma-exposure-based CNS safety margin of 1g was determined to be >8.6-fold (Table 4).
the contrary, two other compounds, 1c and 2i, demonstrated superior CNS safety properties. Although 300 mg/kg administration of compound 1e significantly affected the results of rotarod test at 30 min (p < 0.01), this side effect was attenuated at 120 min (ID₅₀ > 300 mg/kg). Consequently, the plasma exposure-based CNS safety margin of 1c was determined to be >100-fold. Furthermore, the administration of 2i at 300 mg/kg did not affect mice motor activity at all timepoints with ID₅₀ > 300 mg/kg. Consequently, the plasma exposure-based CNS safety margin of 2i was >600-fold.

With promising results, 2i was further evaluated. An in vitro assessment established that 2i did not inhibit other related ion channels, including human Naᵥ1.2, 1.3, 1.4, 1.6, or human ether-a-go-go related gene (hERG). Moreover, in vitro pharmacological activity of 2i on a total of 67 receptors, channels, and transporters was evaluated; 2i inhibited adenosine A₁ and serotonin 5-HT₂B by >50% at 10 µM. These data indicate that 2i is a selective Naᵥ1.7 inhibitor in vitro.

Conclusion

The discovery of quinoline amides as a novel class of state-dependent Naᵥ1,7 inhibitors has been described. The benzazepinone moiety of compound III was replaced with a heteroaromatic ring. Among a variety of heteroaromatic rings examined, quinoline ring was shown to be optimal regarding Naᵥ1.7 liabilities. The modification of acid-unstable Boc group led to the discovery of reverse urethane 2i (DS01171986). Compound 2i inhibited hNaᵥ1.7 at IC₅₀ = 5.2 µM in a state-dependent manner. Notably, 2i inhibited Naᵥ1.7 without affecting other receptors, channels, and transporters, including other Naᵥ subtypes and hERG. Compound 2i demonstrated potent analgesic efficacy in NP model with EC₅₀ = 3.4 mg/kg. A CNS adverse effect evaluation revealed that 2i possessed excellent CNS safety margin (>600 fold).

Experimental

General

Starting reagents were purchased from commercial suppliers and were used without further purification unless otherwise specified. Chromatographic elution was carried under continuous monitoring by TLC using silica gel 60F254 (Merck & Co., Inc., Germany) as the stationary phase; the mobile phase was the elution solvent used in column chromatography. A UV detector was used for detection. Silica gel SK-85 (230–400 mesh) or silica gel SK-34 (70–230 mesh), manufactured by Fuji Sylsia Chemical Ltd. (Japan), was used as the column packing silica gel. ¹H-NMR spectra were obtained on Varian Unity 400- and 500-MHz spectrometers. Spectra were recorded in the indicated solvent at ambient temperature; chemical shifts are reported in ppm (δ) relative to the solvent peak. Resonance patterns are represented with the following notations: br (broad signal), s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). MS analysis was carried out by FAB, electron ionization (EI), or electrospray ionization (ESI). High resolution (HR)MS was carried out using an LC-MS system composed of a Waters Xevo Q-ToF MS and an Agniitutus UHPLC system. Elemental analyses were carried out on a Microcougu JMI0 and a Dionex ICS-1500. The purity of compounds was confirmed as >95% by diode array detector (DAD) and Agilient 1260 LCMS systems. Conditions [column: Develosil Combi-RP-5 2.0 mm × 50 mmL, gradient elution: 0.1% HCO₃⁻–H₂O/0.1% HCO₃⁻–MeCN = 98/2 to 0/100 (v/v), flow rate: 1.2 mL/min, UV detection: 254 nm, column temperature: 40°C, ionization: APC/ESI].

Chemical Synthesis

Compounds 1a–h listed in Table 1 were synthesized by amidation of corresponding amine with N-Boc-α-phenylalanine under usual reaction conditions (Chart 1). The synthesis of thiazolopyridine 7 for 1h was accomplished by the annulation of chloropyridine 5 as a key step.²³ Chart 2 summaries the preparation of compounds 2a–i listed in Table 3. Compounds 2a–2c and 2f were synthesized from amine 8 derived from 1c by the reaction with carbonyl chlorides, whereas urea 2h was prepared from 8 reacted with tert-butyl isocyanate. N-Methyl urethane 2d was prepared by the condensation of 3-aminooquinoline with N-methyl-N-Boc-α-phenylalanine (structures not shown). The synthesis of β-alanine 2e was achieved from key intermediate 11, which was prepared from benzy alcohol 10 and tert-butyl bromoacetate.²⁴ Started from 15,²⁵ neopentyl amide 2g was synthesized in a similar manner. Reverse urethane 2i was prepared from n-phenyllactic acid 18.

General Procedure for Amidation (General Procedure A)

A solution of amine (3.47 mmol), carboxylic acid (3.47 mmol), N,N-diisopropylethylamine (DIPEA) (2.7 mL, 15.6 mmol), N-hydroxybenzotriazole (HOBt)·H₂O (0.84 g, 6.24 mmol) and WSC·HCl (1.20 g, 6.24 mmol) in tetrahydrofuran (THF) (23 mL) was stirred at room temperature overnight under N₂. Water was added to the reaction mixture, and the mixture was extracted with EtOAc several times. The combined organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by silica gel chromatography to obtain 1.

General Procedure for Amidation to Prepare 2 (General Procedure B)

A solution of amine 3 (70 mg, 0.24 mmol) and...
Et₃N (0.10 mL, 0.72 mmol) in CH₂Cl₂ (1.2 mL) was added to the solution of benzyl ester (3.72 mmol) and Pd/C (10%, 300 mg) in EtOH (10 mL) was stirred at room temperature under H₂ for several hours. After being stirred at room temperature for several hours, the mixture was directly purified by silica gel chromatography to obtain 2a–2c or 2f.

**General Procedure for Deprotection by Hydrogeonalysis (General Procedure C)** A solution of benzyl ester (3.72 mmol) and Pd/C (10%, 300 mg) in EtOH (10 mL) was stirred at room temperature under H₂ for several hours. After the removal of the catalyst, the residue was concentrated to obtain carboxylic acid.

**tert-Butyl[(2R)-1-oxo-3-phenyl-1,2,3,4-tetrahydroquinolin-3-ylamino]propan-2-yl]carbamate (1a)** Prepared according to General Procedure A as a diastereomeric mixture. Yield: 99%. HRMS (ESI) m/z: [M + H]⁺ calcd for C₂₃H₂₅N₃O₃: C, 70.57; H, 6.44; N, 10.73. Found C, 70.49; H, 6.19; N, 10.82; δ: 1.45 (9H, s), 3.25 (2H, t, J = 6.0, 14.4 Hz), 4.74 (1H, s), 5.19 (1H, s), 7.07–7.13 (2H, m), 7.18–7.25 (3H, m), 7.32 (1H, t, J = 6.8 Hz), 7.43 (1H, t, J = 8.4 Hz), 7.76 (1H, d, J = 8.0 Hz), 7.83 (1H, d, J = 8.1 Hz), 8.01 (1H, s), 8.09 (1H, s), 8.94 (1H, s); MS (APCI/ESI) m/z: 392 [M + H]⁺; Anal. calcd for C₂₃H₂₅N₃O₃·0.20H₂O: C, 65.82; H, 6.10; N, 10.96. Found C, 65.69; H, 6.19; N, 10.82; [α]_D²⁵ +14.0 (c = 1.007, CHCl₃).

**N-1,3-Benzoxazol-2-yl-Na-(tert-butoxycarbonyl)-o-phenylalaninamide (1d)** Prepared according to General Procedure A. Yield: 90%. H-NMR (400 MHz, CDCl₃) δ: 1.40 (9H, s), 3.08–3.16 (1H, m), 3.22 (1H, dd, J = 6.0, 14.4 Hz), 4.74 (1H, s), 5.19 (1H, s), 7.07–7.13 (2H, m), 7.18–7.25 (3H, m), 7.32 (1H, t, J = 6.8 Hz), 7.43 (1H, t, J = 8.4 Hz), 7.76 (1H, d, J = 8.0 Hz), 7.83 (1H, d, J = 8.1 Hz), 8.01 (1H, s), 8.09 (1H, s), 8.94 (1H, s); MS (APCI/ESI) m/z: 398 [M + H]⁺; Anal. calcd for C₂₂H₂₂N₃O₃·0.20H₂O: C, 62.89; H, 5.88; N, 10.48. Found C, 62.84; H, 5.83; N, 10.56; [α]_D²⁵ +59.9 (c = 1.007, CHCl₃).

**N-1,3-Benzoxazol-2-yl-Na-(tert-butoxycarbonyl)-o-phenylalaninamide (1e)** Prepared according to General Procedure A. Yield: 50%. H-NMR (400 MHz, CDCl₃) δ: 1.41 (9H, s), 3.13 (1H, s), 3.28 (1H, dd, J = 5.9, 14.1 Hz), 4.69 (1H, s), 5.14 (1H, s), 7.20–7.32 (8H, m), 7.45 (1H, d, J = 8.2 Hz); MS (APCI/ESI) m/z: 382 [M + H]⁺; Anal. calcd for C₂₁H₂₁N₃O₃: C, 65.69; H, 6.19; N, 10.82; [α]_D²⁵ +14.0 (c = 1.007, CHCl₃).

**Nα-(tert-Butoxycarbonyl)-N-(1-methyl-1H-benzimidazol-2-yl)-o-phenylalaninamide (1f)** Prepared according to General Procedure A. Yield: 30%. H-NMR (400 MHz, CDCl₃) δ: 1.45 (9H, s), 3.25 (2H, t, J = 5.3 Hz), 3.61 (3H, s), 4.62–4.67 (1H, m), 5.43 (1H, d, J = 7.0 Hz), 7.17–7.31 (9H, m); MS (APCI/ESI) m/z: 395 [M + H]⁺; Anal. calcd for C₂₂H₂₂N₃O₃·0.10H₂O: C, 66.68; H, 6.66; N, 14.14. Found C, 66.56; H, 6.58; N, 14.16; [α]_D²⁵ +73.9 (c = 0.740, CHCl₃).
Na-(tert-Butyroxycarbonyl)-N-(2-methyl-1,3-benzothiazol-5-yl)-o-phenylanilinamide (1g) Prepared according to General Procedure A. Yield: 88%. H-NMR (400 MHz, CDCl₃): δ: 1.44 (9H, s), 2.82 (3H, s), 3.12–3.20 (2H, m), 4.46 (1H, br), 5.14 (1H, br), 7.26–7.34 (5H, m), 7.45 (1H, dd, J = 2.0, 8.6 Hz), 7.71 (1H, d), 7.80 (1H, br), 7.91 (1H, d, J = 2.0 Hz); HRMS (ESI) m/z: [M + H⁺]⁺ calcd for C₂₅H₂₅N₃O₂: 390.1711; found 390.1713; [M + H⁺]⁺ calcd for C₂₅H₂₅N₃O₂·0.3H₂O: C, 73.00; H, 7.04; N, 10.64. Found C, 73.11; H, 7.01; N, 10.64; [α]D → +12.7 (c = 1.008, CHCl₃).

2-Benzyl-N-(2,2-dimethylpropyl)-N’-(quinolin-3-yl)propanediamide (2g) Prepared according to General Procedure A utilizing CH₂Cl₂ as a solvent. Yield: 63%. H-NMR (400 MHz, CDCl₃): δ: 0.85 (9H, s), 3.02 (1H, dd, J = 6.1, 13.1 Hz), 3.09 (1H, dd, J = 6.7, 13.3 Hz), 3.27 (1H, d, J = 8.6, 13.7 Hz), 3.36 (1H, dd, J = 7.0, 13.7 Hz), 3.64 (1H, t, J = 7.6 Hz), 6.89 (1H, t, J = 5.9 Hz), 7.06–7.27 (5H, m), 7.53 (1H, t, J = 7.2 Hz), 7.63 (1H, t, J = 7.0 Hz), 7.77 (1H, d, J = 7.8 Hz), 8.04 (1H, d, J = 8.6 Hz), 8.64 (1H, d, J = 2.3 Hz), 8.76 (1H, d, J = 2.7 Hz), 10.08 (1H, s); MS (APCI/ESI) m/z: 390 [M + H⁺]; Anal. calcd for C₂₅H₂₇N₃O₂·0.2H₂O: C, 73.33; H, 7.03; N, 10.69. Found C, 73.29; H, 7.02; N, 10.66.

α-(tert-Butyloxycarbonyl)-N-(2-methyl-1,3-thiazol-5-yl)-o-phenylanilinamide (2h) A solution of amine 3 (52 mg, 0.18 mmol) and tert-butyloxycarbonyl (40 µL, 0.36 mmol) in toluene (2.3 mL) was stirred at room temperature for 18 h under N₂. The mixture was directly purified by silica gel chromatography to obtain 2h (54.1 mg, 77%) as a mixture of conformers. MS (APCI/ESI) m/z: 391 [M + H⁺; Anal. calcd for C₂₅H₂₇N₃O₂·0.2H₂O: C, 70.10; H, 6.75; N, 14.22. Found C, 69.95; H, 6.73; N, 14.19; [α]D → +6.1 (c = 1.010, CHCl₃).

2-(R)-1-Oxo-3-phenyl-1-(quinolin-3-ylamino)-propan-2-ylcarbamate (2a) Prepared as a mixture of conformers according to General Procedure B utilizing isopropyl carbamoyl chloride as an acetylating reagent. Yield: 46%. H-NMR (400 MHz, CDCl₃): δ: 1.45 (9H, s), 2.85 (3H, s), 3.13–3.25 (2H, m), 4.46–4.54 (1H, m), 5.13 (1H, br), 7.26–7.36 (5H, m), 7.99 (1H, br), 8.39 (1H, d, J = 2.3 Hz), 8.44 (1H, d, J = 2.0 Hz); HRMS (ESI) m/z: [M + H⁺]⁺ calcd for C₂₅H₂₅N₃O₂, 416.1690; found 416.1709; Anal. calcd for C₂₅H₂₅N₃O₂: C, 72.41; H, 6.12; N, 10.21. Found C,63,92; H, 6.36; N, 10.13; [α]D → +4.23 (c = 1.000, CHCl₃).

Ethyl[(2R)-1-oxo-3-phenyl-1-(quinolin-3-ylamino)propan-2-yl]carbamate (2b) Prepared as a mixture of conformers according to General Procedure B utilizing ethyl carbamoyl chloride as an acetylating reagent. Yield: 80%. MS (APCI/ESI) m/z: 364 [M + H⁺]; Anal. calcd for C₂₅H₂₇N₃O₂: 0.50H₂O: C, 67.73; H, 5.95; N, 11.28. Found C, 67.77; H, 5.79; N, 11.26; [α]D → +2.6 (c = 1.002, CHCl₃).

Methyl[(2R)-1-oxo-3-phenyl-1-(quinolin-3-ylamino)propan-2-yl]carbamate (2c) Prepared as a mixture of conformers according to General Procedure B utilizing methyl carbamoyl chloride as an acetylating reagent. Yield: 96%. H-NMR (400 MHz, CDCl₃): δ: 3.16 (1H, dd, J = 7.4, 13.7 Hz), 3.26 (1H, dd, J = 6.7, 13.7 Hz), 3.73 (3H, s), 4.56–4.62 (1H, m), 5.37 (1H, s), 7.25–7.35 (5H, m), 7.52–7.55 (1H, m), 7.61–7.66 (1H, m), 7.79 (1H, d, J = 8.2 Hz), 8.02 (1H, d, J = 8.6 Hz), 8.51 (1H, d, J = 2.7 Hz), 8.64 (1H, d, J = 2.3 Hz); HRMS (ESI) m/z: [M + H⁺]⁺ calcd for C₂₅H₂₅N₃O₂, 350.1510; found 350.1504; [α]D → +6.2 (c = 0.036, CHCl₃).

tert-Butyl Methyl[(2R)-1-oxo-3-phenyl-1-(quinolin-3-ylamino)propan-2-yl]carbamate (2d) Prepared according to General Procedure A utilizing tert-butylcarbonyl-N-methyl-o-phenylalanine as a starting material. Yield: 99%. H-NMR (400 MHz, CDCl₃): δ: 1.44 (9H, s), 2.85 (3H, s), 3.09–3.16 (1H, m), 3.39–3.46 (1H, m), 5.01–5.06 (1H, m), 7.24–7.34 (5H, m), 7.53 (1H, t, J = 7.02 Hz), 7.62 (1H, t, J = 7.4 Hz), 7.79 (1H, d, J = 8.2 Hz), 8.03 (1H, d, J = 8.2 Hz), 8.67 (1H, s), 8.73 (1H, s), 8.91 (1H, s); MS (APCI/ESI) m/z: 406 [M + H⁺; Anal. calcd for C₂₅H₂₇N₃O₂·0.4H₂O: C, 69.85; H, 6.79; N, 10.18. Found C, 69.92; H, 6.74; N, 10.33; [α]D → +64.7 (c = 1.001, CHCl₃).
was concentrated under reduced pressure to obtain crude 7, which was used directly for the next reaction without further purification.

**N-Quinolin-3-yl-o-phenylalaninamide (8)** A solution of 1c (1.20 g, 3.07 mmol) and TFA (6.0 mL in CH₂Cl₂ (30 mL)) was added to 1-butyl bromoacetate (4.40 g, 22.6 mmol) was added to the reaction mixture at 0°C for 30 min. THF (10 mL) solution of the reaction mixture at 0°C under N₂. After being stirred for 20 min at 0°C, THF (20 mL) solution of the catalyst, the residue was obtained after extraction with EtOAc twice. The combined organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc, 90 : 10) to obtain 10 (3.90 g, 91%). 1H-NMR (400 MHz, CDCl₃) δ: 2.69 (2H, t, J = 7.4 Hz), 2.97 (2H, t, J = 7.8 Hz), 5.11 (2H, s), 7.17–7.37 (10H, m).

**Benzyl 3-phenylpropanoate (10)** A solution of benzyl 3-phenylpropanoyl chloride (4, 3.0 g, 17.8 mmol), Et₂N (3.0 mL, 21.5 mmol) and BnOH (2.0 g, 18.5 mmol) in CH₂Cl₂ (40 mL) was added to the reaction mixture at 0°C under N₂. After being stirred for 20 min at 0°C, THF (20 mL) solution of the catalyst, the residue was concentrated to obtain crude 10, the mixture was stirred at room temperature for 2 h. Water was added to the reaction mixture, and the mixture was extracted with EtOAc twice. The combined organic layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc, 90 : 10) to obtain 10 (3.90 g, 91%). 1H-NMR (400 MHz, CDCl₃) δ: 2.69 (2H, t, J = 7.4 Hz), 2.97 (2H, t, J = 7.8 Hz), 5.11 (2H, s), 7.17–7.37 (10H, m).

**1-Benzyl-4-(tert-butyli)-2-benzylbutanedioate (11) n-BuLi (1.57 mmol/L in THF solution, 12.5 mL, 19.6 mmol) was added to a solution of P(NH₂)₂ (2.75 mL, 19.5 mmol) in THF (50 mL) at 0°C under N₂. After being stirred for 20 min at 0°C, THF (20 mL) solution of the catalyst, the residue was obtained after extraction with EtOAc twice. The combined organic layers were washed with water, dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexane/EtOAc, 90 : 10) to obtain 11 (3.29 g, 56%) as a colorless oil. 1H-NMR (400 MHz, CDCl₃) δ: 1.39 (9H, s), 2.35 (1H, dd, J = 5.1, 16.4 Hz), 2.60 (1H, dd, J = 8.6, 16.4 Hz), 2.75–2.82 (1H, m), 3.02 (1H, dd, J = 6.7, 13.7 Hz), 3.12–3.14 (1H, m), 5.06 (1H, d, J = 12.5 Hz), 5.11 (1H, d, J = 12.5 Hz), 7.03–7.17 (10H, m).

**3-Benzyl-4-(benzoxily)-4-oxobutanoic Acid (12)** A solution of 11 (1.60 g, 4.51 mmol) and TFA (15 mL) in CH₂Cl₂ (15 mL) was stirred at room temperature for 18 h. After concentration of the reaction mixture, the mixture was purified by silica gel chromatography (CH₃Cl₂/MeOH, 90 : 10) to obtain 12 (970 mg, 72%) as a colorless oil. 1H-NMR (400 MHz, CDCl₃) δ: 2.46 (1H, dd, J = 5.1, 17.2 Hz), 2.73 (1H, dd, J = 9.8, 17.2 Hz), 2.79 (1H, dd, J = 8.2, 13.7 Hz), 3.07 (1H, dd, J = 6.3, 13.7 Hz), 3.13–3.20 (1H, m), 5.10 (2H, s), 7.11–7.36 (10H, m).

**Benzyl 2-Benzyl-4-(tert-butyli)-4-oxobutanoate (13)** Prepared according to General Procedure A utilizing CH₂Cl₂ as a solvent. Yield: 85%. 1H-NMR (500 MHz, CDCl₃) δ: 1.28 (9H, s), 2.20 (1H, dd, J = 4.9, 14.2 Hz), 2.40 (1H, dd, J = 9.0, 14.9 Hz), 2.83 (1H, dd, J = 7.3, 12.7 Hz), 2.99 (1H, dd, J = 6.8, 13.7 Hz), 3.23–3.29 (1H, m), 5.06 (1H, d, J = 12.1 Hz), 5.11 (1H, d, J = 12.7 Hz), 5.26 (1H, s), 7.11–7.36 (10H, m).
After these pre-compound measurements, the F-head added 3.5 µL of solution from each well of the compound plate to each well on the PatchPlate. After about 5.5 min of incubation, the E-head moved around the PatchPlate to obtain post-compound Na_\text{V} current measurements. The pre- and post-compound Na_\text{V} current amplitude were measured from the peak current response subtracting the baseline current. The degree of Na_\text{V} current block was corrected by vehicle control currents as follows:

\[
\% \text{ inhibition} = 100 \times \left( 1 - \frac{\text{relative current (compound)}}{\text{mean relative current (vehicle)}} \right)
\]

where, relative current (compound) is the value of the post-compound Na_\text{V} current amplitude divided by the respective pre-compound Na_\text{V} current amplitude and mean relative current (vehicle) is the mean value of the post-vehicle Na_\text{V} current amplitude divided by the pre-vehicle Na_\text{V} current amplitude.

Data was fitted with a four parameter logistic equation:

\[
Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{(\text{Log IC}_{50} - X) \times \text{HillSlope}})
\]

The voltage program for each Na_\text{V} subtype or species is summarized in Table 5.

### Table 5. Voltage Program

| Voltage Program                        | hNa_\text{V}1.1\(^a\) | hNa_\text{V}1.5\(^a\) | hNa_\text{V}1.7\(^a\) | hNa_\text{V}1.7\(^b\) | mNa_\text{V}1.1\(^a\) | mNa_\text{V}1.5\(^a\) | mNa_\text{V}1.7\(^a\) |
|----------------------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Holding pulse (for 5 s)                | -100 mV                | -120 mV                | -100 mV                | -100 mV                | -100 mV                | -120 mV                | -100 mV                |
| Second conditioning pulse (for 2 s or 10 s) | -43 mV | -68 mV | -59 mV | -30 mV | -44 mV | -69 mV | -56 mV | -30 mV |
| Holding pulse (for 50 ms)              | -100 mV                |                        | -100 mV                |                        | -100 mV                | -100 mV                |                        |
| Hyperpolarizing pulse (for 20 ms)      | -140 mV                |                        | -140 mV                |                        | -140 mV                |                        |                        |
| Test pulse (for analysis of inactivated-state channels, for 50 ms) | 0 mV | -20 mV | -10 mV | -10 mV | 0 mV | -20 mV | -10 mV | -10 mV |

\(a\) Half-inactivated state. \(b\) \(V_{\text{th}} = -30\) mV.

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