Bioorganic & Medicinal Chemistry 22 (2014) 6899–6907

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Discovery of novel tetrahydroisoquinoline derivatives as orally active N-type calcium channel blockers with high selectivity for hERG potassium channels

Takashi Ogiyama [a,*], Makoto Inoue [a], Shugo Honda [a], Hiroyoshi Yamada [a], Toshihiro Watanabe [a], Takayasu Gotoh [a], Tetsuo Kiso [a], Akiko Koakutsu [b], Shuichiro Kakimoto [a], Jun-ichi Shishikura [a]

[a] Drug Discovery Research, Astellas Pharma Inc., 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan
[b] Drug Discovery Research, Astellas Pharma Inc., 2-1-6 Kashima, Yodogawa-ku, Osaka 532-8514, Japan

ARTICLE INFO

Article history:
Received 9 September 2014
Revised 16 October 2014
Accepted 16 October 2014
Available online 22 October 2014

Keywords:
N-type calcium channel
Pain
hERG

ABSTRACT

N-type calcium channels represent a promising target for the treatment of neuropathic pain. The selective N-type calcium channel blocker ziconotide ameliorates severe chronic pain but has a narrow therapeutic window and requires intrathecal administration. We identified tetrahydroisoquinoline derivative **1a** as a novel potent N-type calcium channel blocker. However, this compound also exhibited potent inhibitory activity against hERG channels. Structural optimizations led to identification of (1*S*)-(1-cyclohexyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[(1-hydroxycyclohexyl)methyl]amino}ethanone ((*S*)-**1h**), which exhibited high selectivity for hERG channels while retaining potency for N-type calcium channel inhibition. (*S*)-**1h** went on to demonstrate in vivo efficacy as an orally available N-type calcium channel blocker in a rat spinal nerve ligation model of neuropathic pain.

1. Introduction

Neuropathic pain is characterized by abnormal pain sensation, such as spontaneous pain, allodynia and hyperalgesia, and is accompanied by dysesthesia and paresthesia. According to the International Association for the Study of Pain, neuropathic pain is defined as that caused by a lesion or disease of the somatosensory nervous system.[1] The prevalence of neuropathic pain is estimated at 7–10% of the world's population,[2] and the unbearable pain suffered by many of these patients reduces their quality of life. Although studies have provided new insights into the mechanism of neuropathic pain, it remains complicated and unclear. In addition, most of the key mediators are not specific for pain pathways and play diverse physiological roles.[3] Therefore, for 40–60% of patients, current medications for neuropathic pain either lack of efficacy or are intolerable due to adverse effects.[4]

Ziconotide is a potent and selective N-type calcium channel blocker and has been used for treatment of severe chronic pain.[5] This drug is a 25-amino acid synthetic conopeptide and requires intrathecal administration via an implanted pump due to lack of oral availability. Of note, intrathecal administration is associated with spinal meningitis due to device-related infection,[5] and ziconotide has been reported to cause substantial side effects, such as nausea, dizziness, confusion and ataxia, which probably result from the blockade of N-type calcium channels that are distributed throughout the central nervous system (CNS). To reduce the risk of serious CNS adverse effects, the United States Food and Drug Administration (US FDA) and European Medicines Evaluation Agency (EMEA) have only approved a slow dosage titration regimen.[5] Although ziconotide is clinically effective in treating chronic pain as an N-type calcium channel blocker, medical problems of intrathecal administration and unwanted CNS side effects limit its clinical use.

N-type calcium channels are localized in the neurons and expressed at a high level in the presynaptic terminals of neurons. In the spinal cord, these channels are involved in the transmission of nociceptive information.[6] N-type calcium channels have at least three different conformational states: closed, open, and inactivated. With prolonged excitation of sensory neurons, the proportion of N-type calcium channels in inactivated state increases. Targeting the inactivated state of N-type calcium channels can preferentially suppress hyperexcitability of sensory neurons in chronic pain and minimize impact on physiological signal transmission. Therefore, the selective blockade of N-type calcium channels in an inactivated state might provide a wider therapeutic window in pain than that of ziconotide, which has little state dependency.[5b,6] Consequently, an orally active and selective N-type calcium channel blocker might

* Corresponding author. Tel.: +81 29 863 6683; fax: +81 29 852 5387.
E-mail address: takashi.ogiyama@astellas.com (T. Ogiyama).

http://dx.doi.org/10.1016/j.bmc.2014.10.020
0968-0896/

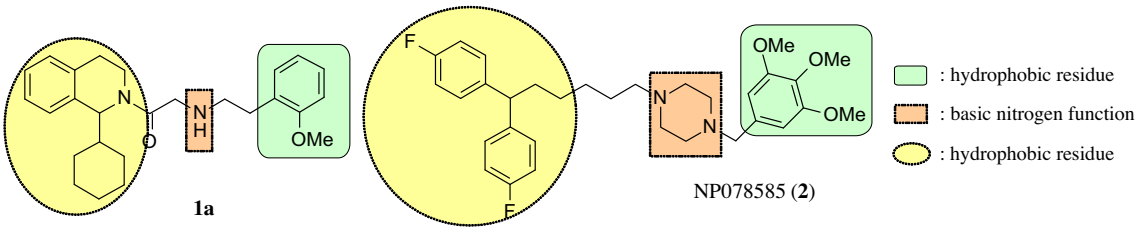


Figure 1. Structures of compound **1a** and NP078585 (**2**).

represent a novel analgesic agent without the disadvantages of ziconotide. Recent studies have focused on identifying small-molecule N-type calcium channel blockers.[7–9]

At the outset of our N-type calcium channel blocker study, we identified compound **1a** as a potent small-molecule N-type calcium channel blocker from our in-house compound collection. The structure of compound **1a** is characterized by a novel tetrahydroisoquinoline scaffold and has some structural similarity with the N-type calcium channel blocker (NP078585 **2**) reported by Zalicus scientists,[9] with both containing a basic nitrogen function linked with hydrophobic residues on both sides (Fig. 1).

Inhibitory activity against N-type calcium channels of compound **1a** is comparable to that of NP078585 (**2**). However, both compound **1a** and NP078585 (**2**) exhibited potent inhibitory activities against human Ether-a-go-go Related Gene (hERG) channels (Table 1). Blockade of the hERG channels can give rise to a prolonged QT interval that is strongly associated with life-threatening cardiac arrhythmias.[10] The structural overlap of these two compounds with general Class III antiarrhythmic agents,[11] which is represented by a phenyl ring linked to a basic nitrogen function through a 1- to 4-atom-long chain, might play a critical role in the compound's high affinity for the hERG channels.

As represented by in vitro profile of compound **3**, Zalicus scientists demonstrated that conversion of the amine of NP078585 (**2**) to an amide and modification of aryl substituents on the benzyl moiety of NP078585 (**2**) significantly decreased hERG channel inhibition while retaining highly potent blockade of N-type calcium channels.[9a] However, our in-house evaluation revealed that these two compounds had low aqueous solubility in the Japanese Pharmacopoeia 2nd fluid for the dissolution test (JP2 pH 6.8). Further, compound **3** also exhibited low aqueous solubility in the Japanese Pharmacopoeia 1st fluid for the dissolution test (JP1 pH 1.2). In contrast, compound **1a** not only exhibited excellent solubility in both JP1 and JP2 but also showed promising properties for further optimization, such as low molecular weight and a simple structure. We therefore conducted a hit-to-lead program to identify novel compounds derived from **1a**. Here, we report the discovery of novel tetrahydroisoquinoline derivatives as orally active small-molecule N-type calcium channel blockers with attenuated blockade of hERG channels.

2. Chemistry

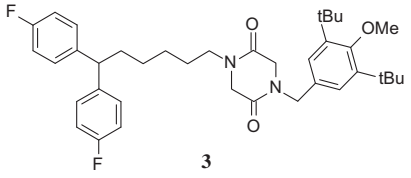
The synthesis of the tetrahydroisoquinoline derivatives **1a**–**1k** is outlined in Scheme 1. Condensation of the tetrahydroisoquinoline **4**[12] with chloroacetyl chloride under Schotten–Baumann reaction condition afforded the chloroacetamide **5**. Substitution of the chloroacetamide **5** with various amines **6a**–**6c**, **6e**, **6g**–**6j** then gave compounds **1a**–**1c**, **1e**, **1g**–**1j**. Amine **6j** was prepared as previously described.[13] Cleavage of methylether of compound **1a** gave compound **1d**. Hydrolysis of the methoxycarbonyl **1e** gave compound **1f**. Optically active compound (*S*)-**1h** was synthesized from (*S*)-tetrahydroisoquinoline **4**[14] and (*R*)-**1h** from (*R*)-tetrahydroisoquinoline **4** in a manner similar to that described for compound **1h**. Acylation of tetrahydroisoquinoline **4** with Boc protected glycine, followed by deprotection of Boc group afforded the amine **7**. An oxirane ring opening reaction between the amine **7** and the oxirane **8**[15] yielded compound **1k**.

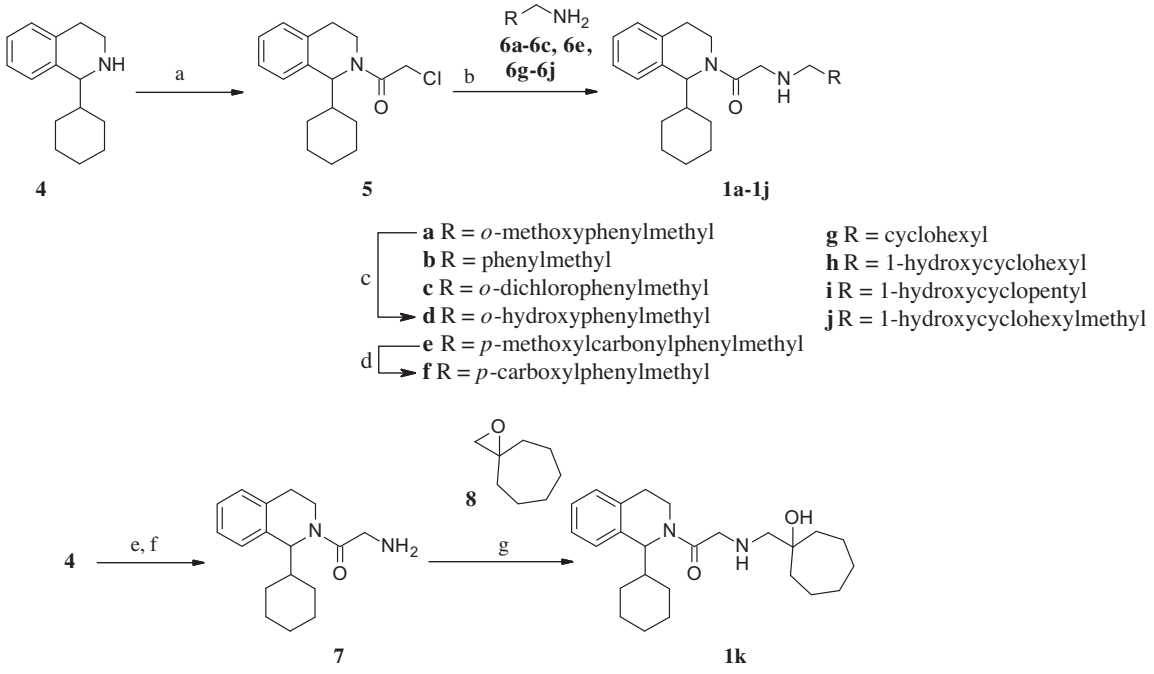
3. Results and discussion

Inhibitory activity against N-type calcium channels was evaluated using a fluorometric imaging plate reader (FLIPR) calcium imaging assay of IMR-32 human neuroblastoma cells. Experiments were conducted in the presence of nitrendipine to block endogenous L-type calcium channels. Inhibitory activity against hERG channels was determined via an Rb efflux assay of Chinese hamster ovary (CHO) cells.

We first investigated the effect of substituents on the benzene moiety of compound **1a** (Table 2). Removal of the methoxy group (**1b**) slightly attenuated N-type calcium channel inhibition, although potent inhibitory activity against hERG channels was retained. Incorporation of an *o*-dichloro substituent (**1c**) into a benzene moiety led to a further 2-fold decrease in N-type calcium channel inhibition compared with compound **1b** while attenuating the blockade of hERG channels. Replacement of the methoxy group (**1a**) with hydroxyl group (**1d**) resulted in a 5-fold decrease in N-type calcium channel blockade. The inhibitory activity of compound **1d** against hERG channels was comparable to that of compound **1b**. Compound **1e**, which has a *p*-methoxy carbonyl group, exhibited weak inhibitory activity against N-type calcium

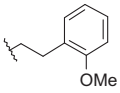
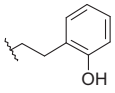
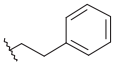
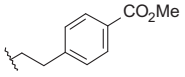
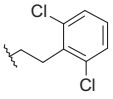
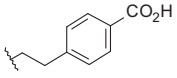
Table 1
In vitro activity, solubility and MW of compound **1a**, NP078585 (**2**) and **3**

Compound	N-type FLIPR IC_{50} (μM)	hERG IC_{50} (μM)	Aqueous solubility JP1/JP2 (μM)	Molecular weight
1a	0.60	8.3	>100/>100	406
NP078585 (**2**)	1.1	3.4	>100/<1	539
 **3**	0.43	>100	<1/<1	619



Scheme 1. Reagents and conditions: (a) chloroacetyl chloride, AcOEt, satd $NaHCO_3$, rt, 1 h; (b) iPr_2NEt, $CHCl_3$, 60 °C, 2 d; (c) BBr_3, CH_2Cl_2, −78 °C to 0 °C, 23 h; (d) 1 M aq NaOH, MeOH, rt, 22 h; (e) [(*tert*-butoxycarbonyl)amino]acetic acid, pivaloyl chloride, 4-methylmorpholine, CH_2Cl_2, rt, 14 h; (f) 4 M HCl/AcOEt, AcOEt, 50 °C, 5 h; and (g) 2-propanol, reflux, 15 h.

Table 2
Effect of phenylethyl substitutions on N-type calcium channel and hERG channel potency

Compound		N-type FLIPR IC_{50} (μM)	hERG IC_{50} (μM) or % inhibition at 10 μM	Compound		N-type FLIPR IC_{50} (μM)	hERG IC_{50} (μM) or % inhibition at 10 μM
1a		0.60	8.3	**1d**		3.0	54.4%
1b		1.3	57.8%	**1e**		2.3	71.7%
1c		2.4	54.7	**1f**		>10	9.0%

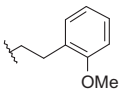
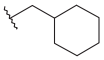
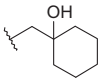
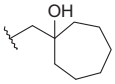
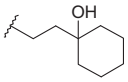
channels, but retained high potency for hERG channels. Introduction of *p*-carboxylic acid (**1f**) to the benzene moiety resulted in a loss of N-type calcium inhibition and also reducing the potency for hERG channels. Taken together, these results demonstrated no correlation between inhibitory activity against hERG channels and the polarity of substituents on the benzene moiety. Further, maintaining the potency for N-type calcium channel inhibition while reducing the inhibitory activity against hERG channels via modification of substituents on the benzene moiety was shown to be relatively difficult.

Based on findings from this preliminary study and due to the structural basis of drugs binding to hERG channels, we next considered a more rational approach that did not involve the incorporation of substituents into the benzene moiety.

Regarding hERG channels, Mitchenson et al. reported that many blockers of sodium and calcium channels bound to residues in the S6 domains.[16a] These authors demonstrated that interactions between the aromatic residues F656 and Y652 of the hERG S6 domain and the drug molecules were crucial for high-affinity binding.[16] They further proposed the disruption of interactions with the aromatic residues of F656 and Y652 to help minimize hERG channel inhibition.[16b]

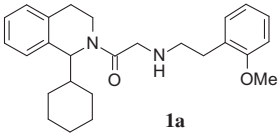
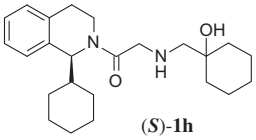
Given these previous findings, we hypothesized that compound **1a** bound to residues in the S6 domains, which is similar to the action of other sodium and calcium channel blockers, to display high affinity for hERG channels. Given that basic nitrogen atoms of typical hERG blockers are considered to be involved in the cation–π interaction with aromatic residues or electrostatic interactions within the pore regions,[17] the conversion of the basic nitrogen atom of compound **1a** might therefore benefit the attenuation of hERG inhibition. However, this nitrogen atom was found to be indispensable for good solubility.[18] We therefore converted

Table 3
Effect of aliphatic ring substitutions on potency for N-type calcium channels and selectivity over hERG channels.

Compound		N-type FLIPR IC_{50} (μM)	hERG IC_{50} (μM)	hERG/N-type selectivity ratio	Compound	N-type FLIPR IC_{50} (μM)	hERG IC_{50} (μM)	hERG/N-type selectivity ratio
1a		0.60	8.3	13.8	(*S*)-**1h**	1.0	98	98.0
1g		1.3	26.0	20.0	(*R*)-**1h**	1.5	76	50.7
1h		0.77	46.1	59.9				
1i		2.8	78.6	28.1				
1k		2.6	50.1	19.6				
1j		1.7	NT	NT				

NT: not tested.

Table 4
Inhibitory activities of compound **1a** and (*S*)-**1h** against five major CYP isoforms

Compound	CYP1A2 IC_{50} (μM)	CYP2C9 IC_{50} (μM)	CYP2C19 IC_{50} (μM)	CYP2D6 IC_{50} (μM)	CYP3A4* Preincubation time	
					0 min (%)	30 min (%)
 **1a**	26	4.8	3.0	0.37	65	53
 (*S*)-**1h**	>50	>50	>50	0.60	87	67

* Residual activities (%) of HLM were evaluated using midazolam as a probe substrate. Details are described in Section 5.

the phenyl moiety of compound **1a** to non-aromatic rings to eliminate the π–π interaction with the aromatic residues, an approach which allowed us to install a hydroxyl moiety on the aliphatic ring to impair hydrophobic interactions (Table 3).

First, we examined the bioisosteric conversion of the phenylethyl group of compound **1a** to a cyclohexylmethyl group.[19] Compound **1g** exhibited 3-fold attenuation of inhibitory activity against hERG channels compared to compound **1a**. Although this conversion resulted in a slight decrease in N-type calcium channel inhibition, compound **1g** exhibited improved selectivity over hERG channel inhibition. Further, the cyclohexylmethyl group was effective regarding N-type calcium potency as a bioisostere of the phenylethyl group (**1b** vs **1g**).

We next investigated the effect of introducing a hydroxyl moiety to the cyclohexyl ring of compound **1g**. As expected, inhibitory activity against hERG channels of compound **1h** was nearly twofold less than that of compound **1g**. Further, compound **1h** retained potent inhibitory activity against N-type calcium channels compared to compound **1a**. Neutral oxygen function, such as the methoxy group of **1a** and the hydroxyl group of **1h**, is involved in the enhancement of potency for N-type calcium inhibition (**1a** vs **1b** and **1g** vs **1h**). In contrast, addition of a phenolic hydroxyl group decreased N-type calcium channel blockade (**1b** vs **1d**).

We also examined the influence of the size of aliphatic rings and the length of linking chain between the cyclohexyl ring and basic nitrogen on compound **1h**. Cyclopentyl (**1i**) and cycloheptyl (**1k**) also exhibited weak inhibitory activity against hERG channels. However, both compounds were less potent as N-type calcium channel blocker than compound **1h**. Introduction of a longer linking chain (**1j**) resulted in an over 2-fold decrease in N-type calcium channel inhibition compared with compound **1h**.

The 1-hydroxycyclohexyl derivative **1h** exhibited highly potent blockade of N-type calcium channels and was optimal for selectivity over hERG channel inhibition. We therefore prepared

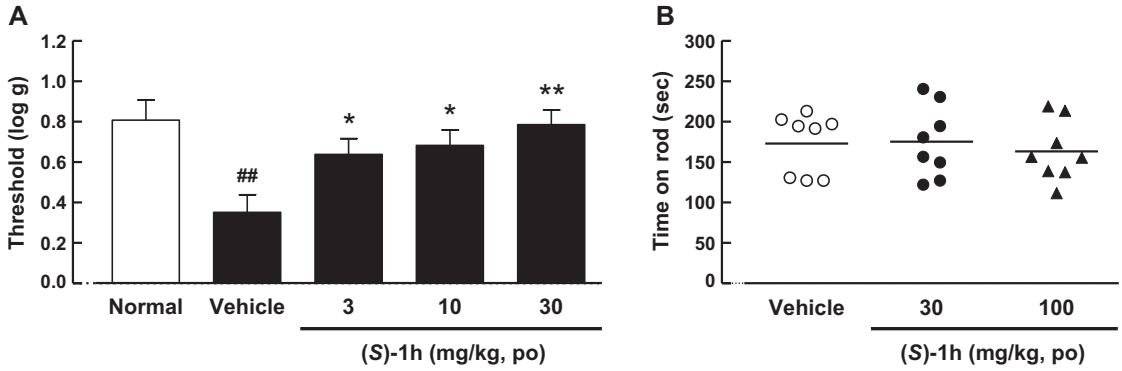


Figure 2. (A) Effect of compound (*S*)-**1h** on mechanical allodynia in SNL model in rats. Each bar represents the mean ± SEM withdrawal threshold of the hindpaw ($n = 8$). $^{\#\#}P < 0.005$, statistically significant compared with normal group (Student's *t*-test). $^{*}P < 0.05$ and $^{**}P < 0.005$, statistically significant compared with vehicle-treated group (Dunnett's test). Closed columns, operated side paw in drug- and vehicle-treated group. Open column, non-operated side paw in vehicle-treated group. (B) Effect of compound (*S*)-**1h** on motor coordination using an accelerating rotarod test in rats. Each symbol represents individual data. Dunnett's test was used to test for statistical significance of differences between drug- and vehicle-treated groups. No significant differences between these groups were noted.

and characterized both enantiomers of compound **1h**. Both enantiomers exhibited potent inhibitory activity against N-type calcium channels[20] and exhibited weak inhibitory activity against hERG channels. Notably, (*S*)-**1h** exhibited higher selectivity over hERG channels than (*R*)-**1h** and was selected for further characterization.

Lead compound (*S*)-**1h** was assessed for the inhibitory activities against five major cytochrome P450 isoforms (CYP1A2, 2C9, 2C19, 2D6 and 3A4) (Table 4). Compared with compound **1a**, (*S*)-**1h** exhibited significantly improved the inhibitory activities against all isoforms, particularly CYP2C9 and CYP2C19. Together, these results prompted us to investigate the in vivo efficacy of (*S*)-**1h**.

The analgesic effect of (*S*)-**1h** was evaluated using an L5/L6 spinal nerve ligation (SNL) model of neuropathic pain in rats (Fig. 2A).[21] (*S*)-**1h** significantly reduced mechanical allodynia with oral administration from 3 to 30 mg/kg. Oral administration of (*S*)-**1h** at 30 mg/kg resulted in maximum reversal of allodynia.

The effect of (*S*)-**1h** on motor coordination was assessed using an accelerating rotarod test in rats (Fig. 2B). (*S*)-**1h** did not decrease the time on the rod at doses up to 100 mg/kg p.o., indicating that (*S*)-**1h** exerted in vivo analgesic effect at 3–30 mg/kg without affecting motor coordination.

State-dependent inhibitory activity of (*S*)-**1h** against N-type calcium channels was evaluated using whole cell patch-clamp recordings at two different membrane potentials in IMR-32 human neuroblastoma cells. Under conditions favoring the inactivated state (at −70 mV), (*S*)-**1h** inhibited N-type calcium channels with an IC_{50} value of 1.7 μM, whereas (*S*)-**1h** exhibited weaker inhibition (IC_{50} = 12.7 μM) under resting state-biased condition (at −90 mV). These results indicate that (*S*)-**1h** is an inactivated state selective N-type calcium channel blocker. This property of inactivated state selective inhibition of (*S*)-**1h** might allow the compound to exert an in vivo analgesic effect without affecting motor coordination.

4. Conclusion

Here, we identified (*S*)-**1h** as a novel small-molecule N-type calcium channel blocker with significantly reduced inhibitory activity against hERG channels from optimization study of **1a**. Attenuation of hERG inhibition was achieved via conversion to an aliphatic ring (elimination of π–π interaction) and incorporation of a hydroxyl moiety (impairment of hydrophobic interaction). (*S*)-**1h** alleviated neuropathic pain in a rat SNL model at doses of 3–30 mg/kg p.o. with no affect on motor coordination. Further structural optimization will be reported in due course.

5. Experimental section

5.1. Chemistry

5.1.1. General

All reactions were carried out using commercially available reagents and solvents without further purification. Column chromatography was performed using a silica gel cartridge Shoko Scientific SI series on a Shoko Scientific Purif-α2. ^{1}H NMR spectra were recorded on a JNM-EX400 spectrometer. Chemical shifts are expressed in δ units (ppm) using tetramethylsilane as an internal standard. Abbreviations used for the signal patterns are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; and br, broad. Mass spectra were recorded on a JEOL LX-2000 or Waters ZQ-2000 mass spectrometer. Elemental analysis was conducted using a Yanaco MT-5 microanalyzer. HPLC analysis was performed using a Daicel OD-H chiral column on a Hitachi HPLC system (L-7000 series), equipped with a UV source (210 nm), utilizing hexane/ethanol/diethylamine as an eluent. Specific rotations were measured using a HORIBA SEPA-300 polarimeter.

5.1.2. 2-(Chloroacetyl)-1-cyclohexyl-1,2,3,4-tetrahydroisoquinoline (5)

To a solution of 1-cyclohexyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (**4**) (14.63 g) in ethyl acetate (90 mL) and saturated aqueous sodium bicarbonate (85 mL) was added dropwise a solution of chloroacetyl chloride (5.95 mL) in ethyl acetate (10 mL) under 4 °C, and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with water, extracted with ethyl acetate, dried over magnesium sulfate and then concentrated in vacuo. The residue was washed with cyclohexane to give the title compound **5** (19.50 g, 98%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): this compound exists as a pair of rotamers at room temperature. δ 0.94–1.30 (5H, m), 1.51–1.92 (6H, m), 2.87–3.13 (2H, m), 3.33–3.43 (minor rotamer, 2H, m), 3.72–3.88 (2H, m), 4.05–4.19 (2H, m), 4.23 (minor rotamer 1H, d, J = 12.0 Hz), 4.32 (minor rotamer, 2H, d, J = 8.0 Hz), 4.34–4.43 (minor rotamer, 1H, m), 5.27 (major rotamer, 1H, J = 8.0 Hz), 7.04–7.08 (minor rotamer, 1H, m), 7.09–7.23 (4H, m); FAB MS m/z 292 $[M+H]^+$.

5.1.3. 1-(1-Cyclohexyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[2-(2-methoxyphenyl)ethyl]amino}ethanone oxalate (1a)

To a solution of compound **5** (2.47 g) in chloroform (50 mL) was added *N*,*N*-diisopropylethylamine (3.77 mL) and [2-(2-methoxyphenyl)ethyl]amine (6.40 g) and the mixture was heated under

reflux for 2 days. The reaction mixture was diluted with water, extracted with chloroform, washed with brine, dried over magnesium sulfate and then concentrated in vacuo. The resulting residue was purified by silica gel column chromatography, eluting with chloroform–ethanol–aqueous ammonia to give a colorless oil (2.98 g). The oil was dissolved in ethyl acetate (20 mL) and ethanol (3 mL). Oxalic acid (660 mg) was then added to the solution. The precipitate was filtered off to obtain the title compound **1a** as a colorless solid (3.37 g, 80%). ^{1}H NMR (400 MHz, DMSO-d_6): this compound exists as a pair of rotamers at room temperature. δ 0.98–1.20 (5H, m) 1.46–1.77 (6H, m), 2.82–3.20 (5H, m), 3.28–3.40 (minor rotamer, 1H, m), 3.52–3.72 (major rotamer, 2H, m), 3.78 (3H, s), 3.94–4.14 (1H, m), 4.16–4.26 (1H, m), 4.30 (minor rotamer, 1H, d, J = 10.0 Hz), 5.11 (major rotamer, 1H, d, J = 9.2 Hz), 6.90 (1H, t, J = 8.0 Hz), 6.99 (1H, d, J = 7.6 Hz), 7.08–7.28 (6H, m); FAB MS m/z 407 [M+H]$^+$. Anal. Calcd for $C_{26}H_{34}N_2O_2{\cdot}C_2H_2O_4$: C, 67.72; H, 7.31; N, 5.64. Found: C, 67.44; H, 7.35; N, 5.62.

5.1.4. 1-(1-Cyclohexyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-[(2-phenylethyl)amino]ethanone oxalate (1b)

Compound **1b** was synthesized using a protocol similar to that for compound **1a**. Compound **1b** was obtained in 57% yield as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): this compound exists as a pair of rotamers at room temperature. δ 0.96–1.22 (5H, m), 1.54–1.68 (6H, m), 2.80–3.05 (5H, m), 3.06–3.24 (2H, m), 3.28–3.42 (minor rotamer, 1H, m), 3.54–3.74 (major rotamer, 2H, m), 4.02–4.12 (1H, m), 4.16–4.26 (1H, m), 4.29 (minor rotamer, 1H, d, J = 9.3 Hz), 5.12 (major rotamer, 1H, d, J = 9.7 Hz), 7.12–7.28 (7H, m), 7.29–7.38 (2H, m); FAB MS m/z 377 [M+H]$^+$.

5.1.5. 1-(1-Cyclohexyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[2-(2,6-dichlorophenyl)ethyl]amino}ethanone oxalate (1c)

Compound **1c** was synthesized using a protocol similar to that for compound **1a**. Compound **1c** was obtained in 82% yield as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): this compound exists as a pair of rotamers at room temperature. δ 0.90–1.37 (5H, m), 1.41–1.85 (6H, m), 2.74–5.01 (11H, m), 5.13 (major rotamer, 1H, d, J = 8.0 Hz), 7.09–7.26 (4H, m), 7.34 (1H, dd, J = 7.6, 7.6 Hz), 7.44–7.54 (2H, m); FAB MS m/z 445 [M+H]$^+$.

5.1.6. Methyl 4-(2-{[2-(1-cyclohexyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-oxoethyl]amino}ethyl)benzoate oxalate (1e)

Compound **1e** was synthesized using a protocol similar to that for compound **1a**. Compound **1e** was obtained in 82% yield as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): this compound exists as a pair of rotamers at room temperature. δ 0.95–1.26 (5H, m), 1.46–1.78 (6H, m), 2.80–3.27 (5H, m), 3.29–3.40 (minor rotamer, 1H, m), 3.53–3.74 (major rotamer, 2H, m), 3.84 (3H, s), 3.96–4.13 (1H, m), 4.15–4.32 (1H, m), 5.11 (major rotamer, 1H, d, J = 9.6 Hz), 7.09–7.26 (4H, m), 7.40 (2H, d, J = 8.0 Hz), 7.92 (2H, d, J = 8.4 Hz); FAB MS m/z 435 [M+H]$^+$.

5.1.7. 1-(1-Cyclohexyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-[(cyclohexylmethyl)amino]ethanone fumarate (1g)

Compound **1g** was synthesized using a protocol similar to that for compound **1a**. Compound **1g** was obtained in 54% yield as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): this compound exists as a pair of rotamers at room temperature. δ 0.79–1.27 (10H, m), 1.43–1.79 (12H, m), 2.44–2.60 (3H, m), 2.75–3.06 (2H, m), 3.22–3.35 (minor rotamer, 1H, m), 3.53–3.79 (3H, m), 3.84 (major rotamer, 1H, d, J = 18.4 Hz), 4.15–4.28 (minor rotamer, 1H, m), 4.41 (minor rotamer, 1H, d, J = 9.2 Hz), 5.12 (major rotamer, 1H, d, J = 10.4 Hz), 6.51 (2H, s, fumaric acid), 7.01–7.32 (4H, m); FAB MS m/z 369 [M+H]$^+$.

5.1.8. 1-(1-Cyclohexyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[(1-hydroxycyclohexyl)methyl]amino}ethanone oxalate (1h)

Compound **1h** was synthesized using a protocol similar to that for compound **1a**. Compound **1h** was obtained in 70% yield as a pale brown solid. ^{1}H NMR (400 MHz, DMSO-d_6): this compound exists as a pair of rotamers at room temperature. δ 0.92–1.80 (21H, m), 2.74–3.07 (5H, m), 3.31–3.41 (minor rotamer, 1H, m), 3.51–3.63 (major rotamer, 1H, m), 3.63–3.73 (major rotamer, 1H, m), 3.73–3.81 (minor rotamer, 1H, m), 3.92–4.04 (2H, m), 4.12 (1H, d, J = 1.6 Hz), 4.15–4.26 (minor rotamer, 1H, m), 4.32 (minor rotamer, 1H, J = 8.0 Hz), 5.11 (major rotamer, 1H, d, J = 8.0 Hz), 7.08–7.28 (4H, m); FAB MS m/z 385 [M+H]$^+$. Anal. Calcd for $C_{24}H_{36}N_2O_2{\cdot}C_2H_2O_4$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.45; H, 8.18; N, 5.85.

5.1.9. (1*S*)-(1-Cyclohexyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[(1-hydroxycyclohexyl)methyl]amino}ethanone oxalate (*S*)-1h

Compound (*S*)-**1h** was synthesized using a protocol similar to that for compound **1h**, except that (1*S*)-cyclohexyl-1,2,3,4-tetrahydroisoquinoline[14] was used as a starting material. Compound (*S*)-**1h** was obtained in 69% yield as a colorless solid.

HPLC (Chiralpak OD-H [0.46 cm I.D. × 15 cm], hexane/ethanol/diethylamine = 80:20:0.1 flow rate 0.5 ml/min, column temp: 40 °C, UV: 210 nm): retention time: t_s = 8.34 min (major), t_r = 9.73 min (minor); 98.4% ee. FAB MS m/z 385 [M+H]$^+$. Anal. Calcd for $C_{24}H_{36}N_2O_2{\cdot}C_2H_2O_4$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.75; H, 8.09; N, 5.90. $[\alpha]_D^{26}$ +10.8° (c 0.1, MeOH).

5.1.10. (1*R*)-(1-Cyclohexyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[(1-hydroxycyclohexyl)methyl]amino}ethanone oxalate (*R*)-1h

Compound (*R*)-**1h** was synthesized using a protocol similar to that for compound **1h**, except that (1*R*)-cyclohexyl-1,2,3,4-tetrahydroisoquinoline[14] was used as a starting material. Compound (*R*)-**1h** was obtained in 57% yield as a colorless solid.

HPLC (Chiralpak OD-H [0.46 cm I.D. × 15 cm], hexane/ethanol/diethylamine = 80:20:0.1 flow rate 0.5 ml/min, column temp: 40 °C, UV: 210 nm): retention time: t_r = 9.73 min (major), t_s = 8.34 min (minor); 98.3% ee. FAB MS m/z 385 [M+H]$^+$. Anal. Calcd for $C_{24}H_{36}N_2O_2{\cdot}C_2H_2O_4$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.70; H, 8.11; N, 5.88. $[\alpha]_D^{26}$ −13.6° (c 0.1, MeOH).

5.1.11. 1-(1-Cyclohexyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[(1-hydroxycyclopentyl)methyl]amino}ethanone oxalate (1i)

Compound **1i** was synthesized using a protocol similar to that for compound **1a**. Compound **1i** was obtained in 78% yield as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): this compound exists as a pair of rotamers at room temperature. δ 0.96–1.26 (5H, m), 1.46–1.82 (14H, m), 2.80–3.10 (4H, m), 3.21–4.66 (6H, m), 5.11 (major rotamer 1H, d, J = 8.0 Hz), 7.14–7.23 (4H, m); FAB MS m/z 371 [M+H]$^+$.

5.1.12. 1-(1-Cyclohexyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[2-(1-hydroxycyclohexyl)ethyl]amino}ethanone oxalate (1j)

Compound **1j** was synthesized using a protocol similar to that for compound **1a**. Compound **1j** was obtained in 48% yield as a colorless solid. ^{1}H NMR (400 MHz, DMSO-d_6): this compound exists as a pair of rotamers at room temperature. δ 0.92–1.84 (25H, m), 2.74–3.11 (4H, m), 3.30–3.40 (major rotamer, 1H, m), 3.53–3.73 (major rotamer, 2H, m), 3.98–4.12 (1H, m), 4.14–4.26 (1H, m), 4.32 (minor rotamer, 1H, J = 9.4 Hz), 5.11 (major rotamer, 1H, d, J = 9.3 Hz), 7.08–7.23 (4H, m); FAB MS m/z 400 [M+2H]$^+$.

5.1.13. 1-(1-Cyclohexyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[2-(2-hydroxyphenyl)ethyl]amino}ethanone oxalate (1d)

To a solution of compound **1a** (449 mg) in dichloromethane (15 mL) was added boron tribromide (6.6 ml of 1 M dichloromethane

solution) at −78 °C. The reaction mixture was stirred at 0 °C for 23 h. Then the reaction was quenched with saturated aqueous sodium bicarbonate. The mixture was diluted with water, extracted with chloroform, dried over magnesium sulfate and then concentrated in vacuo. The resulting residue was purified using silica gel column chromatography, eluting with chloroform–methanol to give a brown foam (439 mg). The foam was dissolved in ethanol (5 mL). Oxalic acid (109 mg) was added to the solution. Precipitate was filtered off to obtain the title compound **1d** as a colorless solid (229 mg, 43%). ^{1}H NMR (400 MHz, DMSO-d_6): this compound exists as a pair of rotamers at room temperature. δ 0.94–1.24 (5H, m), 1.44–1.78 (6H, m), 2.73–3.03 (5H, m), 3.24–3.36 (minor rotamer, 1H, m), 3.55–3.70 (major rotamer, 2H, m), 3.75–3.87 (1H, m), 3.94 (major rotamer, 1H, d, J = 16.8 Hz), 4.16–4.26 (minor 1H, m), 4.36 (minor rotamer, 1H, d, J = 9.6 Hz), 5.12 (major rotamer, 1H, d, J = 9.2 Hz), 6.70 (1H, t, J = 7.2 Hz), 6.77 (1H, d, J = 7.2 Hz), 7.03 (2H, t, J = 7.6 Hz), 7.12–7.26 (4H, m); FAB MS m/z 393 [M+H]$^+$.

5.1.14. 4-(2-{[2-(1-Cyclohexyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-oxoethyl]amino}ethyl)benzoic acid (1f)

To a solution of compound **1e** (298 mg) in methanol (10 mL) was added 1 M aqueous sodium hydroxide (3.5 mL). The reaction mixture was stirred at room temperature for 22 h. Then, 1 M aqueous hydrochloric acid (3.5 mL) was added to the reaction mixture. The reaction mixture was diluted with water, extracted with chloroform, washed with brine, dried over magnesium sulfate and then concentrated in vacuo. The residue was washed with diisopropyl ether and 2-propanol to give the title compound **1f** (227 mg, 95%) ^{1}H NMR (400 MHz, DMSO-d_6): this compound exists as a pair of rotamers at room temperature. δ 0.96–1.20 (5H, m), 1.46–1.76 (6H, m), 2.78–2.99 (5H, m), 3.22–3.32 (minor 1H, m), 3.52–3.84 (4H, m), 4.15–4.25 (minor rotamer, 1H, m), 4.38 (minor rotamer, 1H, d, J = 9.2 Hz), 5.12 (major rotamer, 1H, d, J = 9.6 Hz), 7.10–7.23 (4H, m), 7.28–7.35 (2H, m), 7.86 (2H, d, J = 8.0 Hz); FAB MS m/z 421 [M+H]$^+$.

5.1.15. 2-(1-Cyclohexyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-oxoethanamine (7)

To a solution of [(*tert*-butoxycarbonyl)amino]acetic acid (1.54 g) and 4-methylmorpholine (2.2 mL) in methylene chloride (20 mL), was added pivaloyl chloride (0.98 mL) under 5 °C. The reaction mixture was stirred at room temperature for 30 min and then 1-cyclohexyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (**4**) (1.00 g) was added to the reaction mixture at 4 °C. The reaction mixture was stirred at room temperature for 14 h. The reaction mixture was diluted with water. The mixture was extracted with chloroform, washed with brine, dried over magnesium sulfate and then concentrated in vacuo. The residue was purified by column chromatography, eluting with chloroform–methanol to give *tert*-butyl [2-(1-cyclohexyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-oxoethyl]carbamate (1.49 g 100%) as a colorless oil; ESI MS m/z 373 [M+H]$^+$. To a solution of *tert*-butyl[2-(1-cyclohexyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-oxoethyl]carbamate (1.49 g) in ethyl acetate (20 mL) was added 4 M hydrogen chloride in ethyl acetate (3 mL) under 5 °C. The reaction mixture was stirred at 50 °C for 5 h. Then, the reaction solvent was concentrated in vacuo. Saturated aqueous sodium bicarbonate was added to the residue and extracted with chloroform. The extract was washed with brine and dried over magnesium sulfate. It was concentrated in vacuo and the resulting residue was purified by silica gel column chromatography, eluting with chloroform–methanol to give the title compound **7** (1.09 g, 99%) as a pale yellow oil. ^{1}H NMR (400 MHz, DMSO-d_6): this compound exists as a pair of rotamers at room temperature. δ 0.92–1.29 (5H, m), 1.50–1.96 (6H, m), 2.23 (2H, br s), 2.82–3.12 (2H, m), 3.29–3.71 (4H, m), 4.42–4.51 (minor rotamer, 1H, m), 4.18 (minor rotamer, 1H, d, J = 12.0 Hz), 5.29 (major rotamer, 1H, d, J = 8.0 Hz), 7.00–7.05 (minor rotamer, 1H, m), 7.07–7.25 (4H, m); ESI MS m/z 273 [M+H]$^+$.

5.1.16. 1-(1-Cyclohexyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[(1-hydroxycycloheptyl)methyl]amino}ethanone oxalate (1k)

To a solution compound **7** (200 mg) in 2-propanol (2 mL) was added compound **8**[15] (139 mg) and it was refluxed for 15 h. The reaction solvent was concentrated in vacuo. The residue was purified by silica gel column chromatography, eluting with chloroform–methanol to afford a pale brown oil (89 mg). The oil was dissolved in 2-propanol (3 mL). Oxalic acid (22 mg) was added to the solution at 60 °C. The mixture was evaporated to give the title compound **1k** (72.2 mg, 20%) as a pale brown solid. ^{1}H NMR (400 MHz, DMSO-d_6): this compound exists as a pair of rotamers at room temperature. δ 1.05–1.73 (23H, m), 2.76–3.06 (4H, m), 3.08–3.92 (2H, m), 4.01 (1H, d, J = 16.0 Hz), 4.08–4.26 (1H, m), 4.30 (1H, d, J = 8.0 Hz), 5.11 (major rotamer 1H, d, J = 8.0 Hz), 7.16–7.23 (4H, m); FAB MS m/z 399 [M+H]$^+$.

5.2. Pharmacology

5.2.1. Determination of solubility

The first fluid for disintegration test in Japanese Pharmacopoeia, pH 1.2 (JP1) and the second fluid for disintegration test in Japanese Pharmacopoeia, pH 6.8 (JP2) were used as the aqueous buffer. Small volumes of the compounds in DMSO were diluted to 130 μM by adding the aqueous buffer. After incubation at 25 °C for 20 h, precipitates were separated by filtration, and solubility of each filtrate was determined by HPLC analysis.

5.2.2. Evaluation of inhibitory activity against N-type calcium channel using an in vitro FLIPR assay in IMR-32 human neuroblastoma cells

Human neuroblastoma IMR-32 cells were cultured and differentiated as previously described with some modifications.[22] IMR-32 cells were plated onto poly-L-lysine-coated 96-well assay plates at a density of 6×10^4 cells/well and incubated overnight (37 °C, 5% CO_2). Hank's balanced salt solution without phenol red and containing 20 mM HEPES and 0.5 mM probenecid was prepared on the day of assay and used as assay buffer. Cells were incubated with 100 μL of fluo-3 AM (Dojindo, Kumamoto, Japan) in assay buffer at 37 °C for 1 h. Plates were washed with wash buffer to remove loading buffer, and 100 μL/well of the wash buffer was added. Plates were then placed into a FLIPR system (Molecular Devices, Sunnyvale, CA). Intracellular calcium concentration was measured for 12 min. During fluorescence intensity monitoring, 50 μL/well of the compound, diluted with assay buffer containing 1 μM nitrendipine, was added during the first 30 s, and 50 μL/well KCl solution (final concentration: 50 mM) at 10 min and 10 s after the start of monitoring. The IC_{50} values were determined by in duplicate in one experiment. And the IC_{50} values and 95% confidence intervals were calculated using Sigmoid-Emax nonlinear regression analysis with SAS software (Cary, NC, USA).

5.2.3. Evaluation of hERG inhibition using Rb Efflux assay

CHO cells that stably expressed hERG channels cultured in D-MEM containing 10% FBS, 1% PS, 1% Geneticin. Cell suspension was diluted to 6×10^5 cells/ml. Cells were seeded into 96-well plates and cultured in CO_2 incubator for 24 h. Culture medium was removed and cells were washed with wash buffer (130 mM NaCl, 3 mM KCl, 0.6 mM $MgCl_2$, 0.3 mM $CaCl_2$, 10 mM HEPES, 10 mM D-glucose: pH 7.3). Thereafter 100 μL of Rb loading buffer (130 mM NaCl, 3 mM RbCl, 0.6 mM $MgCl_2$, 0.3 mM $CaCl_2$, 10 mM HEPES, 10 mM D-glucose: pH 7.3) was added. Cells were incubated in CO_2 incubator for 1.5 h. Excess Rb-containing media was aspirated off and washed with wash buffer. Test compounds were

carried in high K buffer (83 mM NaCl, 50 mM KCl, 0.6 mM $MgCl_2$, 0.3 mM $CaCl_2$, 10 mM HEPES, 10 mM D-glucose: pH 7.3), and then 100 μL of the high K buffer was added to the plate. Cells were incubated at room temperature for 10 min. Supernatants were collected and transferred into another well (A). Cells were washed with the wash buffer and treated with 200 μL of lysis buffer (1% Triton-X 100 in wash buffer) to give cell lysate (B). Rb content in the supernatans (A) and the cell lysate (B) were determined using an atomic absorption spectroscopy reader (ICR 8000, Aurora Biosciences, Canada).

5.2.4. Measurement of CYP inhibition

The inhibitory activities of test compounds against CYP1A2, 2C9, 2C19 and 2D6 were determined using fluorescence-based assay. Reaction mixtures containing recombinant human CYP protein, co-factors, fluorogenic substrate, test compound (0.8–50 μM) and potassium phosphate buffer (pH 7.4) were prepared. Fluorogenic substrates and assay conditions for different CYP isoforms are summarized in Table 5. Reactions were initiated by incubation at 37 °C. Incubation times for each isoform was as follows: 15 min (CYP1A2), 45 min (2C9), 30 min (2C19) and 30 min (2D6). After incubation, the reactions were terminated by addition of stop solution (20% 0.5 M Tris base [2-amino-2-hydroxymethyl-1,3-propanediol], 80% acetonitrile). Fluorescence was measured to quantify the metabolite formation, and IC_{50} determined.

For CYP3A4 inhibition assay, midazolam was used as a probe substrate to monitor the changes in CYP3A4 activity during the exposure to each test compound. 0.1 mg protein/mL reaction mixtures containing human liver microsomes (HLM), 1 mM NADPH, 0.1 mM EDTA, 100 mM Na^+–K^+ phosphate buffer (pH 7.4) and 5 μM test compound were prepared and pre-incubated for 0 or 30 min at 37 °C. Reactions were initiated by the addition of 2 μM of midazolam and incubated for additional 20 min before being terminated by addition of 80% acetonitrile with internal standard. Levels of the metabolite of midazolam, 1′-hydroxymidazolam, were measured using LC–MS/MS. Residual metabolic activities for reversible (Eq. 1) and time-dependent (Eq. 2) inhibition were calculated using the following equations:

$$\%\,\text{Residual Activity} = \text{Activity}_{\text{compound},0\,\text{min}} / \text{Activity}_{\text{vehicle},0\,\text{min}} \times 100 \quad (1)$$

$$\%\,\text{ResidualActivity} = \left(\text{Activity}_{\text{compound},30\,\text{min}} / \text{Activity}_{\text{vehicle},30\,\text{min}}\right) / \left(\text{Activity}_{\text{compound},0\,\text{min}} / \text{Activity}_{\text{vehicle},0\,\text{min}}\right) \times 100 \quad (2)$$

where $\text{Activity}_{\text{compound, 0 min}}$ denotes activity obtained in the presence of compound and without pre-incubation, $\text{Activity}_{\text{vehicle, 0 min}}$ denotes activity obtained in the absence of compound and without pre-incubation, $\text{Activity}_{\text{compound, 30 min}}$ denotes that obtained in the presence of compound and with pre-incubation, and $\text{Activity}_{\text{vehicle, 30 min}}$ denotes that obtained in the absence of compound and with pre-incubation.

Table 5
Summary of assay conditions for different CYP450 enzymes

CYP isoform	Protein conc. (pmol/mL)	Substrate	Substrate concd (μM)
1A2	0.06	CEC	5
2C9	1.16	MFC	75
2C19	0.60	CEC	25
2D6	1.88	AMMC	1.5

CEC, 3-cyano-7-ethoxycoumarin; MFC, 7-methoxy-4-trifluoromethylcoumarin; AMMC, 3-[2-(*N*,*N*-diethyl-*N*-methyl amino)-ethyl]-7-methoxy-4-methylcoumarin.

5.2.5. Animal experiments

Male Sprague–Dawley rats (SLC, Hamamatsu, Japan) were used for all in vivo experiments. Animals were group-housed and kept on a 12-h light/dark cycle (lights on from 7:30 AM to 7:30 PM) with free access to food and water. All animal experimental procedures were approved by the Committee for Animal Experiments of Astellas Pharma Inc. and conformed to the International Guiding Principles for Biomedical Research Involving Animals (CIOMS) and Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, 2006). All efforts were made to minimize the number of animals used and their suffering.

The efficacy of compound (*S*)-**1h** in neuropathic pain was evaluated in rat SNL model reported by Chung et al.[21] Effects of compound (*S*)-**1h** on motor coordination was assessed by rotarod test. The solution of (*S*)-**1h** in 10% dimethyl sulfoxide and 10% Cremophol was used in both test. Details of the experimental procedures have been previously described.[23]

5.2.6. The inhibitory effects of (*S*)-1h on N-type calcium channels using the electrophysiological method

For electrical recording, differentiated IMR-32 cells were plated on poly-D-lysine coated glass coverslips at a density of approximately 1.5×10^4 cells/cm^2 and incubated for at least 3 h prior to electrophysiological recording.[24] Whole-cell patch clamp recordings were obtained from differentiated IMR-32 cells using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA, USA). Recording pipettes were constructed from borosilicate glasses (Harvard Apparatus Ltd, Fircroft Way, Edenbridge, UK) with a micropipettes puller (MF-83, Narishige, Tokyo, Japan) and fire-polished in a microforge (PP-83, Narishige, Tokyo, Japan) to obtain electrode resistances ranging from 4 to 6 MΩ. Pipette solution contained 125 mM CsCl, 2 mM $MgCl_2$, 10 mM HEPES, 10 mM EGTA, 4 mM MgATP, and 4 mM phosphocreatine disodium, pH 7.3 with CsOH. Internal cesium chloride was used to suppress potassium currents. Cells were perfused continuously with standard bath solution at room temperature (20–25 °C) containing 120 mM NaCl, 20 mM tetraethyl ammonium chloride, 3 mM KCl, 5 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES, and 10 mM glucose, pH 7.3 with NaOH. To isolate barium currents for whole-cell recording, the standard bath solution was replaced with a Ba^{2+}-containing solution which contained 120 mM NaCl, 20 mM tetraethyl ammonium chloride, 3 mM KCl, 10 mM $BaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES, 10 mM glucose, 2 μM tetrodotoxin, and 1 μM nitrendipine, pH 7.3 with NaOH. Tetrodotoxin was used to block sodium currents and nitrendipine to block L-type calcium currents, respectively. Membrane currents were elicited by 100 ms voltage steps from the holding potential (V_H) of −70 or −90 mV to +10 mV. Test pulses were delivered at 30-s intervals to allow recovery from inactivation. N-type calcium currents were isolated by subtracting ziconotide (1 μM)-insensitive currents.

A multiple-barrel perfusion system was employed to achieve a rapid exchange of bath solutions. The barrels of the perfusion system were directly connected to syringes containing the control and test solutions. The flow rate of extracellular solutions was set at 0.5–1 mL/min. Data were filtered at 2 kHz with a 4-pole Bessel filter, digitized at 50 kHz, and stored on compact disk for off-line analysis using a Digidata 1322A analog/digital interface along with the pClamp 8.0 software (Axon Instruments).

Acknowledgments

We thank Dr. Tatsuya Niimi for his valuable comments regarding the structural basis of drug binding to hERG channels. We also thank Drs. Kenichi Onda, Shinya Nagashima and Susumu Watanuki for their advice and careful reading of the manuscript. In addition,

we thank the staff of the Division of Analytical Science Laboratories for conducting elemental analyses and spectral measurements.

References and notes

1. Jensen, T. S.; Baron, R.; Haanpää, M.; Kalso, E.; Loeser, J. D.; Rice, A. S.; Treede, R.-D. *Pain* **2011**, *152*, 2204.
2. (a) van Hecke, O.; Austin, S. K.; Khan, R. A.; Smith, B. H.; Torrance, N. *Pain* **2014**, *155*, 654; (b) Bouhassira, D.; Lanteri-Minet, M.; Attal, N.; Laurent, B.; Touboul, C. *Pain* **2008**, *136*, 380.
3. Kuner, R. *Nat. Med.* **2010**, *16*, 1258.
4. (a) Dworkin, R. H.; O'Connor, A. B.; Kent, J.; Mackey, S. C.; Raja, S. N.; Stacey, B. R.; Levy, R. M.; Backonja, M.; Baron, R.; Harke, H.; Loeser, J. D.; Treede, R. D.; Turk, D. C.; Wells, C. D. *Pain* **2013**, *154*, 2249; (b) O'Connor, A. B.; Dworkin, R. H. *Am. J. Med.* **2009**, *122*, S22; (c) Dworkin, R. H.; O'Connor, A. B.; Backonja, M.; Farrar, J. T.; Finnerup, N. B.; Jensen, T. S.; Kalso, E. A.; Loeser, J. D.; Miaskowski, C.; Nurmikko, T. J.; Portenoy, R. K.; Rice, A. S. C.; Stacey, B. R.; Treede, R. D.; Turk, D. C.; Wallace, M. S. *Pain* **2007**, *132*, 237.
5. (a) Miljanich, G. P. *Curr. Med. Chem.* **2004**, *11*, 3029; (b) Schmidtkco, A.; Lötsch, J.; Freynhagen, R.; Geisslinger, G. *Lancet* **2010**, *375*, 1569.
6. (a) McGivern, J. G.; McDonough, S. I. *Curr. Drug Targets: CNS Neurol. Disord.* **2004**, *3*, 457; (b) McGivern, J. G. *Drug Discovery Today* **2006**, *11*, 245; (c) Snutch, T. P. *NeuroRx* **2005**, *2*, 662.
7. (a) Yamamoto, T.; Takahara, A. *Curr. Top. Med. Chem.* **2009**, *9*, 377; (b) Barrow, J. C.; Duffy, J. L. *Annu. Rep. Med. Chem.* **2010**, *45*, 2; (c) Lee, M. S. *Prog. Med. Chem.* **2014**, *53*, 147.
8. (a) Beebe, X.; Darczak, D.; Henry, R. F.; Vortherms, T.; Janis, R.; Namovic, M.; Donnelly-Roberts, D.; Kage, K. L.; Surowy, C.; Milicic, I.; Niforatos, W.; Swensen, A.; Marsh, K. C.; Wetter, J. M.; Franklin, P.; Baker, S.; Zhong, C.; Simler, G.; Gomez, E.; Boyce-Rusty, J. M.; Zhu, C. Z.; Stewart, A. O.; Jarvis, M. F.; Scott, V. E. *Bioorg. Med. Chem.* **2012**, *20*, 4128; (b) Beebe, X.; Yeung, C. M.; Darczak, D.; Shekhar, S.; Vortherms, T. A.; Miller, L.; Milicic, I.; Swensen, A. M.; Zhu, C. Z.; Banfor, P.; Wetter, J. M.; Marsh, K. C.; Jarvis, M. F.; Scott, V. E.; Schrimpf, M. R.; Lee, C.-H. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 4857; (c) Subasinghe, N. L.; Wall, M. J.; Winters, M. P.; Qin, N.; Lubin, M. L.; Finley, M. F. A.; Brandt, M. R.; Neeper, M. P.; Schneider, C. R.; Colburn, R. W.; Flores, C. M. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4080; (d) Tyagarajan, S.; Chakravarty, P. K.; Park, M.; Zhou, B.; Herrington, J. B.; Ratliff, K.; Bugianesi, R. M.; Williams, B.; Haedo, R. J.; Swensen, A. M.; Warren, V. A.; Smith, M.; Garcia, M.; Kaczorowski, G. J.; McManus, O. B.; Lyons, K. A.; Li, X.; Madeira, M.; Karanam, B.; Green, M.; Forrest, M. J.; Abbadie, C.; McGowan, E.; Mistry, S.; Jochnowitz, N.; Duffy, J. L. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 869; (e) Shao, P. P.; Ye, F.; Chakravarty, P. K.; Varughese, D. J.; Herrington, J. B.; Dai, G.; Bugianesi, R. M.; Haedo, R. J.; Swensen, A. M.; Warren, V. A.; Smith, M. M.; Garcia, M. L.; McManus, O. B.; Lyons, K. A.; Li, X.; Green, M.; Jochnowitz, N.; McGowan, E.; Mistry, S.; Sun, S. Y.; Abbadie, C.; Kaczorowski, G. J.; Duffy, J. L. *J. Med. Chem.* **2012**, *55*, 9847; (f) Abbadie, C.; McManus, O. B.; Sun, S.-Y.; Bugianesi, R. M.; Dai, G.; Haedo, R. J.; Herrington, J. B.; Kaczorowski, G. J.; Smith, M. M.; Swensen, A. M.; Warren, V. A.; Williams, S.; Arneric, S. P.; Eduljee, C.; Snutch, T. P.; Tringham, E. W.; Jochnowitz, N.; Liang, A.; MacIntyre, D. E.; McGowan, E.; Mistry, S.; White, V. V.; Hoyt, S. B.; London, C.; Lyons, K. A.; Bunting, P. B.; Volksdorf, S.; Duffy, J. L. *J. Pharmacol. Exp. Ther.* **2010**, *334*, 545; (g) Shao, P. P.; Ye, Fe; Chakravarty, P. K.; Herrington, J. B.; Dai, G.; Bugianesi, R. M.; Haedo, R. J.; Swensen, A. M.; Warren, V. A.; Smith, M. M.; Garcia, M. L.; McManus, O. B.; Lyons, K. A.; Li, X.; Green, M.; Jochnowitz, N.; McGowan, E.; Mistry, E.; Sun, S.-Y.; Abbadie, C.; Kaczorowski, G. J.; Duffy, J. L. *ACS Med. Chem. Lett.* **2013**, *4*, 1064; (h) Winters, M. P.; Subashinghe, N.; Wall, M.; Beck, E.; Brandt, M. R.; Finley, M. F. A.; Liu, Y.; Lubin, M. L.; Neeper, M. P.; Qin, N.; Flores, C. M.; Sui, Z. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2053; (i) Winters, M. P.; Subashinghe, N.; Wall, M.; Beck, E.; Brandt, M. R.; Finley, M. F. A.; Liu, Y.; Lubin, M. L.; Neeper, M. P.; Qin, N.; Flores, C. M.; Sui, Z. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2057.
9. (a) Pajouhesh, H.; Feng, Z.-P.; Zhang, L.; Pajouhesh, H.; Jiang, X.; Hendricson, A.; Dong, H.; Tringham, E.; Ding, Y.; Vanderah, T. W.; Porreca, F.; Belardetti, F.; Zamponi, G. W.; Mitscher, L. A.; Snutch, T. P. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4153; (b) Zamponi, G. W.; Feng, Z.-P.; Zhang, L.; Pajouhesh, H.; Ding, Y.; Belardetti, F.; Pajouhesh, H.; Dolphin, D.; Mitscher, L. A.; Snutch, T. P. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6467; AbbVie scientists also reported N-type calcium blockers, which has the structural similarity with NP078585. See: (c) Doherty, G. A.; Bhatia, P.; Vortherms, T. A.; Marsh, K. C.; Wetter, J. M.; Mack, H.; Scott, V. E.; Jarvis, M. F.; Stewart, A. O. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1716.
10. (a) Recanatini, M.; Poluzzi, E.; Masetti, M.; Cavalli, A.; De Ponti, F. *Med. Res. Rev.* **2005**, *25*, 133; (b) Lagrutta, A. A.; Trepakova, E. S.; Salata, J. J. *Curr. Top. Med. Chem.* **2008**, *8*, 1102; (c) Bell, I. M.; Bilodeau, M. T. *Curr. Top. Med. Chem.* **2008**, *8*, 1128.
11. Morgan, T. K.; Sullivan, M. E. *Prog. Med. Chem.* **1992**, *29*, 65.
12. Saitoh, T.; Abe, K.; Ishikawa, M.; Nakatani, M.; Shimizu, S.; Satoh, N.; Yoneda, F.; Taguchi, K.; Horiguchi, Y. *Eur. J. Med. Chem.* **2006**, *41*, 241.
13. Bicking, J. B.; Bock, M. G.; Cragoe, E. J.; DiPardo, R. M.; Gould, N. P.; Holtz, W. J.; Lee, T.-J.; Robb, C. M.; Smith, R. L.; Springer, J. P.; Blaine, E. H. *J. Med. Chem.* **1983**, *26*, 342.
14. Li, C.; Xiao, J. *J. Am. Chem. Soc.* **2008**, *130*, 13208.
15. Ciaccio, J. A.; Drahus, A. L.; Meis, R. M.; Tingle, C. T.; Smrtka, M.; Geneste, R. *Synth. Commun.* **2003**, *33*, 2135.
16. (a) Mitcheson, J. S.; Chen, J.; Lin; Culberson, C.; Sanguinetti, M. C. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12329; (b) Mitcheson, J. S.; Perry, M. D. *Curr. Opin. Drug Discov. Dev.* **2003**, *6*, 667; From the point of view of medicinal chemist, several approaches to attenuate hERG channel inhibition were illustrated using examples by Merck Scientists. See: Ref. 10c. (c) Fernandez, D.; Ghanta, A.; Kauffman, G. W.; Sanguinetti, M. C. *J. Biol. Chem.* **2004**, *279*, 10120; (d) Jamieson, C.; Moir, E. M.; Rankovic, Z.; Wishart, G. *J. Med. Chem.* **2006**, *49*, 5029.
17. (e) Farid, R.; Day, T.; Friesner, R. A.; Pearlstein, R. A. *Bioorg. Med. Chem.* **2006**, *14*, 3160; (f) Boukharta, L.; Keränen, H.; Stary-Weinzinger, A.; Wallin, G.; de Groot, B. L.; Åqvist, J. *Biochemistry* **2011**, *50*, 6146.
18. Compound **1h**, **1i**, **1k**, (*S*)-**1h** and (*R*)-**1h** exhibited good solubility (JP1; >100 μM, JP2; >100 μM).
19. Loser, R.; Abbenante, G.; Madala, P. K.; Halili, M.; Le, G. T.; Fairlie, D. P. *J. Med. Chem.* **2010**, *53*, 2651.
20. The racemate **1h** was more potent than the optically pure compounds. Although we cannot exclude the possibility that this is just an artifact of the error in the assay, this result was probably one of many exceptions to Pfeiffer's rule. See: Pfeiffer, C. C. *Science* **1956**, *124*, 29. Similar results have obtained with 1-substituted tetrahydroisoquinoline derivatives in lead optimization from (*S*)-**1h** (the related article is now in preparation for submission) and also reported by Zalicus scientists. See: Ref. 9a.
21. Kim, S. H.; Chung, J. M. *Pain* **1992**, *50*, 355.
22. Carbone, E.; Sher, E.; Clementi, F. *Pflugers Arch.* **1990**, *416*, 170.
23. Watabiki, T.; Kiso, T.; Kuramochi, T.; Yonezawa, K.; Tsuji, N.; Kohara, A.; Kakimito, S.; Aoki, T.; Matsuoka, N. *J. Pharmacol. Exp. Ther.* **2011**, *336*, 743.
24. Hamill, O. P.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. J. *Pflugers Arch.* **1981**, *391*, 85.