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Discovery of a 1-isopropyltetrahydroisoquinoline derivative as an orally active N-type calcium channel blocker for neuropathic pain

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ABSTRACT

N-type calcium channel blockade is a promising therapeutic approach for the treatment of neuropathic pain. Starting from lead compound (*S*)-**1**, we focused our optimization efforts on potency for N-type calcium channel inhibition and improvement of CYP inhibition profile. 2-{[(1-Hydroxycyclohexyl)methyl]amino}-(1*R*)-(1-isopropyl-6-methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethanone oxalate ((*R*)-**5r**) was identified as a novel orally active small-molecule N-type calcium channel inhibitor with reduced CYP inhibition liability. Oral administration of (*R*)-**5r** improved mechanical allodynia in a spinal nerve ligation model of neuropathic pain in rats with an ED_{50} value of 2.5 mg/kg.

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1. Introduction

N-type (Cav2.2) calcium channels are members of the voltage-dependent calcium channel (VDCC) family, expressed on the plasma membranes of excitable cells and control calcium influx in response to membrane potential.[1] The VDCC family regulates various physiological functions, such as muscular contraction, neuronal excitation, gene expression and hormone secretion. Calcium channels are composed of multimeric protein complexes, named α1, α2δ, β, and γ subunits.[1] The α1 subunit is believed to form the ion-conducting pore and hence determine the fundamental functions of the channel.[1]

N-type calcium channels are found abundantly in the presynaptic terminals of primary afferent nerves in the dorsal horn of the spinal cord. They modulate excitatory neurotransmission and control the release of neurotransmitters, including pain-related amino acids and neuropeptides, such as glutamate, calcitonin gene-related peptide (CGRP), and substance P.[1]

Several groups have reported that mice deficient in the pore-forming α1B subunit of N-type calcium channels exhibit reduced hyperalgesic and allodynic responses to thermal, chemical or mechanical stimuli compared to wild type mice.[2] The validity of targeting N-type calcium channels is further established by the clinical efficacy of ziconotide, a potent and selective N-type calcium channel blocker.[3] Although the synthetic peptide ziconotide has demonstrated marked clinical efficacy in treating severe and chronic pain, the utilization of this drug has been limited, as it requires intrathecal administration and has serious adverse effects on the central nervous system (CNS).[3] To overcome these drawbacks, a large number of studies have been conducted to identify small-molecule N-type calcium channel blockers as novel orally active analgesics.[4,5]

We previously reported the novel tetrahydroisoquinoline (*S*)-**1** as an orally active small-molecule N-type calcium channel blocker.[6] Notably, (*S*)-**1** fully ameliorated mechanical allodynia in a spinal nerve ligation (SNL) model of neuropathic pain in rats at 30 mg/kg po. However, compound (*S*)-**1** also exhibited inhibitory activity against both CYP2D6 and CYP3A4 (Table 1).

CYP2D6 and CYP3A4 play major roles in drug metabolism and are involved in the metabolism of approximately 25% and over 50% of drugs in clinical use, respectively.[7] CYP inhibition-mediated drug–drug interactions (DDIs) might lead to serious adverse events or a decrease in drug efficacy. Notably, the unwanted effects of time-dependent CYP inhibition persist after discontinuation of drug administration. Thus, these DDIs often pose a risk and are extremely difficult to manage in clinical application.

Pregabalin and gabapentin, which are widely prescribed for the treatment of neuropathic pain, have clinical advantages in terms of DDIs compared to other first line drugs, such as tricyclic

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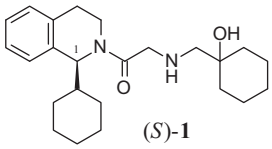
Table 1
In vitro pharmacological properties of (*S*)-**1**

Compound	N-type FLIPR IC_{50} (μM)	CYP2D6 IC_{50} (μM)	CYP3A4[a] preincubation time		*c*Log$D_{7.4}$[b]
			0 min (reversible)	30 min (time-dependent)	
 (*S*)-**1**	1.0	0.60	87%	67%	3.48

[a] Residual activities (%) of HLM were evaluated using midazolam as a probe substrate. Details are described in Section 5.
[b] *c*Log*D* values at pH 7.4 were calculated with ACD/PhysChem Batch (version 12.01).

antidepressants (TCAs) and serotonin and norepinephrine reuptake inhibitors (SNRIs).[8] To mitigate these risks in drug development and improve convenience in clinical use, the minimization of inhibitory activities against CYP2D6 and CYP3A4 in lead optimization from (*S*)-**1** is required.

Here, we describe the design and synthesis of a novel series of tetrahydroisoquinolines and the discovery of (*R*)-**5r** as an orally active small-molecule N-type calcium channel blocker with minimized liability of CYP inhibition.

2. Chemistry

The preparation of the 1-substituted tetrahydroisoquinoline derivatives **5a**–**5t** is outlined in Scheme 1. Acylation of phenylethylamines (**2b**, **2k**–**2n**, **2p**, and **2q**) yielded the amides (**3b**, **3g**, **3h**, **3k**–**3n**, **3p**, and **3q**). These amides (**3b**, **3g**, **3h**, **3k**–**3n**, **3p**, and **3q**) were cyclized utilizing traditional Bischler–Napieralski reaction or a modified version[9] and followed by reduction with sodium borohydride to give tetrahydroisoquinolines (**4b**, **4g**, **4h**, **4k**–**4n**, **4p**, and **4q**). Tetrahydroisoquinolines (**4e**, **4f**, **4i**, **4o**, and **4r**–**4t**) were prepared as previously described.[10,11] (*S*)**-4j** was obtained from a commercial source. Acylation of tetrahydroisoquinolines (**4a**, **4b**, **4e**–**4m**, **4o**, **4p**, and **4r**–**4t**) with chloroacetyl chloride followed by substitution with 1-(aminomethyl)cyclohexanol yielded the tetrahydroisoquinoline derivatives (**5a**, **5b**, **5e**–**5m**, **5o**, **5p**, and **5r**–**5t**). Optically active (*R*)-**5r** was synthesized from tetrahydroisoquinoline (*R*)-**4r**, which was prepared via the diastereomeric salt formation method using racemic **4r** with (*S*)-mandelic acid. (*S*)**-5r** was obtained from tetrahydroisoquinoline (*S*)-**4r** in a similar manner. The absolute stereochemistry of (*R*)-**4r** was determined by single-crystal X-ray crystallography as shown in Figure 1.

The 1-isopropyl-8-substituted tetrahydroisoquinolines (**5n** and **5q**) were also synthesized as shown in Scheme 1. To selectively obtain the 8-substituted tetrahydroisoquinolines, 2-bromophenylethylamines (**2n** and **2q**) were employed as starting materials.[11] 2-Bromophenylethylamines (**2n** and **2q**) were converted to 1-isopropyl-5-bromo-8-substituted tetrahydroisoquinolines (**4n** and **4q**) in a three-step sequence. Acylation of 1-isopropyl-5-bromo-8-substituted tetrahydroisoquinolines (**4n** and **4q**) with chloroacetyl chloride followed by substitution and reductive removal of the bromine gave the 1-isopropyl-8-substituted tetrahydroisoquinolines (**5n** and **5q**).

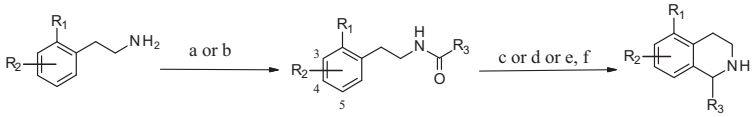
Compounds **5c** and **5d** were synthesized as shown in Scheme 2. A benzyl group was installed on tetrahydroisoquinoline **6** by reductive amination followed by nucleophilic addition with methyl lithium to give the alcohol **7**. Methylation of alcohol **7** and subsequent deprotection of *N*-benzyl group afforded the tetrahydroispquinoline **4c**. Tetrahydroisoquinoline **4c** was converted to compound **5c** in a two-step sequence. Lithiation of *N*-Boc protected tetrahydroispquinoline **8** followed by nucleophilic addition to acetone generated the alcohol. In the presence of trifluoroacetic acid, the alcohol was transformed to the tetrahydroispquinoline **9**.[10a] Acylation of the tetrahydroisoquinoline **9**, substitution with 1-(aminomethyl)cyclohexanol and reductive removal of the pivaloyl group yielded compound **5d**.

3. Results and discussion

Inhibitory activity against N-type calcium channels was evaluated using the fluorometric imaging plate reader (FLIPR) calcium imaging assay in IMR-32 human neuroblastoma cells. Experiments were performed in the presence of nitrendipine to block endogenous L-type calcium channels. Selected compounds were assessed for inhibitory activity against CYP2D6 using a fluorescence substrate. The reversible and time-dependent inhibitory effects of selected compounds were also evaluated using midazolam as a probe substrate to monitor changes in CYP3A4 activity. The calculation of *c*Log*D* values at pH 7.4 was performed with ACD/PhysChem Batch (version 12.01).[12]

Our prior investigation demonstrated that the 1-(aminomethyl)hydroxylcyclohexyl group of compound (*S*)-**1** played an important role in attenuating the blockade of hERG channels while maintaining potency for N-type calcium channels and good aqueous solubility.[6] However, compound (*S*)-**1** exhibited inhibitory activity against both CYP2D6 and CYP3A4 (Table 1). The tendency of highly lipophilic compounds to induce potent inhibitory activities against CYP2D6 and CYP3A4 has also been reported.[13] To improve the CYP profile of lead compound (*S*)-**1**, we focused on the 1-cyclohxyltetrahydroisoquinoline moiety, which is the most lipophilic part of the molecule. We therefore investigated the conversion of the cyclohexyl moiety, which is substituted at C-1 position of tetrahydroisoquinoline, to the less lipophilic groups (Table 2).

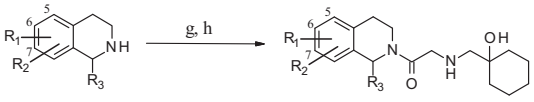
The 1-unsubstituted analogue **5a** resulted in a significant loss of N-type calcium channel inhibitory activity. Ether group substituted analogues (**5b** and **5c**) exhibited an approximately 5-fold decrease in N-type calcium channel potency compared to that of compound **1**. Incorporation of dimethylcarbinol (**5d**) led to a loss of potency for N-type calcium channels. Given that hydrophilic substitution at C-1 position of tetrahydroisoquinoline did not result in potent inhibitory activity against N-type calcium channels, small hydrophobic alkyl substituent, methyl group (**5e**) was introduced but remained insufficient to exert potent inhibitory activity. In contrast, hydrophobic acyclic alkyl substituents such as isopropyl (**5f**), *t*-butyl (**5g**) and 2-ethylpropyl (**5h**) groups exhibited potent inhibitory activities against N-type calcium channels. Hydrophobic cyclic analogues cyclopentyl (**5i**) and (*S*)-phenyl ((*S*)-**5j**) also provided high potency for N-type calcium channels. A degree of lipophilicity at the C-1 position of tetrahydroisoquinoline was therefore required to exert potent inhibitory activity against N-type calcium channels. In this investigation, the 2-isopropyl group was the smallest hydrophobic alkyl substituent to exhibit desirable potency for N-type calcium channels. In a fluorometric assay for CYP2D6 inhibition, no compounds showed



2b: R^1, R^2 = H
(for 3b, 3g, 3h)
2k: R^1 = Me, R^2 = H
2l: R^1 = H, R^2 = 3-Me
2m: R^1 = H, R^2 = 4-Me
2n: R^1 = Br, R^2 = 5-Me
2p: R^1 = H, R^2 = 4-F
2q: R^1 = Br, R^2 = 5-F

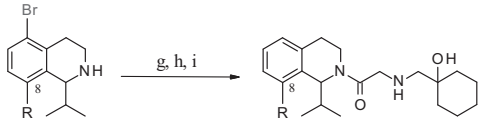
3b: R^1, R^2 = H, R^3 = 4-tetrahydropyrany
3g: R^1, R^2 = H, R^3 = *tert*-butyl
3h: R^1, R^2 = H, R^3 = 1-ethylpropyl
3k: R^1 = Me, R^2 = H, R^3 = isopropyl
3l: R^1 = H, R^2 = 3-Me, R^3 = isopropyl
3m: R^1 = H, R^2 = 4-Me, R^3 = isopropyl
3n: R^1 = Br, R^2 = 5-Me, R^3 = isopropyl
3p: R^1 = H, R^2 = 4-F, R^3 = isopropyl
3q: R^1 = Br, R^2 = 5-F, R^3 = isopropyl

4b: R^1, R^2 = H, R^3 = 4-tetrahydropyrany
4g: R^1, R^2 = H, R^3 = *tert*-butyl
4h: R^1, R^2 = H, R^3 = 1-ethylpropyl
4k: R^1 = Me, R^2 = H, R^3 = isopropyl
4l: R^1 = H, R^2 = 6-Me, R^3 = isopropyl
4m: R^1 = H, R^2 = 7-Me, R^3 = isopropyl
4n: R^1 = Br, R^2 = 8-Me, R^3 = isopropyl
4p: R^1 = H, R^2 = 7-F, R^3 = isopropyl
4q: R^1 = Br, R^2 = 8-F, R^3 = isopropyl



4a: R^1, R^2, R^3 = H
4b: R^1, R^2 = H, R^3 = 4-tetrahydropyranyl
4e: R^1, R^2 = H, R^3 = methyl
4f: R^1, R^2 = H, R^3 = isopropyl
4g: R^1, R^2 = H, R^3 = *tert*-butyl
4h: R^1, R^2 = H, R^3 = 1-ethylpropyl
4i: R^1, R^2 = H, R^3 = cyclopentyl
(*S*)-4j: R^1, R^2 = H, R^3 = *S*-phenyl
4k: R^1 = 5-Me, R^2 = H, R^3 = isopropyl
4l: R^1 = 6-Me, R^2 = H, R^3 = isopropyl
4m: R^1= 7-Me, R^2 = H, R^3 = isopropyl
4o: R^1 = 6-F, R^2 = H, R^3 = isopropyl
4p: R^1 = 7-F, R^2 = H, R^3 = isopropyl
4r: R^1 = 6-MeO, R^2 = H, R^3 = isopropyl
4s: R^1 = 7-MeO, R^2 = H, R^3 = isopropyl
4t: R^1 = 8-MeO, R^2 = H, R^3 = isopropyl

5a: R^1, R^2, R^3 = H
5b: R^1, R^2 = H, R^3 = 4-tetrahydropyranyl
5e: R^1, R^2 = H, R^3 = methyl
5f: R^1, R^2 = H, R^3 = isopropyl
5g: R^1, R^2 = H, R^3 = *tert*-butyl
5h: R^1, R^2 = H, R^3 = 1-ethylpropyl
5i: R^1, R^2 = H, R^3 = cyclopentyl
(*S*)-5j: R^1, R^2 = H, R^3 = *S*-phenyl
5k: R^1 = 5-Me, R^2 = H, R^3 = isopropyl
5l: R^1 = 6-Me, R^2 = H, R^3 = isopropyl
5m: R^1= 7-Me, R^2 = H, R^3 = isopropyl
5o: R^1 = 6-F, R^2 = H, R^3 = isopropyl
5p: R^1 = 7-F, R^2 = H, R^3 = isopropyl
5r: R^1 = 6-MeO, R^2 = H, R^3 = isopropyl
5s: R^1 = 7-MeO, R^2 = H, R^3 = isopropyl
5t: R^1 = 8-MeO, R^2 = H, R^3 = isopropyl



4n: R = Me
4q: R = F

5n: R = Me
5q: R = F

Scheme 1. Reagents and conditions: (a) RCO_2H, WSCD, HOBt, CH_2Cl_2, DMF, rt, 1 d; (b) RCOCl, satd $NaHCO_3$, AcOEt, rt, 3 h; (c) polyphosphate ester, 120 °C, 2 h; (d) (i) $FeCl_3$, $(COCl)_2$, 1,2-DCE, rt, 5 h (ii) H_2SO_4, MeOH, reflux, 12 h; (e) P_2O_5, $POCl_3$, 120 °C, 8 h; (f) $NaBH_4$, MeOH, rt, 1 h; (g) chloroacetyl chloride, K_2CO_3, H_2O, AcOEt, rt, 1 h; (h) 1-(aminomethyl)cyclohexanol, K_2CO_3, CH_3CN, 70 °C, 3 d; (i) 10% Pd/C, H_2, Et_3N, EtOH, rt, 6 h.

improved inhibition liability over compound **1**. In an assay for CYP3A4 inhibition using midazolam, cyclopentyl and (*S*)-phenyl substituted analogues (**5i** and (*S*)**-5j**) only exhibited an improved time-dependent inhibition (TDI) profile over compound **1**. Reducing lipophilicity at the C-1 position of tetrahydroisoquinoline, compound **5f** exhibited notable improvements in both reversible and time-dependent CYP3A4 inhibition profiles, whereas methyl group substituted analogue **5e** reduced CYP3A4 liability despite having low lipophilicity. Notably, isopropyl (**5f**) maintained potent inhibitory activity against N-type calcium channels with an improved CYP3A4 inhibition profile.

Conversion of the cyclohexyl moiety at C-1 position of tetrahydroisoquinoline in (*S*)**-1** to the less lipophilic isopropyl moiety improved the CYP3A4 inhibition profile while maintaining inhibitory activity against N-type calcium channels. However, these conversions did not eliminate CYP2D6 inhibition liability. Many CYP2D6 inhibitors are structurally characterized by the presence of a basic nitrogen function and a planar aromatic ring.[13] Several studies regarding monoamine reuptake inhibitors, whose structures are typical CYP2D6 inhibitors as mentioned above, demonstrated that small modifications of substituents on the benzene moiety had a remarkable impact on inhibitory activity.[14] Therefore, we next investigated the effect of substituents on the benzene ring of the tetrahydroisoquinoline moiety using 1-isopropyltetrahydroisoquinoline **5f** (Table 3).

Compound **5l**, which was substituted at the C-6 position of tetrahydroisoquinoline, exhibited the most favorable potency for N-type calcium channels of the compounds bearing a methyl group (**5k**–**5n**). However, these transformations deteriorated reversible inhibitory activity against CYP3A4 regardless of the position of the methyl substituent (**5k**–**5n**). These results might be related to an increase in lipophilicity of the compounds ($c\log D_{7.4}$ values of **5k**–**5n**: 2.70–2.94 vs **5f**: 2.37). Substitution at the C-6 position with a fluorine group (**5o**) was also more beneficial to N-type calcium channel inhibition than substitution at other positions (**5p** and **5q**). Compound **5o** exhibited potent inhibitory activity against N-type calcium channels comparable to compound **5l**. Incorporation of a methoxy group maintained a satisfactory level of inhibitory activity against N-type calcium channel inhibition regardless of the position of substitution but did not enhance potency (**5r**–**5t**). Regarding the TDI liability of CYP3A4, introduction of substituents at the C-6 position (**5l**, **5o**, and **5r**) maintained a preferable profile, whereas introduction of substituents at the C-8 position (**5n**, **5q**, and **5t**) deteriorated the profile. Of the substituted compounds, **5r** afforded favorable overall CYP3A4 inhibition liability with N-type calcium channel potency. Regarding CYP2D6

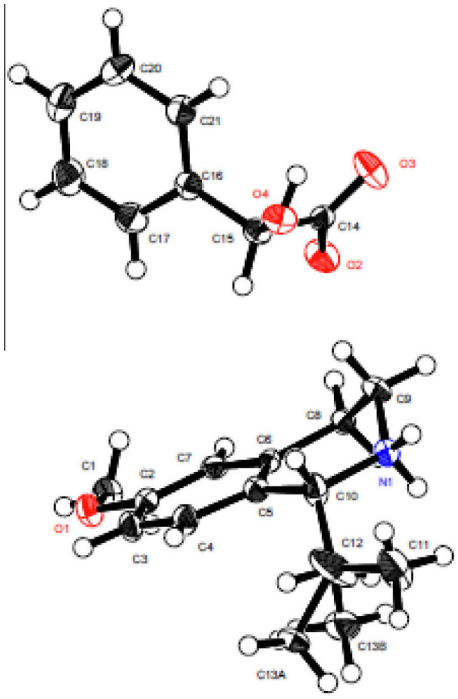


Figure 1. Molecular structure of (*R*)-**4r**, showing 60% probability displacement elipsoids. Disorder present in the isopropyl group is removed for clarity.

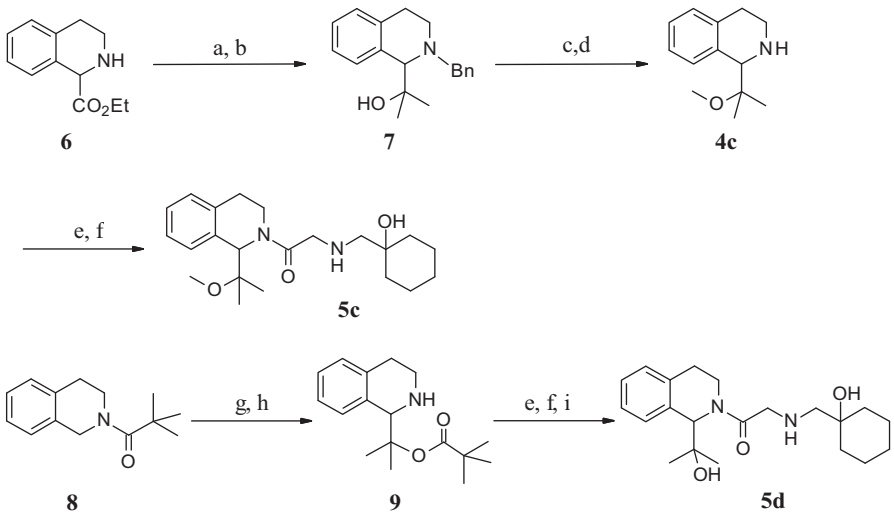
inhibitory activity, only the 6-methoxy group-bearing compound **5r** led to an improvement compared to **5f**. Unexpectedly, introduction of substituents to the benzene ring of the tetrahydroisoquinoline had only minor influence on CYP2D6 inhibition liability.

de Groot et al. investigated monoamine reuptake inhibitors and rationalized their findings on the reduction of CYP2D6 liability via small peripheral modifications with the following explanations: (i) difference in structure and conformation (ii) difference in physicochemical properties (notably, lipophilicity) (iii) (impairment of) specific interaction with residue of CYP2D6 in the active site.[13c]

Taken together, our results may be interpreted as follows. (i) As the isopropyl moiety substituted at the C-1 position of tetrahydroisoquinoline is relatively small, the conformational changes in the relative arrangement, induced by steric repulsion between the isopropyl moiety and substituents introduced to the C-8 position of tetrahydroisoquinoline, might not sufficiently reduce CYP2D6 liability (e.g., compound **5n** and **5t**). In addition, the 6-methoxy group of compound **5r** appeared to have little effect on the conformation of the compound. (ii) Our investigation demonstrated that, whereas CYP3A4 inhibition liability exhibited partial correlation with lipophilicity of a compound, CYP2D6 inhibition liability had a relatively poor relationship. A more dramatic decrease in lipophilicity might be required to improve the CYP2D6 profile, as exemplified by research for CCR3 antagonists.[13a,b] However, this transformation would not allow the compound to exert potent N-type calcium channel inhibition, as demonstrated by the investigation of hydrophilic substitution of the C-1 position of tetrahydroisoquinoline (**5a**–**5e**) and introduction of substituents on the benzene ring of 1-cyclohexyl tetrahydroisoquinoline.[15] (iii) Improvement of compound **5r** in CYP2D6 liability appeared to be mainly due to the impairment of specific interactions with the CYP2D6 enzyme with either or both the methoxy group itself or the effect of this group on the π bonds. Given that introduction of the 6-methoxy moiety (**5r**) resulted in moderate improvement and that introduction of other substituents had minor effect on CYP2D6 inhibition liability, regardless of the polarity or position of the substitution, the impairment effect of substituents appears to be relatively limited.

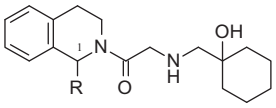
Consequently, the possibility remains that conformational changes, particularly those induced by a steric repulsion of two more bulky substituents between the C-1 and C-8 positions of tetrahydroisoquinoline, reducing CYP2D6 liability. However, compounds with only bulky substituents at the C-1 position of tetrahydroisoquinoline (**1**, **5g**–**5i**, and (*S*)-**5j**) exhibited potent inhibitory activities against CYP2D6. Further, these compounds still exhibited CYP3A4 inhibition liabilities probably due to their high lipophilicity.

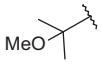
We also prepared and characterized both enantiomers of compound **5r**. Interestingly, in vitro profiles were similar between the enantiomers except for human liver microsomal (HLM) stability (Table 4). Both (*R*)- and (*S*)-**5r** exhibited slight decreases in N-type calcium channel inhibition compared to racemate **5r**. This result might be one of many exceptions to Pfeiffer's rule.[16] In addition, (*R*)-**5r** exhibited slightly weaker CYP2D6 inhibition than racemate **5r** and (*S*)-**5r**.[17] Regarding inhibitory activity against the other four major cytochrome P450 isoforms (CYP1A2, 2C9, 2C19,



Scheme 2. Reagents and conditions: (a) PhCHO, $NaBH(OAc)_3$, AcOH, rt, 15 h; (b) MeLi, THF, −78 °C to 0 °C, 3 h; (c) NaH, MeI, THF, rt, 20 h; (d) 10% Pd/C, H_2, MeOH, rt, 8 h; (e) chloroacetyl chloride, satd $NaHCO_3$, AcOEt, rt, 2 h; (f) 1-(aminomethyl)cyclohexanol, K_2CO_3, 1,4-dioxane, 50 °C, 2 h; (g) (i) *t*-BuLi, TMEDA, THF, −78 °C, 10 min (ii) acetone, −78 °C, 1 h; (h) TFA, rt, 4 h; (i) DIBAH, CH_2Cl_2, −78 °C to 0 °C, 7 h.

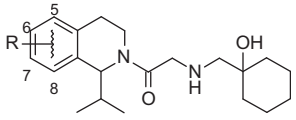
Table 2
Effect of tetrahydroisoquinoline 1-substitutions on N-type calcium channels and CYP2D6 and 3A4 inhibitory activities



Compound no.	R=	N-type FLIPR IC_{50} (μM)	CYP2D6 IC_{50} (μM)	CYP3A4[a] preincubation time		$cLogD_{7.4}$[b]
				0 min (reversible)	30 min (time-dependent)	
1		0.77	0.78	86%	61%	3.48
5a	H	8.0	N.T.[c]	N.T.[c]	N.T.[c]	1.10
5b		4.1	N.T.[c]	N.T.[c]	N.T.[c]	1.51
5c		3.7	N.T.[c]	N.T.[c]	N.T.[c]	1.68
5d		6.1	N.T.[c]	N.T.[c]	N.T.[c]	1.07
5e	Me	2.9	N.T.[c]	65%	33%	1.50
5f		1.1	<0.78	92%	98%	2.37
5g		0.80	<0.78	N.T.[c]	N.T.[c]	2.78
5h		0.73	<0.78	77%	59%	3.39
5i		0.99	<0.39	89%	84%	2.95
(*S*)-**5j**		1.0	<0.39	89%	78%	2.75

[a] Residual activities (%) of HLM were evaluated using midazolam as a probe substrate. Details are described in Section 5.
[b] *c*Log*D* values at pH 7.4 were calculated with ACD/PhysChem Batch (version 12.01).
[c] Not tested.

Table 3
Effect of tetrahydroisoquinoline substitutions on N-type calcium channels and CYP2D6 and 3A4 inhibitory activities



R (compound no.)	N-type FLIPR IC_{50} (μM)	CYP2D6 IC_{50} (μM)	CYP3A4[a] preincubation time		$cLogD_{7.4}$[b]
			0 min (reversible)	30 min (time-dependent)	
H (**5f**)	1.1	<0.78	92%	98%	2.37
5-Me (**5k**)	1.4	<0.78	68%	87%	2.88
6-Me (**5l**)	0.85	<0.78	75%	105%	2.78
7-Me (**5m**)	1.4	<0.78	72%	103%	2.94
8-Me (**5n**)	1.6	<0.78	73%	82%	2.70
6-F (**5o**)	0.89	0.39	86%	101%	2.55
7-F (**5p**)	1.3	0.68	89%	81%	2.24
8-F (**5q**)	1.5	<0.78	100%	85%	1.92
6-MeO (**5r**)	1.3	1.8	94%	95%	2.55
7-MeO (**5s**)	1.6	N.T.[c]	N.T.[c]	N.T.[c]	2.82
8-MeO (**5t**)	1.4	<0.78	72%	81%	2.90

[a] Residual activities (%) of HLM were evaluated using midazolam as a probe substrate. Details are described in Section 5.
[b] *c*Log*D* values at pH 7.4 were calculated with ACD/PhysChem Batch (version 12.01).
[c] Not tested.

and 3A4), (*R*)-**5r** exhibited little or no inhibitory activity. The HLM stability of (*R*)-**5r** was approximately 2-fold superior to that of (*S*)-**5r**. In addition, as might have been expected,[6] (*R*)- and (*S*)-**5r** had no effect on hERG inhibition (IC_{50} >100 μM) and good aqueous solubility (>100 μg/mL) at both pH 6.8 (the Japanese Pharmacopoeia 2nd fluid for the dissolution test) and pH 1.2 (the Japanese Pharmacopoeia 1st fluid for the dissolution test). These results prompted us to investigate in vivo efficacy.

The antinociceptive effect of (*R*)-**5r** was examined using a formalin test in mice. Subcutaneous injection of formalin into a hind paw elicited biphasic pain behavior. The first phase results were due to the direct stimulation of nociceptive nerve terminals, while the late phase results were due to a combination of peripheral input and spinal cord sensitization. Intrathecal administration of (*R*)-**5r** dose-dependently decreased the nociceptive behavior in both the first and second phase of a formalin test (Fig. 2). No abnormal behavior was observed in the test animals. (*R*)-**5r** has a more potent analgesic effect on the second phase than on the first (ED_{50} = 25.4 vs >100 nmol/body), which is consistent with previous findings regarding nociceptive responses using formalin test in α1B subunit deficient mice.[2,18] Therefore, (*R*)-**5r** is considered to induce an antinociceptive effect via N-type calcium channel blockade at a spinal level. In addition, orally administered (*R*)-**5r** exerted potent antinociceptive activity (64% inhibition) in the second phase of formalin test in mice at 100 mg/kg. In contrast, orally administered (*S*)-**5r** showed only moderate antinociceptive activity (34% inhibition). Considering that the HLM stability of (*R*)-**5r** was superior to that of (*S*)-**5r**, the difference in the pharmacokinetic

Table 4
In vitro pharmacological properties of (*R*)-**5r** and (*S*)-**5r**

Compound	N-type FLIPR IC_{50} (μM)	Major CYP isoforms						HLM stability CL (mL/min/kg)
		1A2 IC_{50} (μM)	2C9 IC_{50} (μM)	2C19 IC_{50} (μM)	2D6 IC_{50} (μM)	CYP3A4[a] preincubation time		
						0 min (reversible)	30 min (time-dependent)	
(*R*)-**5r**	1.9	>50	>50	>50	2.9	99%	92%	101.1
(*S*)-**5r**	2.1	>50	>50	N.T.[b]	2.0	96%	99%	187.8

[a] Residual activities (%) of HLM were evaluated using midazolam as a probe substrate. Details are described in Section 5.
[b] Not tested.

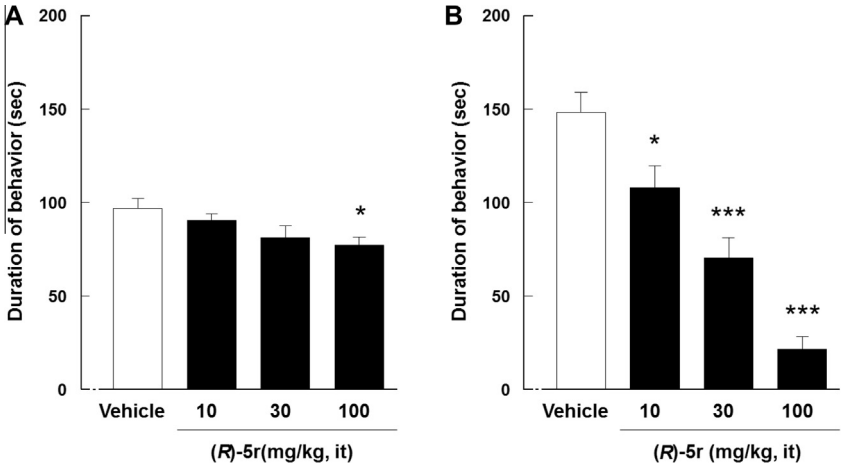


Figure 2. Effect of intrathecal bolus injection of compound (*R*)-**5r** on (A) acute (phase 1: 0–10 min) and (B) persistent (phase 2: 15–25 min) pain behavior in the formalin test in mice. The duration of nociceptive behaviors (lifting/licking time of the hindpaw) induced by subcutaneous injection of 2% formalin (20 μL) into the plantar surface of the hindpaw was measured. Each bar represents the mean ± SEM of the duration of nociceptive behaviors ($n = 6$). $^{*}P<0.05$ and $^{***}P<0.001$, statistically significant compared with vehicle-treated group (Dunnett's test).

properties of these enantiomers might be reflected in their antinociceptive activity.

Encouraged by these results, the oral analgesic effect of (*R*)-**5r** was evaluated in an L5/L6 spinal nerve ligation (SNL) model of neuropathic pain in rats.[19] (*R*)-**5r** dose-dependently reduced mechanical allodynia with oral administration from 1 to 10 mg/kg. Oral administration of (*R*)-**5r** improved mechanical allodynia in a rat SNL model with an ED_{50} value of 2.5 mg/kg (Fig. 3). This result

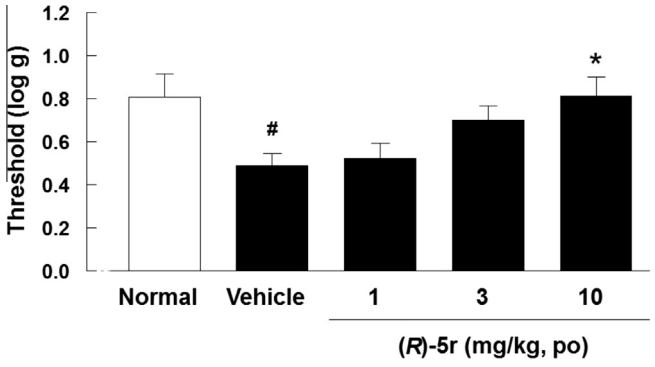


Figure 3. Effect of compound (*R*)-**5r** on mechanical allodynia in SNL model rats. Each bar represents the mean ± SEM withdrawal threshold of the hindpaw ($n = 8$). $^{\#}P<0.05$, statistically significant compared with non-operated side paw in vehicle-treated group (Normal) (Student's *t*-test). $^{*}P<0.05$, statistically significant compared with operated side paw in vehicle-treated group (Dunnett's test). Closed columns, operated side paw in drug-treated and vehicle-treated group. Open column, non-operated side paw in vehicle-treated group.

was also consistent with previous findings regarding the threshold for mechanical stimuli in an SNL model using α1B subunit deficient mice.[2a] Given the results of our formalin test, (*R*)-**5r** probably exerted its effect via suppression of the central sensitization process in the SNL model. The effect of (*R*)-**5r** on motor coordination was assessed using an accelerating rotarod test in rats. (*R*)-**5r** did not significantly decrease the time on the rod at 30 mg/kg po. In contrast, ziconotide had only a narrow margin between the ED_{50} value and TD_{50} value, which represents the dose of agent causing moderate to severe behavioral toxicity in the rat SNL model.[20] This class of N-type calcium channel blockers such as (*R*)-**5r** are expected to exhibit efficacy without adverse effects on the CNS in clinical use, unlike ziconotide.

4. Conclusion

In lead optimization efforts starting from tetrahydoroisoquinoline (*S*)-**1**, we identified 2-{[(1-Hydroxycyclohexyl)methyl]amino}-(1*R*)-(1-isopropyl-6-methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl) ethanone oxalate ((*R*)-**5r**) as a novel orally active small-molecule N-type calcium channel blocker. Reducing the lipophilicity by conversion from a cyclohexyl moiety (*S*)-**1** to isopropyl moiety (**5f**), with substitution at the C-1 position of the tetrahydroisoquinoline moiety, led to improvements in both CYP3A4 reversible and time-dependent inhibition profiles. Further introduction of a 6-methoxy group to the tetrahydroisoquinoline moiety resulted in approximately 5-fold attenuation of CYP2D6 inhibitory activity ((*S*)-**1** vs (*R*)-**5r**). Oral administration of (*R*)-**5r** exhibited marked efficacy in rat SNL model of neuropathic pain with an ED_{50} value of 2.5 mg/kg.

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 Shoko Scientific SI series silica gel cartridge 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; q, quartet; sep., septet; 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 or AD-RH chiral column on a Hitachi HPLC system (L-7000 series), equipped with an UV source (210 or 230 nm). Specific rotations were measured using a HORIBA SEPA-300 polarimeter.

5.1.2. *N*-(2-Phenylethyl)tetrahydro-2*H*-pyran-4-carboxamide (3b)

To a solution of 2-phenylethylamine (2.91 mL) and tetrahydro-2*H*-pyran-4-carboxylic acid (3.0 g) in dichloromethane (30 mL) and *N*,*N*-dimethylformamide (15 mL) were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (4.42 g) and 1-hydroxybenzotriazole (3.11 g). The mixture was stirred at room temperature for 1 day. The reaction mixture was diluted with water, extracted with chloroform, washed with brine, and dried over magnesium sulfate. The resulting mixture was concentrated in vacuo to give compound 3b (4.85 g, 90%) as a colorless solid. ^{1}H NMR ($CDCl_3$): δ 7.11–7.39 (5H, m), 5.46 (1H, br s), 3.92–4.04 (1H, m), 3.53 (2H, q, J = 6.7 Hz), 3.37 (2H, dt, J = 6.7, 12.8 Hz), 2.82 (2H, t, J = 6.8 Hz), 2.26 (sep. J = 5.0 Hz), 1.61–1.83 (m, 4H); APCI MS m/z 234 $[M+H]^+$.

5.1.3. 2,2-Dimethyl-*N*-(2-phenylethyl)propanamide (3g)

To a solution of 2-phenylethylamine (17 mL) and potassium carbonate (37 g) in ethyl acetate (80 mL) and water (150 mL) was added dropwise a solution of 2,2-dimethylpropanoyl chloride (16.3 g) in ethyl acetate (70 mL) at 4 °C. After stirring at room temperature for 1.5 h, the mixture was extracted with ethyl acetate, washed with 1 M aqueous hydrochloric acid, and dried over magnesium sulfate. The resulting mixture was concentrated in vacuo to give compound 3 g (24.6 g, 89%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6): δ 7.50 (1H, br s), 7.24–7.31 (2H, m), 7.14–7.22 (3H, m), 3.20–3.29 (2H, m), 2.71 (2H, t, J = 7.1 Hz), 1.05 (9H, s); FAB MS m/z 206 $[M+H]^+$.

5.1.4. 2-Ethyl-*N*-(2-phenylethyl)butanamide (3h)

Compound **3h** was synthesized in a manner similar to that for compound **3b**. Compound **3h** was obtained at a yield of 76% as a colorless solid. ^{1}H NMR ($CDCl_3$): δ 7.27–7.36 (2H, m), 7.17–7.26 (3H, m), 5.42 (1H, br s), 3.56 (2H, q, J = 6.8 Hz), 2.83 (2H, t, J = 6.9 Hz), 1.71–1.81 (1H, m), 1.51–1.65 (2H, m), 1.37–1.49 (2H, m), 0.85 (6H, t, J = 7.4 Hz); ESI MS m/z 220 $[M+H]^+$.

5.1.5. 2-Methyl-*N*-[2-(2-methylphenyl)ethyl]propanamide (3k)

Compound **3k** was synthesized in a manner similar to that for compound **3g**. Compound **3k** was obtained at a quantitative yield as a pale brown oil. ^{1}H NMR (DMSO-d_6): δ 7.75–7.88 (1H, br), 7.05–7.17 (3H, m), 3.33 (3H, s), 3.20 (2H, q, J = 8.0 Hz), 2.69 (2H, t, J = 8.0 Hz), 2.22–2.37 (4H, m), 0.98 (6H, d, J = 4.0 Hz); EI MS m/z 205 $[M]^+$.

5.1.6. 3-Methyl-*N*-[2-(2-methylphenyl)ethyl]propanamide (3l)

Compound **3l** was synthesized in a manner similar to that for compound **3g**. Compound **3l** was obtained at a yield of 90% as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.20 (1H, t, J = 7.3 Hz), 6.95–7.08 (3H, m), 5.42 (1H, br s), 3.50 (2H, q, J = 6.8 Hz), 2.78 (2H, t, J = 6.8 Hz), 2.33 (3H, s), 2.28 (1H, sep., J = 6.8 Hz), 1.12 (6H, d, J = 6.8 Hz); FAB MS m/z 206 $[M+H]^+$.

5.1.7. 4-Methyl-*N*-[2-(2-methylphenyl)ethyl]propanamide (3m)

Compound **3m** was synthesized in a manner similar to that for compound **3g**. Compound **3m** was obtained at a yield of 60% as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.05–7.15 (4H, m), 5.40 (1H, br s), 3.49 (2H, q, J = 6.8 Hz), 2.77 (2H, t, J = 6.8 Hz), 2.33 (3H, s), 2.27 (1H, sep., J = 6.8 Hz) 1.11 (6H, d, J = 7.1 Hz); FAB MS m/z 206 $[M+H]^+$.

5.1.8. *N*-[2-(2-Bromo-5-methylphenyl)ethyl]-2-methylpropanamide (3n)

Compound **3n** was synthesized in a manner similar to that for compound **3g** using **2n**[21] instead of **2b**. Compound **3n** was obtained 83% yield as a red solid. ^{1}H NMR (DMSO-d_6): δ 7.73–7.89 (1H, m), 7.43 (1H, d, J = 8.4 Hz), 7.09 (1H, br s), 6.92–7.01(1H, m), 3.26 (2H, q, J = 7.3 Hz), 2.79 (2H, t, J = 7.1 Hz), 2.31 (1H, sep., J = 6.9 Hz), 2.23 (3H, s), 0.97 (6H, d, J = 6.9 Hz); CI MS m/z 284 $[M]^+$.

5.1.9. 4-Fluoro-*N*-[2-(2-methylphenyl)ethyl]propanamide (3p)

Compound **3p** was synthesized in a manner similar to that for compound **3g**. Compound **3p** was obtained at a yield of 99% as a pale yellow solid. ^{1}H NMR (DMSO-d_6): δ 7.76 (1H, br s), 7.16–7.28 (2H, m), 7.04–7.14 (2H, m), 3.24 (2H, q, J = 7.3 Hz), 2.69 (2H, t, J = 7.1 Hz), 2.30 (1H, sep., J = 6.8 Hz), 0.96 (6H, d, J = 6.8 Hz); FAB MS m/z 210 $[M+H]^+$.

5.1.10. *N*-[2-(2-Bromo-5-fluorophenyl)ethyl]-2-methylpropanamide (3q)

Compound **3q** was synthesized in a manner similar to that for compound **3g** using **2q**[21] instead of **2b**. Compound **3q** was obtained 98% yield as a colorless solid. ^{1}H NMR (DMSO-d_6): δ 7.82 (1H, br s), 7.61 (1H, dd, J = 5.4, 8.8 Hz), 7.15 (1H, dd, J = 3.1, 9.8 Hz), 7.05 (1H, dt, J = 3.0, 8.4 Hz), 3.31 (2H, q, J = 6.8 Hz), 2.83 (2H, t, J = 7.0 Hz), 2.30 (1H, sep., J = 6.8 Hz), 0.96 (6H, d, J = 6.8 Hz); FAB MS m/z 288 $[M+H]^+$.

5.1.11. 1-(Tetrahydro-2*H*-pyran-4-yl)-1,2,3,4-tetrahydroisoquinoline (4b)

Compound **3b** (3.0 g) was combined with polyphosphate ester (15 mL) and stirred at 120 °C for 2 h. The reaction mixture was diluted with water, basified with ammonia, extracted with ethyl acetate, washed with water and brine, and dried over magnesium sulfate. The resulting mixture was concentrated in vacuo to obtain 1-(tetrahydro-2*H*-pyran-4-yl)-3,4-dihydroisoquinoline (2.61 g, 94%), which was used in the next step without further purification. APCI MS m/z 216 $[M+H]^+$. To a solution of 1-(tetrahydro-2*H*-pyran-4-yl)-3,4-dihydroisoquinoline (2.61 g) in methanol (15 mL) was added sodium borohydride (458 mg) at 4 °C. The reaction mixture was stirred at room temperature for 1 h. The reaction was quenched by the addition of water, extracted with ethyl acetate, washed with water and brine, and dried over magnesium sulfate. The resulting mixture was concentrated in vacuo to give compound **4b** (1.61 g, 61%) as a brown solid. ^{1}H NMR ($CDCl_3$): δ 7.03–7.36 (4H, m), 3.82–4.19 (2H, m), 3.18–3.58 (2H, m), 2.56–3.07 (2H, m), 1.39–1.85 (3H, m), 1.26–1.38 (1H, m); APCI MS m/z 218 $[M+H]^+$.

5.1.12. 1-*tert*-Butyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (4g)

To a solution of compound **3g** (10.0 g) in toluene (100 mL) was added phosphorus pentoxide (13.83 g) and stirred at 70 °C. Phosphoryl chloride (7 mL) was then added to the mixture, warmed to 120 °C and stirred for 8 h. After cooling to room temperature, the supernatant was removed. 20% aqueous sodium hydroxide was added to the residue, extracted with ethyl acetate, washed with brine, and dried over magnesium sulfate. The mixture was concentrated in vacuo to obtain 1-*tert*-butyl-3,4-dihydroisoquinoline (4.9 g, 53%), which was used in the next step without further purification. EI MS: *m*/*z*: 186 $[M-H]^+$. To a solution of 1-*tert*-butyl-3,4-dihydroisoquinoline (4.83 g) in ethanol (40 mL) and toluene (5 mL) was added sodium borohydride (1.10 g) at 4 °C. After stirring at room temperature for 1 h, 1 M aqueous hydrochloric acid (20 mL) was added to the mixture and stirred at room temperature for 30 min. The resulting mixture was basified with 1 M aqueous sodium hydroxide (40 mL), extracted with ethyl acetate, dried over magnesium sulfate, and concentrated in vacuo. The residue was dissolved in ethyl acetate (20 mL) and ether (60 mL) and 4 M hydrogen chloride in ethyl acetate (7 mL) was then added to the mixture. The precipitate was filtered off to give compound **4g** (5.82 g, 23%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6): δ 10.5 (1H, br s), 8.26 (1H, br s), 7.23–7.32 (4H, m), 4.31 (1H, br s), 3.48–3.56 (1H, m), 2.69–2.91 (3H, m), 1.00 (9H, s); ESI MS *m*/*z* 190 $[M+H]^+$.

5.1.13. 1-(Pentan-3-yl)-1,2,3,4-tetrahydroisoquinoline hydrochloride (4h)

Compound **4h** was synthesized in a manner similar to that for compound **4g**. Compound **4h** was obtained at a yield of 80% as a colorless solid. ^{1}H NMR (DMSO-d_6): δ 9.66 (1H, br s), 8.42 (1H, br s), 7.18–7.34 (4H, m), 4.61 (1H, br s), 3.36–3.46 (1H, m) 3.03–3.25 (2H, m), 2.85–2.96 (1H, m), 1.95 (1H, br s), 1.22–1.60 (3H, m), 1.07–1.19 (1H, m), 1.00 (3H, t, *J* = 7.6 Hz), 0.80 (3H, t, *J* = 7.5 Hz); ESI MS *m*/*z* 204 $[M+H]^+$.

5.1.14. 1-Isopropyl-5-methyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (4k)

To a solution of compound **3k** (7.64 g) in dichloromethane (50 mL) was added oxalyl chloride (5.0 mL) at 4 °C. After stirring at room temperature for 1.5 h, ferric chloride (6.7 g) was added to the mixture at 4 °C. The mixture was then stirred at room temperature for 4.5 h. The reaction was quenched by the addition of 1 M aqueous hydrochloric acid, extracted with chloroform, dried over magnesium sulfate and concentrated in vacuo. The residue was washed with ether, and the precipitate was filtered off to obtain 10*b*-isopropyl-7-methyl-6,10*b*-dihydro-5*H*-[1,3]oxazolo[2,3-*a*]isoquinoline-2,3-dione (5.25 g, 55%) as a colorless solid. FAB MS *m*/*z* 260 $[M+H]^+$. To a solution of concentrated sulfuric acid (3.0 mL) in methanol (30 mL) was added 10*b*-isopropyl-7-methyl-6,10*b*-dihydro-5*H*-[1,3]oxazolo[2,3-*a*]isoquinoline-2,3-dione (5.22 g) and the mixture was heated under reflux for 4 days. The mixture was concentrated in vacuo, diluted with water and washed with ether. The aqueous phase was neutralized with aqueous ammonia, extracted with toluene, dried over magnesium sulfate and concentrated in vacuo to obtain 1-isopropyl-5-methyl-3,4-dihydroisoquinoline (3.66 g, 97%) as a brown oil. FAB MS *m*/*z* 188 $[M+H]^+$. To a solution of 1-isopropyl-5-methyl-3,4-dihydroisoquinoline (3.61 g) in ethanol (40 mL) and toluene (10 mL) was added sodium borohydride (0.80 g) at 4 °C. After stirring at room temperature for 14 h, the reaction was quenched by the addition of 1 M aqueous hydrochloric acid. The mixture was basified with 1 M aqueous sodium hydroxide, extracted with toluene, and dried over magnesium sulfate. 4 M hydrogen chloride in ethyl acetate (6 mL) was added to the mixture. The precipitate was filtered off to give compound **4k** (4.19 g, 96%) as a colorless solid. ^{1}H NMR (DMSO-d_6): δ 9.85 (1H, br s), 8.77 (1H, br s), 7.02–7.26 (3H, m), 4.36–4.45 (1H, m), 3.39–3.63 (1H, m), 3.08–3.21 (1H, m), 2.78–2.98 (2H, m), 2.34–2.55 (1H, m), 2.22 (3H, s), 1.09 (3H, d, *J* = 7.1 Hz), 0.84 (3H, d, *J* = 6.9 Hz); FAB MS *m*/*z* 190 $[M+H]^+$.

5.1.15. 1-Isopropyl-6-methyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (4l)

Compound **4l** was synthesized in a manner similar to that for compound **4g**. Compound **4l** was obtained at a yield of 88% yield as a colorless solid. ^{1}H NMR (DMSO-d_6): δ 9.85 (1H, br s), 8.72 (1H, br s), 7.17 (1H, d, *J* = 8.1 Hz), 7.08 (1H, d, *J* = 8.1 Hz), 7.03 (1H, s), 4.37 (1H, br s), 3.38–3.47 (1H, m), 2.98–3.16 (2H, m), 2.80–2.91 (1H, m), 2.40–2.48 (1H, m), 2.27 (3H, s), 1.09 (3H, d, *J* = 6.9 Hz), 0.84 (3H, d, *J* = 7.0 Hz); CI MS *m*/*z* 190 $[M+H]^+$.

5.1.16. 1-Isopropyl-7-methyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (4m)

Compound **4m** was synthesized in a manner similar to that for compound **4g**. Compound **4m** was obtained at a yield of 74% as a colorless solid.^{1}H NMR (DMSO-d_6) δ 9.89 (1H, br s), 8.72 (1H, br s), 6.82–7.26 (3H, m), 4.38 (1H, br s), 3.38–3.46 (1H, m), 2.97–3.14 (2H, m), 2.79–2.90 (1H, m), 2.41–2.52 (1H, m), 2.28 (3H, s), 1.10 (3H, d, *J* = 7.1 Hz), 0.84 (3H, d, *J* = 7.0 Hz); CI MS *m*/*z* 190 $[M+H]^+$.

5.1.17. 5-Bromo-1-isopropyl-8-methyl-1,2,3,4-tetrahydroisoquinoline (4n)

Compound **4n** was synthesized in a manner similar to that for compound **4g**. Compound **4n** was obtained at a yield of 8% as a brown oil. ^{1}H NMR ($CDCl_3$): δ 7.32 (1H, d, *J* = 8.1 Hz), 6.87 (1H, d, *J* = 8.1 Hz), 4.00 (1H, d, *J* = 5.9 Hz), 3.26–3.39 (1H, m), 2.83–3.01 (2H, m), 2.56–2.69 (1H, m), 2.15–2.37 (4H, m), 2.02–2.13 (1H, m), 0.98 (3H, d, *J* = 6.8 Hz), 0.83 (3H, d, *J* = 6.8 Hz); CI MS *m*/*z* 268 $[M]^+$.

5.1.18. 7-Fluoro-1-isopropyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (4p)

Compound **4p** was synthesized in a manner similar to that for compound **4g**. Compound **4p** was obtained at a yield of 12% as a pale yellow solid.^{1}H NMR (DMSO-d_6): δ 10.07 (1H, br s), 8.90 (1H, br s), 7.25–7.34 (1H, m), 7.07–7.24 (1H, m), 4.46 (1H, br s), 3.43 (1H, br s), 2.82–3.15 (4H, m), 1.12 (3H, d, *J* = 7.1 Hz), 0.83 (3H, d, *J* = 7.1 Hz); FAB MS *m*/*z* 194 $[M+H]^+$.

5.1.19. 5-Bromo-8-fluoro-1-isopropyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (4s)

Compound **4s** was synthesized in a manner similar to that for compound **4k**. Compound **4s** was obtained at a yield of 7% as a colorless solid. ^{1}H NMR (DMSO-d_6): δ 9.95 (1H, br s), 9.09 (1H, br s), 7.71 (1H, dd, *J* = 5.1, 8.8 Hz), 7.20 (1H, t, *J* = 9.3 Hz), 4.52 (1H, d, *J* = 6.9 Hz), 3.39–3.51 (1H, m), 3.22–3.38 (1H, m), 2.89–2.06 (2H, m), 2.19–2.35 (1H, m), 1.00 (3H, d, *J* = 6.8 Hz), 0.94 (3H, dd, *J* = 2.5, 6.8 Hz); ESI MS *m*/*z*: 272 $[M+H]^+$.

5.1.20. 1-(3,4-Dihydroisoquinolin-2(1*H*)-yl)-2-{[(1-hydroxycyclohexyl)methyl]amino}ethanone oxalate (5a)

To a solution of 1,2,3,4-tetrahydroisoquinoline (2.0 g) in toluene (10 mL) and water (10 mL) was added potassium carbonate (3.2 g) and chloroacetyl chloride (1.4 mL) at 4 °C. The reaction mixture was stirred at room temperature for 23 h and then diluted with water, extracted with ethyl acetate, washed with 1 M aqueous hydrochloric acid and brine, dried over magnesium sulfate, and concentrated in vacuo to give 2-chloro-1-(3,4-dihydroisoquinolin-2(1*H*)-yl)ethanone (2.7 g, 86%) as a pale yellow solid. EI MS *m*/*z* 209 $[M]^+$. To a solution of 2-chloro-1-(3,4-

dihydroisoquinolin-2(1*H*)-yl)ethanone (0.35 g) in acetonitrile (5 mL) were added 1-(aminomethyl)cyclohexanol hydrochloride (0.7 g) and potassium carbonate (0.7 g). The mixture was stirred at 70 °C for 3 days and then diluted with water, extracted with ethyl acetate, washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was purified by column chromatography, eluting with chloroform–methanol, and dissolved in 2-propanol (4 mL). A solution of oxalic acid (54 mg) in ether (10 mL) was added to the solution. The precipitate was filtered off to obtain compound **5a** (194 mg, 30%) as a colorless solid. ^{1}H NMR (DMSO-d_6): δ 7.62 (1H, br s), 7.13–7.27 (4H, m), 4.66 (1H, s), 4.60 (1H, s), 4.03–4.14 (2H, m), 3.73 (1H, t, J = 6.1 Hz), 3.60 (1H, t, J = 6.0 Hz), 2.86–2.95 (3H, m), 2.81 (1H, t, J = 6.0 Hz), 1.18–1.64 (11H, m); FAB MS m/z 303 [M+H]$^+$.

5.1.21. 2-{[(1-Hydroxycyclohexyl)methyl]amino}-1-[1-(tetrahydro-2*H*-pyran-4-yl)-3,4-dihydroisoquinolin-2(1*H*)-yl]ethanone oxalate (5b)

Compound **5b** was synthesized in a manner similar to that for compound **5a**. Compound **5b** was obtained at a yield of 44% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.12–7.27 (4H, m), 5.14 (major rotamer 1H, t, J = 9.7 Hz), 4.39 (minor rotamer 1H, d, J = 9.8 Hz), 4.21–4.30 (1H, m), 4.14 (major rotamer 1H, d, J = 16.2 Hz), 3.30–4.07 (7H, m), 3.07–3.28 (2H, m), 2.82–3.03 (4H, m), 1.80–2.00 (1H, m), 1.19–1.65 (15H, m); FAB MS m/z 387 [M+H]$^+$.

5.1.22. 2-{[(1-Hydroxycyclohexyl)methyl]amino}-1-(1-methyl-3,4-dihydroisoquinolin-2(1*H*)-yl)ethanone oxalate (5e)

Compound **5e** was synthesized in a manner similar to that for compound **5a**. Compound **5e** was obtained at a yield of 8% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.11–7.29 (4H, m), 5.46 (major rotamer 1H, q, J = 6.8 Hz), 4.96 (minor rotamer 1H, q, J = 6.7 Hz), 4.38–4.47 (minor rotamer 1H, m), 3.97–4.27 (2H, m), 3.62–3.73 (major rotamer 1H, m), 3.44–3.55 (major rotamer 1H, m), 3.10–3.21 (minor rotamer 1H, m), 2.69–3.01 (4H, m), 1.18–1.65 (13H, m); FAB MS m/z 317 [M+H]$^+$.

5.1.23. 2-{[(1-Hydroxycyclohexyl)methyl]amino}-1-(1-isopropyl-3,4-dihydroisoquinolin-2(1*H*)-yl)ethanone oxalate (5f)

Compound **5f** was synthesized in a manner similar to that for compound **5a**. Compound **5f** was obtained at a yield of 20% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.12–7.27 (4H, m), 5.09 (major rotamer 1H, d, J = 9.3 Hz), 4.31 (minor rotamer 1H, d, J = 9.4 Hz), 4.18–4.27 (minor rotamer 1H, m), 4.12 (major rotamer 1H, d, J = 16.2 Hz), 3.94–4.04 (1H, m), 3.64–3.74 (major rotamer 1H, m), 3.53–3.63 (major rotamer 1H, m), 3.32–3.42 (minor rotamer 1H, m), 2.80–3.02 (4H, m), 1.92–2.10 (1H, m), 1.17–1.65 (10H, m), 0.78–1.02 (6H, m); FAB MS m/z 345 [M+H]$^+$; Anal. Calcd for $C_{21}H_{32}N_2O_2{\cdot}C_2H_2O_4$: C, 63.57; H, 7.89; N, 6.45. Found: C, 63.44; H, 7.92; N, 6.49.

5.1.24. 1-(1-*tert*-Butyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[(1-hydroxycyclohexyl)methyl]amino}ethanone oxalate (5g)

Compound **5g** was synthesized in a manner similar to that for compound **5a**. Compound **5g** was obtained at a yield of 20% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.12–7.31 (4H, m), 5.33 (major rotamer 1H, s), 4.48–4.59 (minor rotamer 1H, m), 4.46 (minor rotamer 1H, s), 4.08 (2H, q, J = 11.7 Hz), 3.62–3.43 (2H, m), 3.30–3.43 (minor rotamer 1H, m), 2.76–3.08 (4H, m), 1.19–1.66 (11H, m), 0.98 (9H, s), 0.94 (9H, s); FAB MS m/z 359 [M+H]$^+$.

5.1.25. 2-{[(1-Hydroxycyclohexyl)methyl]amino}-1-[1-(pentan-3-yl)-3,4-dihydroisoquinolin-2(1*H*)-yl]ethanone oxalate (5h)

Compound **5h** was synthesized in a manner similar to that for compound **5a**. Compound **5h** was obtained at a yield of 50% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.13–7.31 (4H, m), 5.32 (major rotamer 1H, d, J = 9.4 Hz), 4.43 (minor rotamer 1H, d, J = 9.9 Hz), 3.84–4.19 (2H, m), 3.55–3.72 (2H, m), 3.38 (minor rotamer 1H, q, J = 6.9 Hz), 2.78–3.05 (4H, m), 1.13–1.77 (14H, m), 0.72–0.93 (6H, m); FAB MS m/z 373 [M+H]$^+$.

5.1.26. 1-(1-Cyclopentyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[(1-hydroxycyclohexyl)methyl]amino}ethanone oxalate (5i)

Compound **5i** was synthesized in a manner similar to that for compound **5a**. Compound **5i** was obtained at a yield of 58% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.63 (1H, br s), 7.09–7.26 (9H, m), 5.24 (major rotamer 1H, d, J = 10.1 Hz), 4.49 (minor rotamer 1H, d, J = 9.8 Hz), 4.14 (major rotamer 1H, d, J = 10.1 Hz), 3.95–4.08 (1H, m), 3.55–3.74 (major rotamer 2H, m), 3.27–3.40 (minor rotamer 1H, m), 2.78–3.05 (4H, m), 2.07–2.31 (1H, m), 1.15–1.79 (19H, m); FAB MS m/z 371 [M+H]$^+$.

5.1.27. 2-{[(1-Hydroxycyclohexyl)methyl]amino}-1-[(1*S*)-1-phenyl-3,4-dihydroisoquinolin-2(1*H*)-yl]ethanone oxalate ((*S*)-5j)

Compound (*S*)-**5j** was synthesized in a manner similar to that for compound **5a**. Compound (*S*)-**5j** was obtained at a yield of 36% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.14–7.42 (9H, m), 6.66 (major rotamer 1H, s), 6.16 (minor rotamer 1H, s), 4.34 (minor rotamer 1H, d, J = 16.2 Hz), 4.19 (major rotamer 1H, d, J = 16.2 Hz), 3.99–4.13 (1H, m), 3.84 (minor rotamer 1H, d, J = 16.2 Hz), 3.41–3.70 (2H, m), 2.73–3.08 (5H, m), 1.18–1.65 (11H, m); ESI MS m/z 379 [M+H]$^+$; Anal. Calcd for $C_{24}H_{30}N_2O_2{\cdot}C_2H_2O_4$: C, 66.65; H, 6.88; N, 5.98. Found: C, 66.63; H, 6.88; N, 5.94. $[\alpha]_D^{26}$ +129.6°(c 0.1, MeOH).

5.1.28. 2-{[(1-Hydroxycyclohexyl)methyl]amino}-1-(1-isopropyl-5-methyl-3,4-dihydroisoquinolin-2(1*H*)-yl)ethanone oxalate (5k)

Compound **5k** was synthesized in a manner similar to that for compound **5a**. Compound **5k** was obtained at a yield of 68% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.03–7.12 (3H, m), 5.06 (major rotamer 1H, d, J = 9.4 Hz), 4.37–4.47 (minor rotamer 1H, m), 4.27 (minor rotamer 1H, d, J = 9.4 Hz), 4.16 (major rotamer 1H, d, J = 16.2 Hz), 3.91–4.08 (1H, m), 3.58–3.75 (2H, m), 3.25–3.36 (minor rotamer 1H, m), 2.83–2.98 (1H, m), 2.72–2.82 (3H, m), 2.21 (major rotamer 3H, s), 2.19 (minor rotamer 3H, s), 1.92–2.11 (1H, m), 1.16–1.67 (10H, m), 0.82–1.01 (6H, m); FAB MS m/z 359 [M+H]$^+$.

5.1.29. 2-{[(1-Hydroxycyclohexyl)methyl]amino}-1-(1-isopropyl-6-methyl-3,4-dihydroisoquinolin-2(1*H*)-yl)ethanone oxalate (5l)

Compound **5l** was synthesized in a manner similar to that for compound **5a**. Compound **5l** was obtained at a yield of 66% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 6.95–7.11 (3H, m), 5.04 (major rotamer 1H, d, J = 9.2 Hz), 4.26 (minor rotamer 1H, d, J = 9.6 Hz), 4.17–4.24 (minor rotamer 1H, m), 4.14 (major rotamer 1H, d, J = 16.2 Hz), 3.95–4.05 (1H, m), 3.51–3.71 (2H, m), 3.29–3.38 (minor rotamer 1H, m), 2.77–2.98 (4H, m), 2.26 (3H, s), 1.88–2.08 (1H, m), 1.15–1.68 (10H, m), 0.82–1.00 (6H, m); FAB MS m/z 359 [M+H]$^+$.

5.1.30. 2-{[(1-Hydroxycyclohexyl)methyl]amino}-1-(1-isopropyl-7-methyl-3,4-dihydroisoquinolin-2(1*H*)-yl)ethanone oxalate (5m)

Compound **5m** was synthesized in a manner similar to that for compound **5a**. Compound **5m** was obtained at a yield of 44% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 6.97–7.12 (3H, m), 5.04 (major rotamer 1H, d, J = 9.2 Hz), 4.25 (minor rotamer 1H, d, J = 9.5 Hz), 4.15–4.22 (minor rotamer 1H, m), 4.11 (major rotamer 1H, d, J = 16.2 Hz), 3.92–4.07 (1H, m), 3.50–3.71 (major rotamer 2H, m), 3.29–3.40 (minor rotamer 1H, m), 2.77–2.97 (4H, m), 2.27 (major rotamer 3H, s), 2.28 (minor rotamer 3H, s), 1.90–2.12 (1H, m), 1.18–1.65 (10H, m), 0.83–0.99 (6H, m); FAB MS m/z 359 [M+H]$^+$.

5.1.31. 1-(6-Fluoro-1-isopropyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[(1-hydroxycyclohexyl)methyl]amino}ethanone oxalate (5o)

Compound **5o** was synthesized in a manner similar to that for compound **5a**. Compound **5o** was obtained at a yield of 62% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.25 (1H, dd, J = 5.9, 8.5 Hz), 6.98–7.12 (2H, m), 5.11 (major rotamer 1H, d, J = 9.3 Hz), 4.36 (minor rotamer 1H, d, J = 9.4 Hz), 4.18–4.26 (minor rotamer 1H, m), 4.13 (major rotamer 1H, d, J = 16.3 Hz), 3.97–4.06 (1H, m), 3.55–3.71 (2H, m), 3.29–3.38 (minor rotamer 1H, m), 2.83–3.02 (4H, m), 1.90–2.09 (1H, m), 1.16–1.64 (10H, m), 0.80–0.98 (6H, m); FAB MS m/z 363 [M+H]$^+$.

5.1.32. 1-(7-Fluoro-1-isopropyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[(1-hydroxycyclohexyl)methyl]amino}ethanone oxalate (5t)

Compound **5t** was synthesized in a manner similar to that for compound **5a**. Compound **5t** was obtained at a yield of 53% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.02–7.36 (3H, m), 5.14 (major rotamer 1H, d, J = 9.2 Hz), 4.38 (minor rotamer 1H, d, J = 9.4 Hz), 4.09–4.26 (1H, m), 3.96–4.05 (1H, m), 3.54–3.71 (major rotamer 2H, m), 3.30–3.44 (minor rotamer 1H, m), 2.80–3.00 (4H, m), 1.94–2.12 (1H, m), 1.18–1.63 (10H, m), 0.81–0.99 (6H, m); FAB MS m/z 363 [M+H]$^+$.

5.1.33. 2-{[(1-Hydroxycyclohexyl)methyl]amino}-1-(1-isopropyl-6-methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethanone oxalate (5r)

Compound **5r** was synthesized in a manner similar to that for compound **5a**. Compound **5r** was obtained at a yield of 60% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.07–7.15 (1H, m), 6.71–6.83 (2H, m), 5.03 (major rotamer 1H, d, J = 9.3 Hz), 4.54 (minor rotamer 1H, d, J = 9.7 Hz), 4.08–4.22 (1H, m), 3.95–4.05 (1H, m), 3.73 (3H, s), 3.51–3.70 (major rotamer 2H, m), 3.29–3.38 (minor rotamer 1H, m), 2.82–2.98 (4H, m), 1.88–2.04 (1H, m), 1.19–1.63 (10H, m), 0.83–0.97 (6H, m); FAB MS m/z 375 [M+H]$^+$; Anal. Calcd for $C_{22}H_{34}N_2O_3{\cdot}C_2H_2O_4$: C, 62.05; H, 7.81; N, 6.03. Found: C, 61.96; H, 7.91; N, 6.05.

5.1.34. 2-{[(1-Hydroxycyclohexyl)methyl]amino}-(1*R*)-(1-isopropyl-6-methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethanone oxalate ((*R*)-5r)

Compound (*R*)-**5r** was synthesized in a manner similar to that for compound **5r**, except that (*R*)-**4r** was used instead of **4r**. Compound (*R*)-**5r** was obtained at a yield of 64% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.07–7.14 (1H, m), 6.72–6.82 (2H, m), 5.03 (major rotamer 1H, d, J = 9.3 Hz), 4.25 (minor rotamer 1H, d, J = 9.5 Hz), 4.07–4.23 (1H, m), 3.94–4.06 (1H, m), 3.73 (3H, s), 3.50–3.70 (major rotamer 2H, m), 3.28–3.39 (minor rotamer 1H, m), 2.79–3.00 (4H, m), 1.88–2.06 (1H, m), 1.18–1.66 (10H, m), 0.81–1.07 (6H, m); HPLC (Chiralpak OD-H [0.46 cm I.D. × 25 cm], hexane/ethanol/diethylamine = 90:10:0.1 flow rate 0.5 mL/min, column temp: 40 °C UV: 230 nm): retention time: tr = 14.96 min (major), ts = 11.76 min (minor); 98.4% ee. FAB MS m/z 375 [M+H]$^+$; Anal. Calcd for $C_{22}H_{34}N_2O_3{\cdot}C_2H_2O_4$: C, 62.05; H, 7.81; N, 6.03. Found: C, 61.80; H, 7.83; N, 5.96. $[\alpha]_D^{26}$ +31.8°(c 0.1, MeOH).

(R)-4r was prepared via the following procedure: To a solution of 4r (12.7 g) in 2-propanol (150 mL) was added (2*S*)-mandelic acid (9.20 g) and a small portion of the seed crystal. After stirring for 30 min, the precipitate was filtered off to obtain rough crystal (75% ee). Further recrystallization using 2-propanol (150 mL) was repeated three times to furnish enantiomerically pure (1*R*)-isopropyl-6-methoxy-1,2,3,4-tetrahydroisoquinoline (2*S*)-mandelate (6.11 g, 28%, >98% ee) as a colorless solid. ^{1}H NMR (DMSO-d_6): δ 7.37 (2H, d, J = 7.4 Hz), 7.26 (2H, t, J = 7.7 Hz), 7.18 (1H, t, J = 7.3 Hz), 7.13 (1H, d, J = 8.6 Hz), 6.78 (1H, dd, J = 2.7, 8.6 Hz), 6.72 (1H, d, J = 2.5 Hz), 4.68 (1H, s), 4.13 (1H, d, J = 4.0 Hz), 3.73 (3H, s), 3.23–3.35 (1H, m), 2.82–3.01 (2H, m), 2.67–2.79 (1H, m), 2.24–2.37 (1H, m), 0.99 (3H, d, J = 7.0 Hz), 0.73 (3H, d, J = 6.8 Hz); HPLC (Chiralpak AD-RH [0.46 cm I.D. × 15 cm], acetonitrile: 20 mM borate buffer (pH 9.0) = 40/60 flow rate 0.5 mL/min, column temp: 40 °C UV: 210 nm): retention time: tr = 18.31 min (major), ts = 14.30 min (minor); >98% ee. FAB MS m/z 206 [M+H]$^+$.

5.1.35. 2-{[(1-Hydroxycyclohexyl)methyl]amino}-(1*S*)-(1-isopropyl-6-methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethanone oxalate ((*S*)-5r)

Compound (*S*)-**5r** was synthesized in a manner similar to that for compound **5r**, except that (*S*)-**4r** was used instead of **4r**. Compound (*S*)-**5r** was obtained at a yield of 70% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.04–7.14 (1H, m), 6.72–6.82 (2H, m), 5.03 (major rotamer 1H, d, J = 9.3 Hz), 4.25 (minor rotamer 1H, d, J = 9.4 Hz), 4.06–4.23 (1H, m), 3.93–4.05 (1H, m), 3.73 (3H, s), 3.51–3.70 (major rotamer 2H, m), 3.28–3.38 (minor rotamer 1H, m), 2.79–3.00 (4H, m), 1.88–2.07 (1H, m), 1.18–1.65 (10H, m), 0.82–1.07 (6H, m); HPLC (Chiralpak OD-H [0.46 cm I.D. × 25 cm], hexane/ethanol/diethylamine = 90:10:0.1 flow rate 0.5 mL/min, column temp: 40 °C UV: 230 nm): retention time: ts = 11.76 min (major), tr = 14.96 min (minor). FAB MS m/z 375 [M+H]$^+$; Anal. Calcd for $C_{22}H_{34}N_2O_3{\cdot}C_2H_2O_4$: C, 62.05; H, 7.81; N, 6.03. Found: C, 61.83; H, 7.86; N, 6.00. $[\alpha]_D^{23}$ −24.3°(c 0.1, MeOH).

(*S*)-**4r** was prepared in a manner similar to that for (*R*)-**4r**, except that (2*R*)-mandelic acid was used instead of (2*S*)-mandelic acid. Compound (*S*)-**4r** was obtained at a yield of 29% as a colorless solid. ^{1}H NMR (DMSO-d_6): δ 7.37 (2H, d, J = 7.1 Hz), 7.24–7.28 (2H, m), 7.17–7.21 (1H, m), 7.13 (1H, d, J = 8.6 Hz), 6.78 (1H, dd, J = 2.8, 8.7 Hz), 6.71 (1H, d, J = 2.6 Hz), 4.70 (1H, s), 4.08 (1H, d, J = 4.1 Hz), 3.72 (3H, s), 3.24–3.30 (1H, m), 2.83–2.97 (2H, m), 2.68–2.75 (1H, m), 2.26–2.34 (1H, m), 1.00 (3H, d, J = 7.0 Hz), 0.72 (3H, d, J = 6.9 Hz); HPLC (Chiralpak AD-RH [0.46 cm I.D. × 15 cm], acetonitrile: 20 mM borate buffer (pH 9.0) = 40/60 flow rate 0.5 mL/min, column temp: 40 °C UV: 210 nm): retention time: ts = 14.30 min (major), tr = 18.31 min (minor); >99% ee. ESI MS m/z 206 [M+H]$^+$.

5.1.36. 2-{[(1-Hydroxycyclohexyl)methyl]amino}-1-(1-isopropyl-7-methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethanone oxalate (5s)

Compound **5s** was synthesized in a manner similar to that for compound **5a**. Compound **5s** was obtained at a yield of 62% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.06–7.16 (1H, m), 6.74–6.86 (2H, m), 5.08 (major rotamer 1H, d, J = 9.2 Hz), 4.30 (minor rotamer 1H, d, J = 9.4 Hz), 4.07–4.22 (1H, m), 3.93–4.03 (1H, m), 3.74 (major

rotamer 3H, s), 3.73 (minor rotamer 3H, s), 3.61–3.70 (major rotamer 1H, m), 3.51–3.60 (major rotamer 1H, m), 3.29–3.12 (minor rotamer 1H, m), 2.79–2.94 (4H, m), 1.92–2.06 (1H, m), 1.19–1.63 (10H, m), 0.83–0.98 (6H, m); FAB MS *m*/*z* 375 $[M+H]^+$.

5.1.37. 2-{[(1-Hydroxycyclohexyl)methyl]amino}-1-(1-isopropyl-8-methoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethanone oxalate (5t)

Compound **5t** was synthesized in a manner similar to that for compound **5a**. Compound **5t** was obtained at a yield of 56% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.16–7.24 (1H, m), 6.78–6.92 (2H, m), 5.58 (major rotamer 1H, d, *J* = 9.8 Hz), 4.30 (minor rotamer 1H, d, *J* = 9.6 Hz), 4.00–4.17 (1H, m), 3.95 (major rotamer 1H, d, *J* = 16.3 Hz), 3.85 (minor rotamer 1H, d, *J* = 16.1 Hz), 3.81 (minor rotamer 3H, s), 3.78 (major rotamer 3H, s), 3.68–3.79 (1H, m), 3.36–3.52 (1H, m), 2.98–3.13 (1H, m), 2.80–2.96 (3H, m), 2.05–2.18 (minor rotamer 1H, m), 1.90–2.03 (1H, m), 1.17–1.65 (10H, m), 0.98 (minor rotamer 3H, d, *J* = 6.8 Hz), 0.93 (major rotamer 3H, d, *J* = 6.8 Hz), 0.81 (minor rotamer 3H, d, *J* = 6.7 Hz), 0.78 (major rotamer 1H, d, *J* = 6.6 Hz); FAB MS *m*/*z* 375 $[M+H]^+$.

5.1.38. 2-{[(1-Hydroxycyclohexyl)methyl]amino}-1-(1-isopropyl-8-methyl-3,4-dihydroisoquinolin-2(1*H*)-yl)ethanone oxalate (5n)

1-(5-Bromo-1-isopropyl-8-methyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[(1-hydroxycyclohexyl)methyl]amino}ethanone was synthesized in a manner similar to that for compound **5a**. 1-(5-Bromo-1-isopropyl-8-methyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[(1-hydroxycyclohexyl)methyl]amino}ethanone was obtained at a yield of 63% as a brown oil. FAB MS *m*/*z* 437 $[M]^+$.

To a mixture of 1-(5-Bromo-1-isopropyl-8-methyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[(1-hydroxycyclohexyl)methyl]amino}ethanone (116 mg) and triethylamine (0.05 mL) in ethanol (10 mL) was added 10% palladium on charcoal (10 mg) under an argon atmosphere. The mixture was stirred at room temperature under a hydrogen atmosphere for 6 h. After the mixture was filtered through a celite pad, the filtrate was concentrated in vacuo. The residue was dissolved with chloroform, washed with 1 M aqueous sodium hydroxide, dried over magnesium sulfate, and then concentrated in vacuo. The residue was purified by column chromatography, eluting with chloroform–methanol to give a pale yellow oil (85 mg). The oil was dissolved in 2-propanol (0.8 mL). Oxalic acid (23 mg) and ether (5 mL) were added to the solution. The precipitate was filtered off to obtain compound **5n** (66 mg, 56%) as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 6.99–7.20 (3H, m), 5.46 (major rotamer 1H, d, *J* = 10.4 Hz), 4.41 (minor rotamer 1H, d, *J* = 10.2 Hz), 4.19 (minor rotamer 1H, d, *J* = 15.8 Hz), 4.05 (major rotamer 1H, d, *J* = 16.5 Hz), 3.90–4.00 (1H, m), 3.81 (1H, t, *J* = 9.0 Hz), 3.48–3.59 (minor rotamer 1H, m), 3.32–3.43 (1H, m), 3.10–3.23 (1H, m), 2.82–2.97 (3H, m), 2.36 (minor rotamer 3H, s), 2.29 (major rotamer 3H, s), 1.94–2.07 (1H, m), 1.20–1.64 (10H, m), 1.03 (minor rotamer 3H, d, *J* = 6.7 Hz), 0.99 (major rotamer 3H, d, *J* = 6.6 Hz), 0.74–0.84 (3H, m); ESI MS *m*/*z* 359 $[M+H]^+$.

5.1.39. 1-(8-Fluoro-1-isopropyl-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-{[(1-hydroxycyclohexyl)methyl]amino}ethanone oxalate (5q)

Compound **5q** was synthesized in a manner similar to that for compound **5n**. Compound **5q** was obtained at a yield of 45% as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.26–7.32 (1H, m), 7.04–7.14 (2H, m), 5.44 (major rotamer 1H, d, *J* = 9.9 Hz), 4.49 (minor rotamer 1H, d, *J* = 10.0 Hz), 4.14–4.23 (minor rotamer 1H, m), 4.09 (1H, d, *J* = 16.3 Hz), 3.97 (1H, d, *J* = 16.4 Hz), 3.34–3.92 (2H, m), 2.78–3.16 (4H, m), 1.95–2.18 (1H, m), 1.18–1.63 (10H, m), 0.99 (minor rotamer 3H, d, *J* = 6.8 Hz), 0.96 (major rotamer 3H, d, *J* = 6.6 Hz), 0.88 (minor rotamer 3H, dd, *J* = 3.1, 6.6 Hz), 0.84 (major rotamer 3H, dd, *J* = 2.9, 6.7 Hz); ESI MS *m*/*z* 363 $[M+H]^+$.

5.1.40. 2-(2-Benzyl-1,2,3,4-tetrahydroisoquinolin-1-yl)propan-2-ol (7)

To a mixture of compound **6** (as a hydrochloride salt 4.98 g) and benzaldehyde (2.72 g) in acetic acid (50 mL) was added sodium triacetoxyborohydride (6.11 g) at 4 °C. After stirring at room temperature for 15 h, the mixture was basified with 1 M aqueous sodium hydroxide solution, extracted with chloroform, dried over magnesium sulfate and concentrated in vacuo. The residue was purified by column chromatography, eluting with hexane-ethyl acetate to obtain 2-benzyl-1,2,3,4-tetrahydroisoquinoline-1-carboxylate (1.99 g, 33%) as a colorless oil. ESI MS *m*/*z* 297 $[M+H]^+$. To a solution of 2-benzyl-1,2,3,4-tetrahydroisoquinoline-1-carboxylate (1.99 g) in tetrahydrofuran (20 mL) was added a solution of 1.04 M methyl lithium in hexane (16.2 mL) at −78 °C under an argon atmosphere. After stirring at −78 °C for 30 min, the mixture was warmed to 0 °C and stirred for 1 h. The mixture was then cooled back to −78 °C, and a solution of 1.04 M methyl lithium in hexane (3.24 mL) was added to the mixture. After stirring at −78 °C for a further 30 min, the mixture was warmed to 0 °C and stirred for 1 h. The reaction was then quenched by the addition of water, extracted with ethyl acetate, washed with brine, dried over magnesium sulfate and then concentrated in vacuo. The residue was purified by column chromatography, eluting with hexane-ethyl acetate to obtain compound **6** (1.27 g, 67%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.24–7.39 (5H, m), 7.11–7.23 (4H, m), 4.36 (1H, br s), 3.98 (1H, d, *J* = 13.2 Hz), 3.76 (1H, d, *J* = 13.2 Hz), 3.68 (1H, s), 3.26–3.35 (1H, m), 2.77–2.87 (1H, m), 2.66–2.75 (1H, m), 2.57–2.65 (1H, m), 1.37 (3H, s), 0.97 (3H, s); ESI MS *m*/*z* 283 $[M+H]^+$.

5.1.41. 1-(2-Methoxypropan-2-yl)-1,2,3,4-tetrahydroisoquinoline (4c)

To a suspension of sodium hydride (60% dispersion in mineral oil, 199 mg) in tetrahydrofuran (5 mL) was added dropwise a solution of 2-(2-benzyl-1,2,3,4-tetrahydroisoquinolin-1-yl)propan-2-ol (1.27 g) in THF (7 mL) at 4 °C and stirred at room temperature for 30 min. Methyl iodide (0.42 mL) was then added to the mixture at 4 °C. After stirring at room temperature for 8 h, additional sodium hydride (60% dispersion in mineral oil, 199 mg) and methyl iodide (0.42 mL) were added to the mixture, followed by stirring at room temperature for 12 h. The reaction was quenched by the addition of water, extracted with ethyl acetate, washed with brine, dried over magnesium sulfate, and then concentrated in vacuo. The residue was purified by column chromatography, eluting with hexane–ethyl acetate to obtain 2-benzyl-1-(2-methoxypropan-2-yl)-1,2,3,4-tetrahydroisoquinoline (1.17 g, 88%) as a pale brown oil. ESI MS *m*/*z*: 296 $[M+H]^+$. To a mixture of 2-benzyl-1-(2-methoxypropan-2-yl)-1,2,3,4-tetrahydroisoquinoline (1.17 g) and in methanol (12 mL) was added 10% palladium on charcoal (300 mg) under an argon atmosphere. The mixture was stirred at room temperature under a hydrogen atmosphere for 8 h. The mixture was filtered through a celite pad, and the filtrate was concentrated in vacuo to obtain compound **4c** (770 mg, 95%) as a yellow gum. ^{1}H NMR ($CDCl_3$): δ 7.39–7.48 (1H, m), 7.03–7.20 (3H, m), 4.23 (1H, s), 3.25–3.38 (4H, m), 2.80–2.92 (2H, m), 2.61–2.70 (1H, m), 1.16 (3H, s), 1.12 (3H, s); ESI MS *m*/*z* 206 $[M+H]^+$.

5.1.42. 2-{[(1-Hydroxycyclohexyl)methyl]amino}-1-[1-(2-methoxypropan-2-yl)-3,4-dihydroisoquinolin-2(1*H*)-yl]ethanone oxalate (5c)

Compound **5c** was synthesized in a manner similar to that for compound **5a**. Compound **5c** was obtained at a yield of 69% as a

colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.29 (1H, d, J = 7.1 Hz), 7.11–7.24 (3H, m), 5.44 (major rotamer 1H, s), 4.63 (minor rotamer 1H, s), 4.28–4.38 (minor rotamer 1H, m), 4.15 (1H, d, J = 16.2 Hz), 3.98–4.10 (1H, m), 3.89–3.78 (1H, m), 3.60–3.70 (1H, m), 3.50–3.59 (1H, m), 3.07 (minor rotamer 3H, s), 3.04 (major rotamer 3H, s), 2.78–3.02 (4H, m), 1.30–1.64 (9H, m), 1.25 (major rotamer 3H, s), 1.21 (minor rotamer 3H, s), 1.17 (minor rotamer 3H, s), 1.12 (major rotamer 3H, s); FAB MS m/z 375 $[M+H]^+$.

5.1.43. 2-(1,2,3,4-Tetrahydroisoquinolin-1-yl)propan-2-yl pivalate (9)

To a mixture of compound **8** (3.0 g) and tetramethylethylenediamine (2.2 mL) in tetrahydrofuran (40 mL) was added to a solution of 1.64 M *tert*-butyllithium in *n*-pentane (12 mL) at −78 °C under an argon atmosphere. After stirring at −78 °C for 10 min, acetone (1.8 mL) was added to the mixture and stirred at −78 °C for 1 h. The reaction was quenched by the addition of acetic acid (2 mL), warmed to room temperature, and then concentrated in vacuo. The residue was dissolved with ethyl acetate and washed with water, aqueous 5% citric acid solution, saturated aqueous sodium bicarbonate, and brine. The resulting mixture was dried over magnesium sulfate and concentrated in vacuo. The residue was purified by column chromatography, eluting with hexane–ethyl acetate to give 1-[1-(2-hydroxypropan-2-yl)-3,4-dihydroisoquinolin-2(1*H*)-yl]-2,2-dimethylpropan-1-one (3.45 g, 68%) as a colorless solid. FAB MS m/z: 276 $[M+H]^+$.

Trifluoroacetic acid (2.9 mL) was combined with 1-[1-(2-hydroxypropan-2-yl)-3,4-dihydroisoquinolin-2(1*H*)-yl]-2,2-dimethylpropan-1-one (300 mg) and stirred at room temperature for 4 h. The mixture was concentrated in vacuo, basified with saturated aqueous sodium bicarbonate, extracted with ethyl acetate, washed with water, and dried over magnesium sulfate. The resulting solution was concentrated in vacuo to obtain compound **8** (294 mg 98%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.34 (1H, d, J = 7.3 Hz), 7.06–7.20 (3H, m), 4.60 (1H, br s), 3.25–3.35 (1H, m), 2.83–2.99 (2H, m), 2.63–2.74 (1H, m), 1.53 (3H, s), 1.45 (3H, s), 1.15 (9H, br s); FAB MS m/z 276 $[M+H]^+$.

5.1.44. 2-{[(1-Hydroxycyclohexyl)methyl]amino}-1-[1-(2-hydroxypropan-2-yl)-3,4-dihydroisoquinolin-2(1*H*)-yl]ethanone oxalate (5d)

2-(2-{*N*-[(1-Hydroxycyclohexyl)methyl]glycyl}-1,2,3,4-tetrahydroisoquinolin-1-yl)propan-2-yl pivalate was synthesized in a manner similar to that for compound **5a**. 2-(2-{*N*-[(1-hydroxycyclohexyl)methyl]glycyl}-1,2,3,4-tetrahydroisoquinolin-1-yl)propan-2-yl pivalate was obtained at a yield of 81% as a colorless oil. APCI MS m/z 445 $[M+H]^+$.

To a solution of 2-(2-{*N*-[(1-hydroxycyclohexyl)methyl]glycyl}-1,2,3,4-tetrahydroisoquinolin-1-yl)propan-2-yl pivalate (1.43 g) in dichloromethane (15 mL) was added a solution of 1.01 M diisobutyl aluminum hydride in hexane (9.55 mL) at −78 °C under an argon atmosphere. After stirring at −78 °C for 5 h, the mixture was warmed to 0 °C for 2 h. The reaction was then quenched by the addition of saturated aqueous potassium sodium tartrate solution, stirred for 20 min and filtered through a celite pad. The filtrate 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 a colorless oil (118 mg), which was dissolved in ethanol (4 mL). Oxalic acid (32 mg) was added to the solution and concentrated in vacuo. The residue was washed with acetonitrile and filtered off to obtain compound **5d** (120 mg, 8%) as a colorless solid. ^{1}H NMR (DMSO-d_6): This compound exists as a pair of rotamers at room temperature. δ 7.25–7.34 (1H, m), 7.24–7.10 (2H, m), 5.30 (major rotamer 1H, s), 4.56 (minor rotamer 1H, s), 4.22–4.32 (minor rotamer 1H, m), 4.08–4.20 (1H, m), 3.91–4.07 (2H, m), 3.53–3.70 (1H, m), 3.07–3.19 (major rotamer 1H, m), 2.77–2.94 (minor rotamer 1H, m), 2.77–2.94 (3H, m), 1.07–1.66 (16H, m); FAB MS m/z 361 $[M+H]^+$.

5.2. Pharmacology

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

Details of experimental procedures were as previously described.[6] IC_{50} values were determined in duplicate in one experiment. IC_{50} values and 95% confidence intervals were calculated using Sigmoid-Emax nonlinear regression analysis with SAS software (Cary, NC, USA).

5.2.2. Evaluation of inhibitory activity against hERG potassium channels using an in vitro Rb Efflux assay in Chinese hamster ovary (CHO) cells

The inhibitory activities of (*R*)-**5r** against hERG potassium channels were evaluated using an in vitro Rb efflux assay of Chinese hamster ovary (CHO) cells. Details of experimental procedures were as previously described.[6]

5.2.3. Measurement of CYP inhibition

The inhibitory activities of test compounds against CYP1A2, 2C9, 2C19, and 2D6 were determined using fluorescence substrates. Details of experimental procedures were as previously described.[6] For the CYP3A4 inhibition assay, midazolam was used as a probe substrate to monitor the changes in CYP3A4 activity during the exposure to each test compound. Details of experimental procedures were also as previously described.[6] Residual metabolic activities for reversible (Eq. 1 and time-dependent (Eq. 2 inhibition were calculated using the following equations:

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

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

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.

5.2.4. In vitro DDI assay for CYP 2D6

Dextromethorphan was used as a probe substrate for CYP2D6. Reaction mixtures containing 100 mM Na^+–K^+ phosphate buffer (pH 7.4), 0.4 mg protein/mL human liver microsomes, 1 mM NADPH, 0.1 mM EDTA, and 0.3–100 μM of test compounds were prepared and pre-incubated for 0 or 30 min at 37 °C. Reactions were initiated by the addition of 7 μM of dextromethorphan and incubated for an additional 20 min. Reactions were terminated by the addition of 80% acetonitrile with internal standard. The metabolite of dextromethorphan, dextrophan, was measured by LC–MS/MS, and IC_{50} values were determined for 0 (reversible inhibition) and 30 minutes (time-dependent inhibition) pre-incubation.

5.2.5. In vitro metabolic stability in human liver microsomes

Reaction mixtures containing 100 mM Na^+–K^+ phosphate buffer (pH 7.4), 0.2 mg protein/mL human liver microsomes, 1 mM NADPH and 0.1 mM EDTA were pre-incubated for 5 min at 37 °C. The reactions were initiated by the addition of test compound solution. The final concentration of test compound was 0.2 μM. After 0, 15, 30 and 45 min incubations at 37 °C, reactions were terminated by the addition of acetonitrile with internal standard. The reaction mixtures were centrifuged and supernatants injected into the LC–MS/MS system to determine the residual ratio of the test compound.

5.2.6. Calculation of in vitro intrinsic clearance

In vitro intrinsic clearance (CLint, in vitro) was calculated using Eq. 3, which is based on the time course of the residual ratio of the test compounds, as determined using least-squares linear regression as follows:[22]

$$\text{CLint, in vitro } (\mu\text{L/min/mg protein}) = k_e/\text{microsomal protein concentration} \quad (3)$$

where k_e denotes the disappearance rate constant.

5.2.7. Animal experiments

Male ddY mice or Sprague-Dawley rats (SLC, Hamamatsu, Japan) were used for in vivo experiments. Animals were group-housed and kept on a 12-hour 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.

We examined the antinociceptive effect of compound (*R*)-**5r** in mice formalin test previously published by Hunskaar et al.[23] with slight modifications. Briefly, 2% formalin (20 μL) was subcutaneously injected 15 min after intrathecal bolus administration of compound (*R*)-**5r**. The summation of time spent in lifting/licking of the paw that received injections was measured. The duration of responses in the first 10 min and that from 15 to 25 min represent the first and second phases, respectively. In addition, the efficacy of compound (*R*)-**5r** in neuropathic pain was evaluated in rat SNL model reported by Chung et al.[19] Effects of compound (*R*)-**5r** on motor coordination was assessed via the rotarod test. Details of the experimental procedures were as previously described.[24]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.05.030.

References and notes

1. (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.
2. (a) Saegusa, H.; Kurihara, T.; Zong, S.; Kazuno, A.; Matsuda, Y.; Nonaka, T.; Han, W.; Toriyama, H.; Tanabe, T. *EMBO J.* **2001**, *20*, 2349; (b) Kim, C.; Jun, K.; Lee, T.; Kim, S.; McEnery, M.; Chin, H.; Kim, H.; Park, J.; Kim, D.; Jung, S.; Kim, J.; Shin, H. *Mol. Cell. Neurosci.* **2001**, *18*, 235; (c) Hatakeyama, S.; Wakamori, M.; Ino, M.; Miyamoto, N.; Takahashi, E.; Yoshinaga, T.; Sawada, K.; Imoto, K.; Tanaka, I.; Yoshizawa, T.; Nishizawa, Y.; Mori, Y.; Niidome, T.; Shoji, S. *NeuroReport* **2001**, *12*, 2423.
3. Schmidtkco, A.; Lötsch, J.; Freynhagen, R.; Geisslinger, G. *Lancet* **2010**, *375*, 1569.
4. (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.
5. (a) 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; (b) Shao, P. P. *ACS Med. Chem. Lett.* **2013**, *1064*, 4; (c) 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; (d) 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.
6. Ogiyama, T.; Inoue, M.; Honda, S.; Yamada, H.; Watanabe, T.; Kiso, T.; Goto, T.; Koakutsu, A.; Kakimoto, S.; Shishikura, J. *Bioorg. Med. Chem.* **2014**, *22*, 6899.
7. (a) Zhou, S.-F.; Liu, J.-P.; Lai, X.-S. *Curr. Med. Chem.* **2009**, *16*, 2661; (b) Zhou, S.-F. *Curr. Pharm. Des.* **2008**, *14*, 990.
8. (a) Bockbrader, H. N.; Wesche, D.; Miller, R.; Chapel, S.; Janiczek, N.; Burger, P. *Clin. Pharmacokinet.* **2010**, *49*, 661; (b) Spina, E.; Trifiro, G.; Caraci, F. *CNS Drugs* **2012**, *26*, 39; (c) Wernicke, J. F.; Gahimer, J.; Yalcin, I.; Wulster-Radcliff, M.; Viktrup, L. *Expert Opin. Drug Saf.* **2005**, *4*, 987; (d) Shin, J.-G.; Park, J.-Y.; Kim, M.-J.; Shon, J.-H.; Yoon, Y.-R.; Cha, I.-J.; Lee, S.-S.; Oh, S.-W.; Kim, S.-W.; Flockhart, D. A. *Drug Metab. Dispos.* **2002**, *30*, 1102.
9. Larsen, R.; Reamer, R.; Corley, E.; Davis, P.; Grabowski, E.; Reider, P.; Shinkai, I. *J. Org. Chem.* **1991**, *56*, 6034.
10. The synthesis of compound **4e** and **4f**: (a) Seebach, D.; Lohmann, J.-J.; Syfrig, M. A.; Yoshifuji, M. *Tetrahedron* **1983**, *39*, 1963; The synthesis of compound **4i**: (b) Kitabatake, M.; Nagai, J.; Abe, K.; Tsuchiya, Y.; Ogawa, K.; Yokoyama, T.; Mohri, K.; Taguchi, K.; Horiguchi, Y. *Eur. J. Med. Chem.* **2009**, *44*, 4034.
11. The synthesis of compound **4o**, **4r**–**4t**: Watanuki, S.; Matsuura, K.; Tomura, Y.; Okada, M.; Okazaki, T.; Ohta, M.; Tsukamoto, S. *Chem. Pharm. Bull.* **2011**, *59*, 1029.
12. The measured log*D* value at pH 7.4 of (*R*)-**5r** was 2.4, while the calculated one was 2.55.
13. (a) Kumar, S.; Sharma, R.; Roychowdhury, A. *Curr. Med. Chem.* **2012**, *19*, 3605; (b) Bourdonnec, B.; Leister, L. *Curr. Med. Chem.* **2009**, *16*, 3093; (c) de Groot, M. J.; Wakenhut, F.; Whitlock, G.; Hyland, R. *Drug Discovery Today* **2009**, *14*, 964.
14. (a) Fray, M. J.; Bish, G.; Fish, P. V.; Stobie, A.; Wakenhut, F.; Whitlock, G. A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4349; (b) Fish, P. V.; Fray, M. J.; Stobie, A.; Wakenhut, F.; Whitlock, G. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2022; (c) Hudson, S.; Kiankarimi, M.; Eccles, W.; Dwight, W.; Mostofi, Y. S.; Genicot, M. J.; Fleck, B. A.; Gogas, K.; Aparicio, A.; Wang, H.; Wen, J.; Wade, W. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4491; (d) Pontillo, J.; Wu, D.; Ching, B.; Hudson, S.; Genicot, M. J.; Gao, Y.; Ewing, T.; Fleck, B. A.; Gogas, K.; Aparicio, A.; Wang, H.; Wen, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6151; (e) Ishichi, Y.; Kimura, E.; Honda, E.; Yoshikawa, M.; Nakahata, T.; Terao, Y.; Suzuki, A.; Kawai, T.; Arakawa, Y.; Ohta, H.; Kanzaki, N.; Nakagawa, H.; Terauchi, J. *Bioorg. Med. Chem.* **2013**, *21*, 4600.
15. Ogiyama, T.; Yonezawa, K.; Inoue, M.; Katayama, N.; Watanabe, T.; Yoshimura, S.; Gotoh, T.; Kiso, T.; Koakutsu, A.; Kakimoto, S.; Shishikura, J. *Bioorg. Med. Chem.* **2015**, *23*, 4638.
16. Compound **1** exhibited a similar result among the racemate (IC_{50} = 0.77 μM) and the optically pure compounds ((*S*)-**1** IC_{50} = 1.0 μM and (*R*)-**1** IC_{50} = 1.5 μM). In addition, similar results were also reported by Zalicus scientists. See: Refs. 6,17a. Additional refferences are therein.
17. The reversible and time-dependent inhibitory effects of (*R*)-**5r** against CYP2D6 were scrutinized by the HLM-LC–MS assay using dextromethorphan as a probe substrate (details of experimental procedures are described in Section 5). Surprisingly, (*R*)-**5r** showed relatively weak inhibitory activity (IC_{50} = 37 μM) although the reason for the discrepancy in results between the fluorometric assay and HLM-LC–MS assay is not clear. In addition, (*R*)-**5r** had little effect on TDI of CYP2D6 (IC_{50} = 41 μM), suggesting little concern of CYP2D6 inhibition-mediated drug-drug interactions. Further evaluation is ongoing at present. Discussion about the disparity in inhibitory activities between the fluorometric assay and HLM-LC–MS assay reported by Wyeth (now part of Pfizer) scientists might be informative. See: Di, L.; Kerns, E. H.; Li, S. Q.; Carter, G. T. *Int. J. Pharm.* **2007**, *335*, 1.
18. Zalicus scientists also reported similar results using their small-molecule N-type calcium channel blockers. See: (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.
19. Kim, S. H.; Chung, J. M. *Pain* **1992**, *50*, 355.
20. Scott, D. A.; Wright, C. E.; Angus, J. A. *Eur. J. Pharmacol.* **2002**, *451*, 279.
21. Compound **2n** and **2q** were prepared as previously described. See: PCT Int. Appl. WO2008143263, 2008. Compound **2n** (as a HCl salt): ^{1}H NMR (DMSO-d_6): δ 8.27 (3H, br s), 7.49 (1H, d, *J* = 8.1 Hz), 7.21 (1H, d, *J* = 1.8 Hz), 7.04 (1H, dd, *J* = 2.1, 8.2 Hz), 2.92–3.06 (4H, m), 2.27 (3H, s); FAB MS *m*/*z* 214 $[M+H]^+$.

Compound **2q** (as a HCl salt): ^{1}H NMR (DMSO-d_6): δ 8.43 (3H, br s), 7.65 (1H, dd, J = 5.5, 8.9 Hz), 7.34 (1H, d, J = 3.2, 9.7 Hz), 7.13 (1H, dt, J = 3.3, 8.7 Hz), 2.99–3.14 (4H, m); FAB MS m/z 218 $[M+H]^+$.

22. Naritomi, Y.; Terashita, S.; Kimura, S.; Suzuki, A.; Kagayama, A.; Sugiyama, Y. *Drug Metab. Dispos.* **2001**, *29*, 1316.
23. Hunskaar, S.; Fasmer, O. B.; Hole, K. *J. Neurosci. Methods* **1985**, *14*, 69.
24. 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.