Design, Synthesis, and Evaluation of a Novel 4-Aminomethyl-4-fluoropiperidine as a T-Type Ca^{2+} Channel Antagonist

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Abstract: The novel T-type antagonist (*S*)-**5** has been prepared and evaluated in in vitro and in vivo assays for T-type calcium ion channel activity. Structural modification of the piperidine leads **1** and **2** afforded the fluorinated piperidine (*S*)-**5**, a potent and selective antagonist that displayed in vivo CNS efficacy without adverse cardiovascular effects.

Calcium is an important physiological signaling molecule, and control of intracellular calcium concentration is a tightly regulated process. Calcium ions (Ca^{2+}) can be released from intracellular stores or enter the cell via ligand-gated or voltage-gated calcium channels, initiating a wide range of physiological responses.[1] Historically, voltage-gated calcium channels have been divided by functional descriptors of the Ca^{2+} currents (L-type, N-type, T-type, etc.), but more recently molecular cloning of the critical pore forming α subunit has resulted in three main classes denoted $Ca_v1.x$ (L-type), $Ca_v2.x$ (N, P/Q, R-type), and $Ca_v3.x$ (T-type).[2] The $Ca_v3.x$ family has three members ($Ca_v3.1$, $Ca_v3.2$, and $Ca_v3.3$), and these channels activate at more negative membrane potential than members of the $Ca_v1.x$ and $Ca_v2.x$ families and produce smaller and more transient currents. They are widely expressed in CNS and peripheral tissues and may have potential for the treatment of various indications such as hypertension, epilepsy, pain, regulation of arousal states, tinnitus, and cancer.[3] In the brain, T-type channels are found in the thalamic and cortical regions where they have an important role in the function of the thalamocortical loop, a neural network structure implicated in absence epilepsy, arousal state, and movement disorders such as essential tremor.[4]

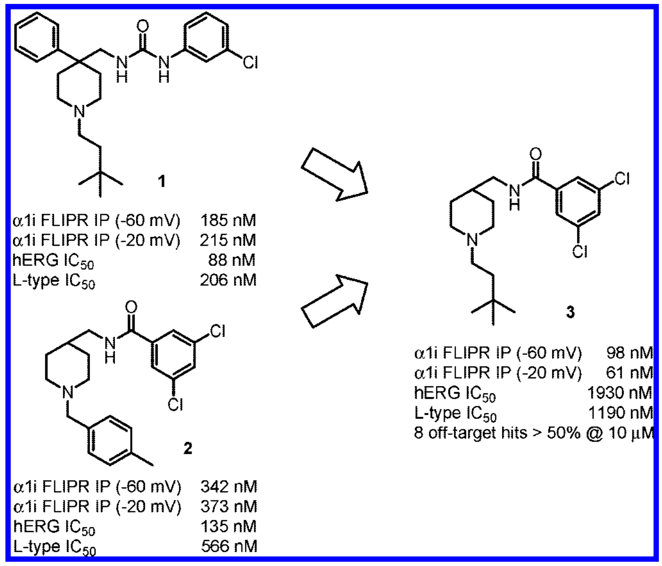

Figure 1. Profiles of 4-aminomethyl piperidine leads **1**−**3**.

Mibefradil, often described as the first selective T-type antagonist, demonstrated efficacy as an antihypertensive.[5] Shortly after its introduction, mibefradil was withdrawn from the U.S. market in 1998 because of drug−drug interactions,[6] but in lieu of other compounds, it remains the prototype T-type calcium channel blocker. Additionally, ethosuximide has T-type antagonist properties (among others) and is used to suppress absence seizures.[7] Potent and selective agents are needed to better elucidate the true functional role of T-type calcium channels in these and other disease states. With increasing knowledge of the localization, function, and properties of T-type calcium channels, there has been heightened activity for the discovery of novel inhibitors in recent years.[8] Here, we detail the discovery of a novel, orally bioavailable piperidine T-type calcium channel antagonist that evolved via optimization of piperidines **1** and **2** and its in vivo evaluation in cardiovascular and CNS models.

Screening efforts identified piperidines **1** and **2** as T-type Ca^{2+} channel antagonists (Figure 1). Their ability to block the $Ca_v3.3$ (α1I) channel was assessed using a high-throughput cell-based calcium flux assay (FLIPR[a]) at a membrane potential of −20 mV (depolarized) or −60 mV (hyperpolarized).[9] High-voltage activated Ca^{2+} channel antagonists can have preferential affinity for one state over another, and these properties can profoundly affect the efficacy and safety profiles of the compounds.[10] Although it is unknown whether these properties would impact the physiological effect of T-type antagonists, the state-dependent properties of all described compounds were also characterized by standard voltage-clamp electrophysiological assays. The compounds were counterscreened for activity on

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[a] Abbreviations: CSF, cerebrospinal fluid; DMF, dimethylformamide; ECG, electrocardiography; EEG, electroencephalography; FLIPR, fluorometric imaging plate reader; hERG, human ether-a-go-go-related gene; HOBt, *N*-hydroxybenzotriazole; HR, heart rate; MAP, mean arterial blood pressure; MP-BH(OAc)$_3$, macroporous polystyrene-bound triacetoxyborohydride; MP-carbonate, macroporous polystyrene-bound carbonate; PS-carbodiimide, polystyrene-bound carbodiimide; WAG/Rij, Wistar albino Glaxo/Rijswijk.

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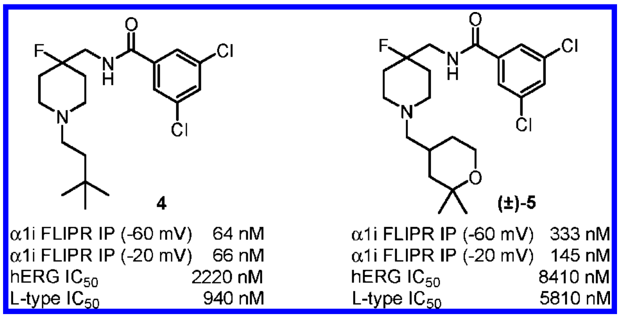
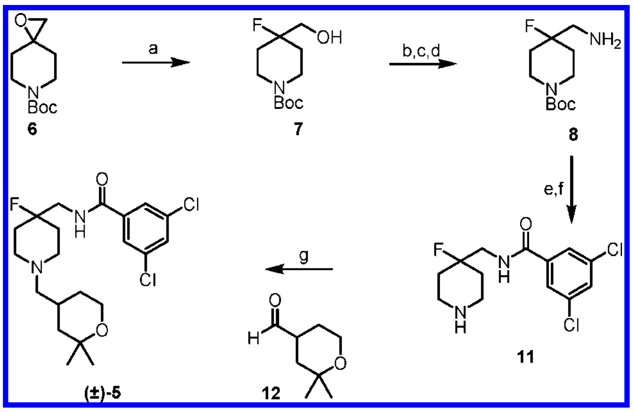


Figure 2. Profiles of piperidines **4** and **5**.

Scheme 1[a]



[a] Conditions: (a) 70% HF·pyridine, −10 °C → room temp; (b) *p*-TsCl, pyridine, 0 °C → room temp; (c) potassium phthalimide, DMF, 150 °C; (d) ethanolamine, 60 °C; (e) HOBt, 3,5-dichlorobenzoic acid, *i*-Pr_2EtN, PS-carbodiimide, then MP-carbonate; (f) 4 N HCl/dioxane, CH_2Cl_2; (g) **12**, MP-$BH(OAc)_3$, CH_2Cl_2.

the hERG K^+ and L-type Ca^{2+} channels using binding assays.[11,12] While **1** and **2** showed good T-type potency, a concern was the lack of selectivity observed with respect to the hERG and L-type ion channels. Blocking hERG or L-type ion channels could have unwanted cardiac effects for a CNS therapeutic agent; therefore, an early goal of the program was to improve this selectivity margin.

Initial SAR showed the neohexyl group of **1** and the 3,5-dichlorobenzoyl group of **2** to be preferred structural features from a potency viewpoint. Combining those structural elements of these leads about a 4-aminomethylpiperidine scaffold produced **3**, which showed improved T-type potency and selectivity with respect to the hERG and L-type ion channels. However, submission of the compound to a broad external screen revealed eight off-target activities with greater than 50% activity at 10 μM, reflecting its moderate selectivity versus other ion channels and GPCRs.

Fluorinated piperidine analogues were then prepared. The potential benefit of this strategy was 2-fold: (1) by reduction of the basicity of the ring nitrogen atom, the ancillary pharmacological profile might be improved; (2) the fluorinated piperidine ring might exhibit enhanced metabolic stability.[13] Compound **4**, bearing a fluorine atom at the 4-position, possessed a potency and selectivity profile similar to those of **3** (Figure 2), but improved selectivity was observed by maintaining this fluorine and varying the N-substituent. After extensive investigation, the tetrahydropyran (±)-**5** displayed the best improvement on the ion channel selectivity over analogues **3** and **4**.

The synthesis of (±)-**5** proceeded as outlined in Scheme 1. Regioselective epoxide opening of **6**[14] with HF·pyridine yielded the fluorinated alcohol **7**.[15] A straightforward three-step sequence was employed to convert **7** to primary amine **8**.[16] Amide **11** was obtained by carbodiimide-mediated coupling of **8** to 3,5-dichlorobenzoic acid and subsequent removal of the *tert*-butyloxycarbonyl protective group. Reductive amination with tetrahydropyranyl aldehyde **12**[17] provided the racemic tertiary amine **5**.

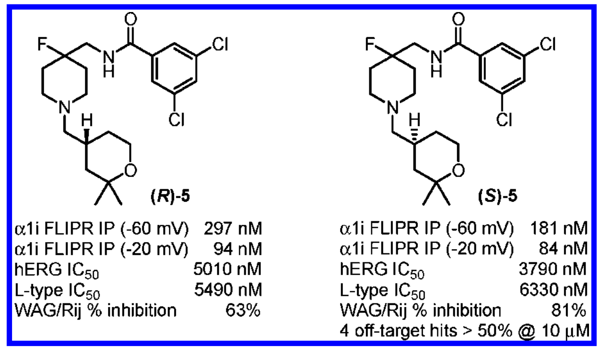


Figure 3. Profiles of piperidines (*R*)-**5** and (*S*)-**5**.

Racemic (±)-**5** was resolved by HPLC with a ChiralPak AD column so that the biological activities of the enantiomers could be individually evaluated. Little distinction could be made between (*R*)-**5** and (*S*)-**5**[18] on the basis of ion channel data (Figure 3), but (*S*)-**5** was consistently superior in a WAG/Rij rat model of absence epilepsy (vide infra) and was selected for full characterization.

In agreement with the profile observed in the calcium flux assays, the in vitro potencies of (*S*)-**5** determined in a standard voltage-clamp electrophysiological assay at membrane holding potentials of −80 and −100 mV (115 and 84 nM, respectively) confirmed that compounds in this piperidine series are state-independent antagonists. Similar electrophysiology experiments with $Ca_v3.1$ (α1G, 93 nM at −100 mV) and $Ca_v3.2$ (α1H, 196 nM at −100 mV) indicated that the compound potently inhibits all T-type family members.

The pharmacokinetic parameters for (*S*)-**5** following intravenous and oral doses in preclinical species are shown in Table 1. The compound exhibited significant species differences in clearance and volume of distribution but relatively short half-life (∼2−5 h) across species. The oral bioavailability of (*S*)-**5** was reasonably good (>35%) in all species.

P-Glycoprotein (P-gp) mediated transport of (*S*)-**5** was found to be minimal (B-A/A-B ≤ 2), and the compound showed good passive cellular permeability (P_{app} = 34 × 10^{-6} cm/s). Consistent with the in vitro data, (*S*)-**5** showed good brain penetration in rats (brain/plasma concentration ratio of 6.0 and CSF/unbound plasma ratio of 2.8 at 9 h after dosing), supporting its potential therapeutic use as a CNS agent.

To investigate the in vivo CNS activity of these compounds, we employed a genetic model of absence epilepsy using Wistar albino Glaxo rats bred in Rijswijk, The Netherlands (WAG/Rij). The WAG/Rij rats display cortical EEG patterns and physical behaviors characteristic of an epileptic condition, including frequent seizures.[19] Since T-type calcium channels are involved in the regulation of thalamocortical rhythms, measurement of EEG in these animals serves as a relevant pharmacodynamic readout of brain penetration and T-type channel activity. (*S*)-**5** exhibited a dose- and exposure-dependent decrease in the total seizure time during the 4 h period following oral dosing (Figure 4). Notably, (*S*)-**5** demonstrated robust CNS efficacy in this model at a dose of 10 mg/kg with a plasma level of 1 μM.

Another pathophysiological condition that may result from thalamocortical dysfunction is essential tremor;[20] therefore, (*S*)-**5**

Table 1. Pharmacokinetic Profile of (*S*)-**5**

			iv (in DMSO)			po (in 1% methylcellulose)			
species	plasma protein binding (% bound)	dose (mg/kg)	CL (mL/min/kg)	$t_{1/2}$ (h)	V_{dss} (L/kg)	C_{max} (μM)	T_{max} (h)	AUC_{0-24h} (μM·h)	*F* (%)
rat	94.3	1	75 ± 21	1.8 ± 0.1	10 ± 2				
		10				0.48 ± 0.12	0.7 ± 0.3	1.94 ± 0.43	36 ± 8
dog	99.0	0.5	2.3	4.7	1.0				
		1				2.18 ± 1.97	3.1 ± 4.3	15.8 ± 10.9	87 ± 64
monkey	97.5	0.5	13.6	3.3	3.8				
		2				0.58	1.5	3.2	44

was also evaluated in the rat harmaline model[21] of essential tremor. Harmaline-induced tremor activity in the 6−12 Hz range was monitored and quantified to assess tremor suppression. (*S*)-**5** was found to significantly reduce tremor activity in a dose-dependent manner (Figure 5), suggesting a possible role for T-type antagonists in the treatment of movement disorders such as essential tremor.

To gain further insight into potential ancillary pharmacology, (*S*)-**5** was submitted to a large external screen, which revealed only four off-target activities with greater than 50% activity at 10 μM. Most prominent was σ receptor binding (<100 nM), but follow-up in a vas deferens functional assay[22] revealed only weak antagonist activity (30% at 30 μM). Also noted was sodium channel site 2 binding (IC_{50} = 1.9 μM), but voltage clamp studies with the $Na_v1.5$ sodium channel showed an IC_{50} of 84 μM.[23] (*S*)-**5** also showed little inhibition of $Ca_v2.2$ in a counterscreen (18% inhibition at 10 μM).[24] hERG potassium channel binding was also evident in the external screen (IC_{50} = 2.9 μM), so we examined the effect of compound on cardiovascular function in a conscious dog model.[25] Three dogs were infused intravenously with (*S*)-**5** first at a rate of 1.29 (mg/kg)/60 min and then 4.30 (mg/kg)/60 min, resulting in mean plasma levels of 1.5 ± 0.3 μM at the end of the first infusion and 4.6 ± 1.7 μM at the end of the second. No behavioral effects were noted during the study, and there were no significant changes in mean arterial blood pressure (Figure 6), heart rate (Figure 7), or ECG intervals (PR, QRS, QT, and QTc).

Since T-type calcium channels are present in renal vascular and tubular tissues,[26] (*S*)-**5** was evaluated for side effects on renal function. Four dogs were administered a single oral dose (20 mg/kg) of (*S*)-**5** and observed for 3 h after treatment.[27] No effects were observed on glomerular filtration rate, effective

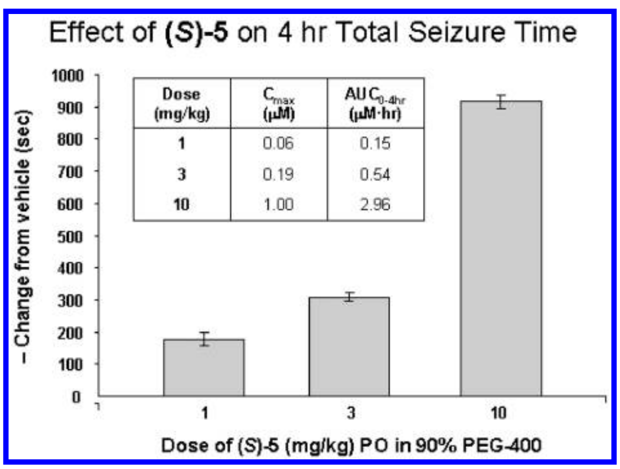


Figure 4. Dose−response of (*S*)-**5** in WAG/Rij rat model of absence epilepsy, $n \geq 6$ rats per dose with PK taken from satellite animals, n = 3 per group except 3 mg/kg group where n = 1.

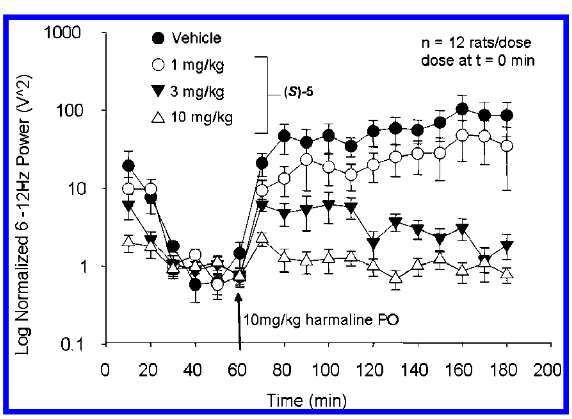


Figure 5. Dose−response of (*S*)-**5** in harmaline-induced rat model of essential tremor.

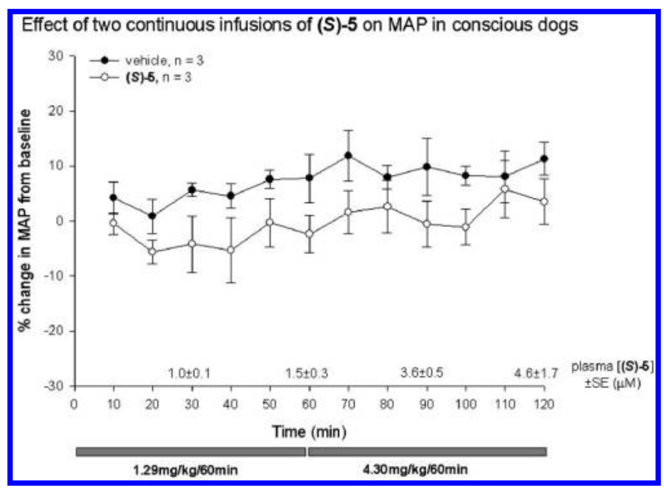


Figure 6. Effect of (*S*)-**5** on mean arterial blood pressure (MAP) in conscious dogs.

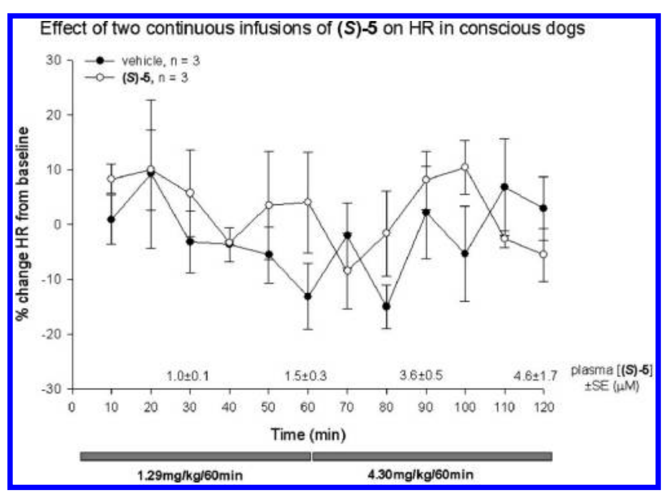


Figure 7. Effect of (*S*)-**5** on heart rate (HR) in conscious dogs.

renal plasma flow, urine flow, urinary excretion of sodium and potassium, or plasma electrolytes. While blood pressure and ECG intervals were unaffected in this assay, increased heart rate and minor behavioral changes (panting) were observed. Peak plasma concentrations of (*S*)-**5** were 34.5 ± 11.0 μM during the experiment.

In conclusion, we identified (*S*)-**5**, a potent and selective T-type calcium channel antagonist, starting from 1,4-disubstituted piperidine HTS leads. The introduction of a fluorine atom at the 4-position served to improve the ancillary pharmacological profile and to enhance metabolic stability. Robust efficacy was seen in WAG/Rij epilepsy and harmaline-induced tremor models at plasma levels well below the no effect level seen in cardiovascular dog experiments. This suggests a good margin between CNS and peripheral effects of selective T-type calcium channel antagonists. Compounds such as (*S*)-**5** hold promise for the treatment of a diverse set of neurological indications without adversely affecting cardiovascular function.

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Supporting Information Available: Spectral and analytical data for new compounds, a scheme detailing the preparation of **5**, FLIPR assay protocol, $Na_v1.5$ electrophysiology measurements, dog renal function assay protocol, and expanded references. This material is available free of charge via the Internet at http://pubs.acs.org.

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