Discovery of 1,4-Substituted Piperidines as Potent and Selective Inhibitors of T-Type Calcium Channels

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The discovery of a novel series of potent and selective T-type calcium channel antagonists is reported. Initial optimization of high-throughput screening leads afforded a 1,4-substituted piperidine amide **6** with good potency and limited selectivity over hERG and L-type channels and other off-target activities. Further SAR on reducing the basicity of the piperidine and introducing polarity led to the discovery of 3-axial fluoropiperidine **30** with a significantly improved selectivity profile. Compound **30** showed good oral bioavailability and brain penetration across species. In a rat genetic model of absence epilepsy, compound **30** demonstrated a robust reduction in the number and duration of seizures at 33 nM plasma concentration, with no cardiovascular effects at up to 5.6 μM. Compound **30** also showed good efficacy in rodent models of essential tremor and Parkinson's disease. Compound **30** thus demonstrates a wide margin between CNS and peripheral effects and is a useful tool for probing the effects of T-type calcium channel inhibition.

Introduction

Voltage-gated calcium channels regulate the entry of Ca^{2+} into cells in response to membrane depolarization. The consequences of Ca^{2+} influx include further depolarization of the cell membrane, muscle contraction, neurotransmitter release, and many others. Early electrophysiology studies classified calcium channels as either high- or low-voltage-activated. The former class includes L-, N-, P/Q-, and R-types. The last is designated as T-type owing to their fast inactivation (transient) and small conductance. Molecular cloning studies of all high and low voltage-gated calcium channels identified 10 genes encoding the main pore-forming α1 subunit.[1] As a result, the T-type Ca^{2+} channel family has three members: $Ca_v3.1$ (α1G), $Ca_v3.2$ (α1H), and $Ca_v3.3$ (α1I). The α1H subtype is found in many tissues, including brain, liver, kidney, heart, and smooth muscle,[2] and on the basis of this distribution, the T-type channel has been proposed to play a role in cardiac pace-making and blood pressure regulation. Researchers at Roche identified the dual T-/L-type calcium channel antagonist mibefradil (**2**, Figure 1), which was briefly marketed as an antihypertensive agent.[3] Studies have shown that **2** is a potent T-type blocker with 10- to 30-fold selectivity for T- over L-type channels.[4,5] Therefore, it has been widely used as a pharmacological tool for studying T-type channels. Some have suggested its antihypertensive effect

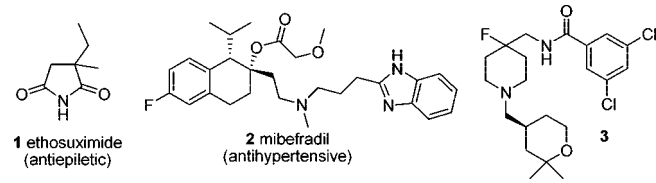

Figure 1

is due to its T-type activity. However, a more recent study using conditional L-type knockout mice indicates that the antihypertensive effects of **2** depend on its L-type antagonist properties.[6] In this study, mean arterial blood pressure in control mice was reduced significantly by a relevant dose of **2** but not changed in conditional L-type KO mice. **2** also blocks voltage-gated sodium and potassium channels,[7] so conclusions based on in vivo observations with **2** should be viewed with caution.

The α1G and α1I subtypes are primarily expressed in CNS neurons.[8] Recent evidence has linked T-type Ca^{2+} channels to many potential therapeutical indications, including epilepsy,[9,10] pain,[11−15] movement disorders,[16] tinnitus/hearing loss,[17] arousal states,[18] overactive bladder,[19] and cancer.[20] In the brain, T-type channels are found throughout the thalamus and cortex regions and play an important role in the integration of neuronal firing. The proper function of thalamocortical signaling is critical for generating a normal rhythmic pattern of oscillatory activities.[21] Alternations in the normal functioning of the thalamocortical signaling have been attributed to many neurological disorders, including aforementioned epilepsy, pain, and movement disorders.[22] In fact, ethosuximide (**1**, Figure 1), a marketed drug for epilepsy has been extensively investigated and found to block T-type calcium channels at therapeutic concentrations.[23,24]

Despite their huge potential, the physiological roles of T-type channels have remained elusive because of the lack of truly selective blockers.[25] Efforts in several laboratories have focused

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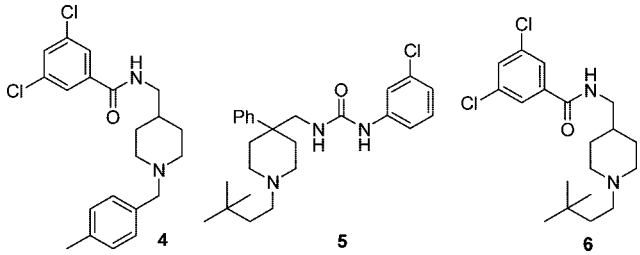


Figure 2. T-Type calcium channel antagonist leads.

Table 1. Piperidine Leads

compd	T-type, FLIPR IP (nM)[a]	hERG, IP (nM)[b]	L-type binding, IC_{50} (nM)[c]
1	>5 000 000		
2	126 ± 52	806	7
4	281 ± 80	135	566
5	245 ± 38	88	206
6	61 ± 16	1934	1191

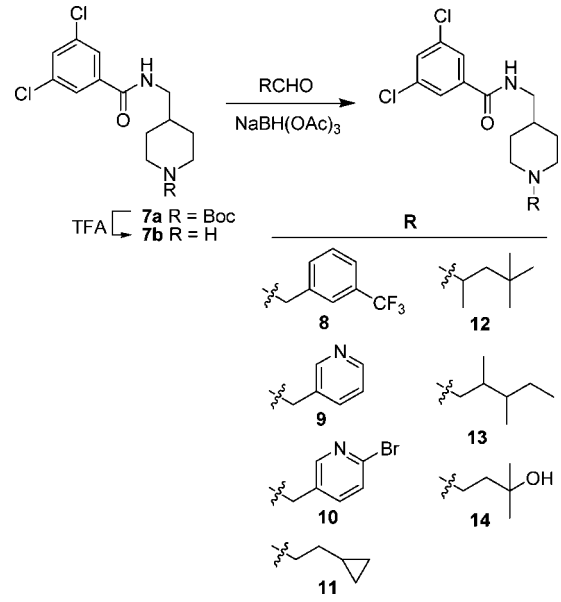
[a] All values are the mean ± standard deviation of at least $n = 3$ measurements. [b] All values are the average of $n = 2$. [c] Inhibition of diltiazem binding to L-type calcium channels in rabbit muscle cells. All values are the average of $n = 2$.

on the identification of new classes of selective agents, but these reports are restricted to in vitro studies.[26] Recently we reported the in vitro and in vivo evaluation of a novel class of selective and potent T-type channel antagonists based on a piperidine scaffold exemplified by **3**.[27] Compound **3** has good potency, selectivity, brain penetration, and pharmacokinetic profile and demonstrated excellent separation between CNS target-engagement and adverse CV effects. Herein, we expand on the SAR and in vitro and in vivo properties of this promising series of T-type calcium channel antagonists.

Results and Discussion

An HTS campaign was initiated, and screening of Merck sample collection led to identification of two structurally similar leads **4** and **5** (Figure 2). The former is a 1,4-substituted piperidine amide, while the latter is a 1,4,4-substuituted piperidine urea. Their activity in blocking the α1i subtype of the T-type channel was evaluated using a high-throughput cell-based calcium flux assay (FLIPR[a]).[28,29] Compound **4** showed good potency with a FLIPR IP value of 281 nM (Table 1). Compound **5** is slightly more active, with an IP value of 245 nM. Counterscreening of these compounds, however, showed that they provided no selectivity over human ether-a-go-go related gene (hERG) and L-type Ca^{2+} channels. Blocking either hERG K^+ or L-type Ca^{2+} channels could raise serious cardiovascular issues. The hERG channel is responsible for the I_{Kr} current, a critical component in ventricular repolarization. Its blockade can lead to increased QTc interval and potentially to ventricular arrhythmias.[30] Blockers of L-type channel, like nifedipine, can cause hypotension. In hERG[31,32] and L-type calcium channel (diltiazem ligand)[33] binding assays, **4** showed IC_{50} values of 135 and 566 nM, respectively, while **5** gave IC_{50} of 88 and 206 nM against these two channels. While measurement of selectivity by comparison of FLIPR functional T-type potency versus binding assays for hERG and the L-type channel is not optimal, this method provided robust SAR in each assay that would be later confirmed in vivo. Combining two leads together provided a hybrid lead **6**, which is not only more potent but also more selective against hERG and L-type channel screening. Compound **6** displayed an IP value of 61 nM, which is approximately a 5-fold improvement over **4**. Its hERG binding liability was reduced by more than 10-fold (IC_{50} = 1.9 μM) compared to **4**. Compound **6** also displayed a 2-fold improvement on L-type binding (IC_{50} = 1.2 μM). Thus, a new potent lead was identified, and it is significantly more selective over hERG and L-type channels.

Scheme 1



Accordingly, a more detailed profiling of **6** was initiated. We employed a rat genetic model of absence epilepsy to evaluate the pharmacodynamics of T-type channel blockers. These rats belong to a strain of Wistar albino Glaxo rats from Rijswijk (WAG/Rij).[34] They display abnormal thalamocortical oscillatory activities, which show a characteristic EEG pattern of excessive seizures. Because of the role of T-type channels in the regulation of thalamocortical rhythms, the WAG/Rij model provided a quick readout of compounds' efficacy on T-type channels. In this model, compound **6** showed a robust effect on reducing rat seizure count and time (data reported as 68% inhibition of seizure duration over a 4 h period after dosing). A pharmacokinetic analysis of the compound in rat revealed that compound **6** was highly bioavailable (100% *F*). Compound **6** was evaluated in a P-glycoprotein (P-gp) assay and proved not to be a P-gp substrate but did have good cell permeability, properties consistent with good brain penetration. A broad based screen of 170 enzymes, receptors, and ion channels (MDS Pharma Services, Bothell, WA) identified eight off-target activities of <10 μM, suggesting further improvement on selectivity was needed.

Because of the positive in vivo profile of **6**, we set out to optimize this structural series to improve further on potency and selectivity. We first screened the *N*-alkyl group of the piperidine. Some representative examples are shown in Scheme 1. Their FLIPR data as well as hERG and L-type binding activities were determined and listed in Table 2. One notable trend of the SAR on FLIPR was that lipophilicity at the terminal end is crucial for potency. Introduction of a nonsubstituted

[a] Abbreviations: CSF, cerebrospinal fluid; DAST, diethylaminosulfur trifluoride; DIEA, diisopropylethylamine; DMF, dimethylformamide; ECG, electrocardiography; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; EEG, electroencephalography; FLIPR, fluorometric imaging plate reader; hERG, human ether-a-go-go-related gene; HOAt, 1-hydroxy-7-azabenzotriazole; HR, heart rate; MAP, mean arterial blood pressure; MP-$BH(OAc)_3$, macroporous polystyrene-bound triacetoxyborohydride; PBSF, perfluoro-1-butanesulfonyl fluoride; THF, tetrahydrofuran; WAG/Rij, Wistar albino Glaxo/Rijswijk.

Table 2. SAR on the *N*-Alkyl Group

compd	T-type, FLIPR IP (nM)[a]	hERG, IP (nM)[b]	L-type binding, IC_{50} (nM)[c]
8	93 ± 9	483	279
9	7613	ND[d]	ND[d]
10	552 ± 213	655	1556
11	771 ± 253	ND[d]	ND[d]
12	109 ± 30	2208	438
13	38 ± 4	883	254
14	3480	ND[d]	ND[d]

[a] All values are the mean ± standard deviation of at least $n = 3$ measurements except for **9** and **14**, where $n = 1$. [b] All values are the average of $n = 2$. [c] Inhibition of diltiazem binding to L-type calcium channels in rabbit muscle cells. All values are the average of $n = 2$. [d] ND = not determined.

Scheme 2

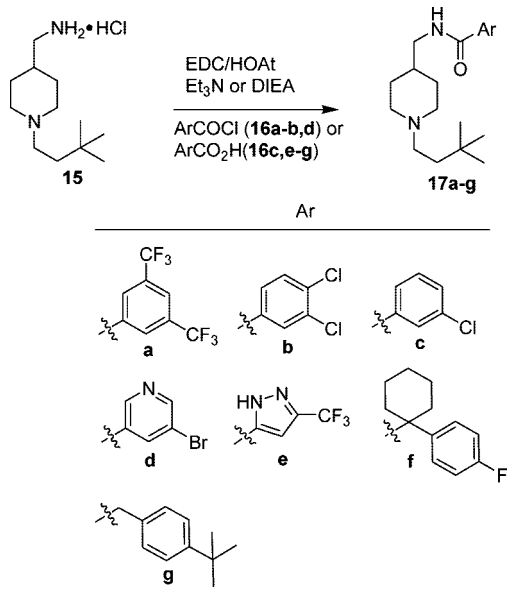


Table 3. SAR on the Benzoic Acid Moiety

compd	T-type, FLIPR IP (nM)[a]	hERG, IP (nM)[b]	L-type binding, IC_{50} (nM)[c]
17a	41 ± 4	1799	739
17b	45 ± 7	1047	616
17c	351 ± 98	4823	2946
17d	2552 ± 1110	ND[d]	ND[d]
17e	1683 ± 591	ND[d]	ND[d]
17f	99 ± 34	3674	984
17g	27 ± 3	1021	944

[a] All values are the mean ± standard deviation of at least $n = 3$ measurements. [b] All values are the average of $n = 2$. [c] Inhibition of diltiazem binding to L-type calcium channels in rabbit muscle cells. All values are the average of $n = 2$. [d] ND = not determined.

pyridine (**9**) or terminal hydroxyl group (**14**) resulted in >50-fold loss of potency vs **6**. Even replacement of *tert*-butyl with a cyclopropyl group led to greater than 10-fold reduction of potency (**11** vs **6**). In addition, none of these analogues showed improvement on selectivity as judged by hERG and L-type binding values.

Next, we shifted our focus on optimizing the benzoic acid moiety. Some representative examples are shown in Scheme 2, and their FLIPR, hERG, and L-type binding data are listed in Table 3. We found that both 3,5- and 3,4-disubstituted benzoic amides helped potency (**17a**,**b**), while mono-Cl analogue at 3-position resulted in a 5-fold decrease of FLIPR potency (**17c**). Heterocyclic replacements were not tolerated (**17d**−**e**). Interestingly, substitution with 4-substituted phenylacetyl group retained

Scheme 3

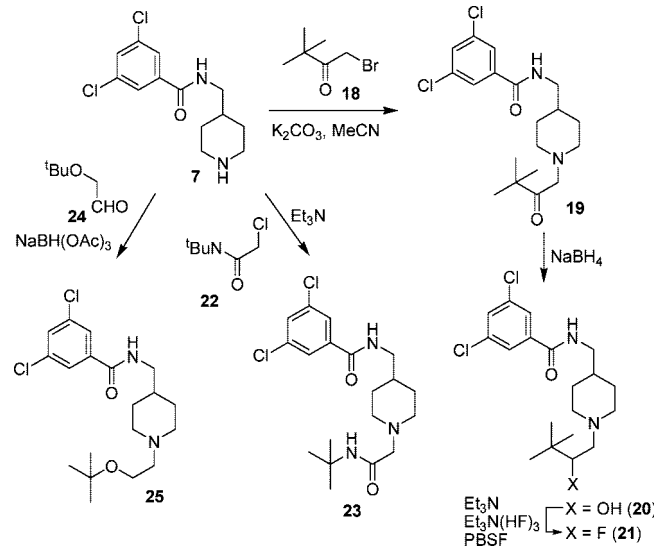


Table 4. Introducing Polarity and Reducing pK_a

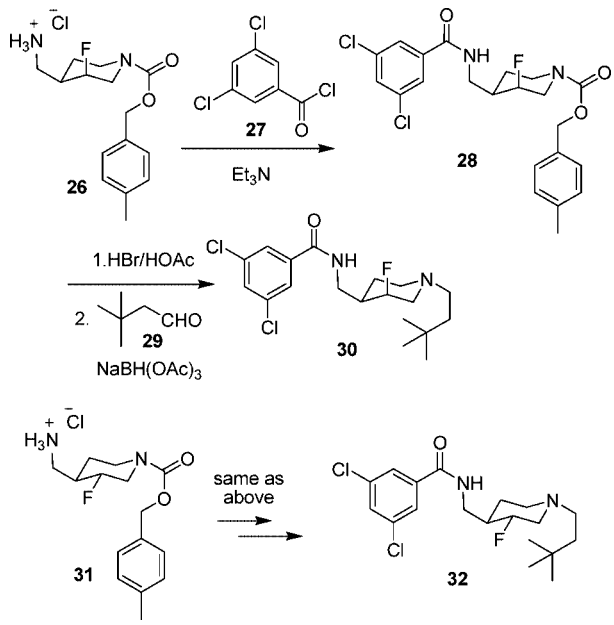
compd	T-type, FLIPR IP (nM)[a]	hERG, IP (nM)[b]	L-type binding, IC_{50} (nM)[c]	pK_a[d]	calcd pK_a[e]
19	97 ± 40	2047	5097	8.0	7.3
20	252 ± 166	4810	3891	9.2	8.8
21	169 ± 64	4731	1526		8.1
23	22 ± 8	>10000	1416	7.6	7.4
25	42 ± 13	1260	1567		8.6
30	**32** ± 10	4114	2134	7.9	7.5
32	411 ± 112	3034	1049	6.7	7.5
6	61 ± 16	1934	1191	8.7	9.5
3	82 ± 27	3790	6330	7.9	7.5

[a] All values are the mean ± standard deviation of at least $n = 3$ measurements. [b] All values are the average of $n = 2$. [c] Inhibition of diltiazem binding to L-type calcium channels in rabbit muscle cells. All values are the average of $n = 2$. [d] Measured pK_a by potentiometric titrations. [e] Calculated pK_a using ACD/pK_a database.

potency (**17f**,**g**). However, none of these modifications improved selectivity on hERG and L-type channels.

Previously it has been proposed that basic amine-containing hERG blockers are involved in a π−cation interaction with Y652[35,36] and thus suggested a need to reduce the basicity of piperidine N.[37,38] Several electron-withdrawing groups, including hydroxyl, fluorine, and ether, were placed onto the *N*-alkyl chain at β-position, while a keto or amide group was incorporated at the α-position. Chemistry for preparation of these molecules was straightforward (Scheme 3). Alkylation of piperidine precursor **7** with α-bromoketone **18** under basic conditions gave the keto derivative **19**. Reduction of **15** with $NaBH_4$ provided the corresponding alcohol **20**. Conversion of the hydroxyl group to fluorine proved to be nontrivial. Common fluorinating reagents, such as DAST or bis(2-methyoxyethyl)-aminosulfur trifluoride, failed to give any product, presumably because of the presence of the basic amine. Under a one-pot procedure developed by the Merck process group,[39] we were able to convert **20** to its fluorinated counterpart **21** by treatment with Et_3N, $Et_3N(HF)_3$, and perfluoro-1-butanesulfonyl fluoride (PBSF) in THF. Treatment of **7** with α-chloroacetamide **22** yielded the bis-amide **23**. Finally, the ether derivative **25** was prepared from **7** under reductive amination of aldehyde **24**. The pK_a of these compounds was either measured or calculated using ACD/pK_a DB. In comparison to lead **6**, all of these analogues proved to be less basic (Table 4). However, only the bis-amide **23** displayed a significant improvement on selectivity over hERG. It is a potent T-type blocker with IP value of 17 nM in

Scheme 4



the depolarized FLIPR assay, while its hERG binding IC_{50} is greater than 10 μM. Unfortunately, this compound is a P-gp substrate and still possessed significant L-type channel binding activity.

We then shifted our focus on incorporating fluorine into the piperidine ring. Previously, we have had success with 4-F derivative such as compound **3**.[27] Here, we focused our efforts on introducing 3-F at the piperidine. Both cis (single enantiomer) and trans isomers (racemic mixture) were prepared using standard transformations from intermediates **26** or **28** previously published by Merck (Scheme 4).[40] As expected, the trans isomer **32** is a much weaker base than the cis counterpart **30**. The pK_a value of **32** is 2 log units lower than that of **6**, while **30** (like its 4-fluoro counterpart **3**) is 1 log unit lower. In depolarized FLIPR assay, compound **30** is 2-fold more potent than nonfluorinated **6**, while **32** is 8-fold weaker than **6**. To our surprise, cis isomer **30** (pK_a = 7.9) is more selective than the less basic trans isomer **32** (pK_a = 6.7) on hERG and L-type channels. Overall, compound **30** is 4-fold more selective on T-type than hERG and L-type channels than **6**. In addition, **30** showed only four off-target activities below 10 μM in a broad based screen of 170 enzymes, receptors, and ion channels (MDS Pharma Services, Bothell, WA), representing another significant improvement over **6**. Among those four off-target activities, the most prominent were σ1 and σ2 receptor binding (IC_{50} < 20 nM), but follow-up in a tissue functional assay[41] revealed only weak antagonism (30% inhibition at 30 μM)). The others included IC_{50} = 1.4 μM on 5HT$_4$, IC_{50} = 8 μM on muscarinic M$_2$, and IC_{50} = 6 μM on histamine H$_1$). Thus, a highly potent and selective T-type channel antagonist, **30**, was identified.

Overall Profile and Cardiovascular Effect of 30. The FLIPR potency of **30** on T-type calcium channels was confirmed using standard voltage clamp electrophysiology assays (see Supporting Information for assay protocol). It had similar potency on the α1I subtype at two different holding potentials (−100 and −80 mV), suggesting a state-independent profile. It also had similar potency on another T-type submember, α1G, indicating that it is not subtype selective. In functional patch clamp, compound **30** showed similar hERG activity as predicated by hERG binding screening assay, with an IC_{50} of 2.4 μM. However, in an L-type voltage clamp assay, **30** had reduced activity ($IC_{50} \approx 15$ μM), which was not predicted by our high-throughput L-type binding assay (IC_{50} = 2.1 μM). This is not uncommon because of multiple binding sites for L-type channels or voltage-dependence of the inhibitors[42] and underscores the need to check high-throughput binding results with functional assays.

Table 5. Ion-Channel Selectivity of **30**

α1I VC[a]		α1G VC[a]			
−100 mV, nM	−80 mV, nM	−100 mV, nM	−80 mV, nM	L-type VC,[a] −100 mV, nM	hERG EP, nM
43	25	62	44	14900	2400

[a] Obtained from whole-cell patch-clamp recordings on HEK-293 cells expressing human Ca_v 3.1 and 3.3 T-type or Ca_v 1.2 L-type calcium channels.

Table 6. Pharmacokinetic Parameters of **30**[a]

species	iv dose (mg/kg)	Cl_p ((mL/min)/kg)	$T_{1/2}$ (h)	po dose (mg/kg)	AUC (μM h)	C_{max} (nM)	F (%)	plasma protein binding (% bound)
rat	2	41	4.9	10	3.1	432	30	93
				1	0.19	33		
				0.3	~0.01			
dog	1	9.1	10.8	3	9.5	1175	67	97
rhesus	1	9.6	4.6	3	2.4	184	18	97

[a] Plasma levels from PK experiments carried out as described in Supporting Information.

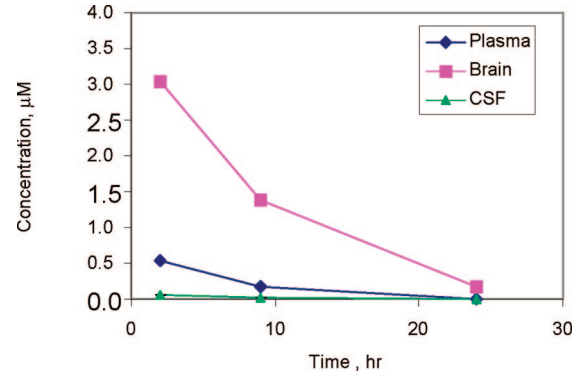


Figure 3. Brain and plasma level of **30** following an oral dose (10 mpk) to rats.

The pharmacokinetic parameters for compound **30** following intravenous and oral administration in preclinical species are shown in Table 6. The compound exhibited moderate clearance and moderate to good bioavailability across all three species. P-Glycoprotein (P-gp) mediated transport of **30** was minimal (B-A/A-B < 2 in both human and rat), and the compound showed good passive cellular permeability ($P_{app} = 37 \times 10^{-6}$ cm/s). Consistent with the in vitro data, **30** showed excellent brain penetration in rats (brain/plasma concentration ratio of 9 and CSF/plasma ratio of 0.14 at 9 h after oral dosing) (Figure 3), supporting its therapeutic potential as a CNS agent.

The in vivo efficacy of compound **30** was first evaluated in the WAG/Rij rat absence epilepsy model.[33] The WAG/Rij rats display cortical EEG patterns and physical behaviors characteristic of an epileptic condition, including frequent seizures. Since T-type calcium channels are involved in the regulation of thalamocortical rhythms, this model served as a relevant pharmacodynamic readout. The data are reported as total seizure time reduction (in seconds) over the 4 h period after dosing orally compared with vehicle control. As shown in Figure 4a, T-type channel blocker **30** exhibited a nice dose-dependent reduction of seizure time, evident as a brain-penetrant, centrally

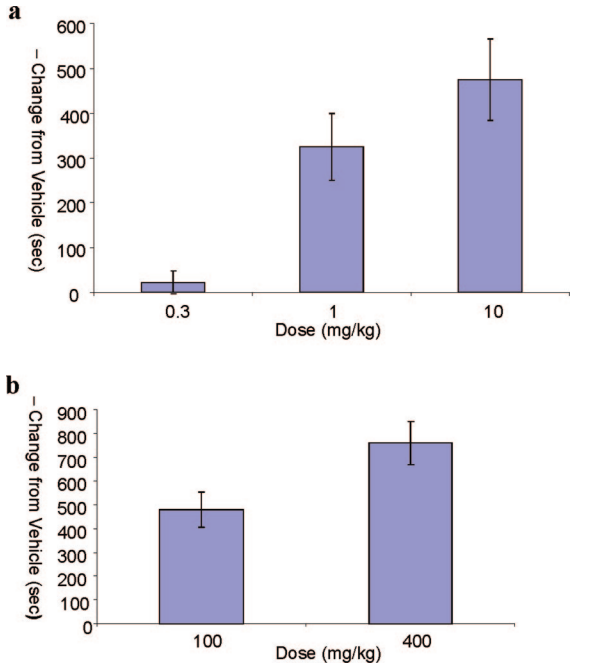


Figure 4. Dose response (po in 90% PEG400) in WAG/Rij rat model of absence epilepsy of **30** (a) and ethosuximide (b), $n \geq 5$ rats per dose. Values represent decrease in total seconds spent in seizure during the 4 h period after dosing compared to vehicle.

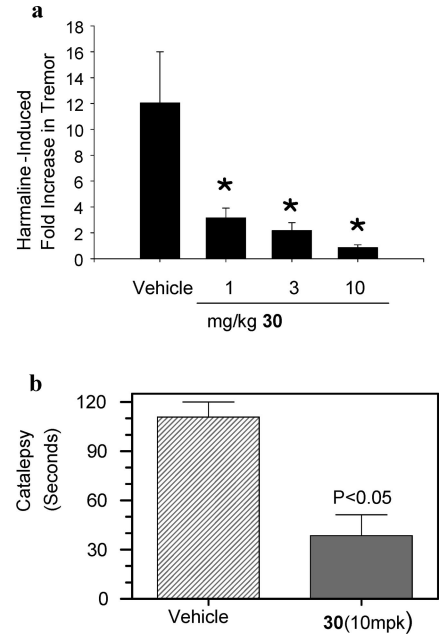


Figure 5. (a) Dose response of **30** in harmaline-induced rat model of essential tremor after po dosing in 90% PEG200. (b) Effect of **30** on haloperidol induced catalepsy after po dosing.

active blocker of T-type channels. The exposures at each dose were determined and listed in Table 6. Robust seizure suppression was observed at 1 mpk dose (AUC = 0.19 μM·h) with $C_{max} \approx IC_{50}$. In comparison, ethosuximide suppressed seizure to a similar degree at a much higher dose (100 mpk, Figure 4b), consistent with its reduced potency on the T-type channel.

Like absence epilepsy, essential tremor has also been correlated to thalamocortical dysfunction;[43] therefore, **30** was evaluated in rat harmaline model[44] of essential tremor. Harmaline-induced tremor activity in the 6−12 Hz range was monitored and quantified to assess tremor suppression. **30** was found to significantly reduce tremor activity in a dose-dependent manner (Figure 5a), highlighting the potential role of T-type antagonists as a novel treatment for movement disorders such as essential tremor.

Given the success of **30** in the essential tremor model, it was examined in a rat model of Parkinson's disease, another potential indication of T-type calcium blockers. In this mode, a cataleptic state is induced by treatment of rats with the dopamine antagonist haloperidol as previously described,[45] and therapeutic agents are evaluated for their ability to reverse this state. Compound **30** was dosed orally in 90% PEG400 to rats 1 h after haloperidol administration; after an additional 30 min, catalepsy was measured by placing rats on a vertical grid and measuring latency to forepaw movement. As shown in Figure 5b, in the vehicle control group, the level of haloperidol induced catalepsy is reflected by a latency to forepaw movement of approximately 110 s. Compound **30** when dosed at 10 mpk significantly reduced haloperidol induced catalepsy, which is encouraging for potential palliative treatment of the motor deficits of Parkinson's disease. Notably, counterscreening showed that the compound does not bind to dopamine receptors or the dopamine transporter (D1, D2, D3, D4, DAT < 50% inhibition at 10 μM). Thus, T-type blockers present a potential novel mechanism for treatment of Parkinson's disease.

Bearing in mind that compound **30** still has micromolar hERG and L-type activities, we evaluated potential cardiovascular effects using a CV dog model. The three dogs used are anesthetized, ventilated, and vagotomized. In this assay, systolic/diastolic and mean arterial pressures, heart rate, and blood flow were measured, as well as PR, QRS, QT/QTc intervals. The compound was dosed in 30 min consecutive iv infusions (i.e., three rising doses). T-Type blocker **30** did not alter the intervals of QTc and QRS up to 33 μM plasma levels, despite having an IC_{50} of 2.4 μM in hERG functional clamp assay. This could be partially due to that fact that compound **30** is highly protein-bound in dogs (97%). Compound **30** had no effect on PR interval and displayed modest bradycardia (5−15% up to 33 μM) and hypotension (8−13% at 1−3 mg/kg; 32% at 33 μM). In fact, the hypotension effect of **30** became significant only at plasma exposures greater than 5.6 μM, possibly because of its activity on the L-type channel. In comparison, the mixed T- and L-type blocker **2** has significant effects on PR interval (+30%), heart rate (−22%), and blood pressure (−27%) at lower plasma exposures (6.4 μM) in this assay (see Supporting Information for detailed data).

Since T-type channels are expressed in renal vascular and tubular tissues, compound **30** was evaluated for potential side effects on renal function. Four dogs were administered a single oral dose (10 mpk) of **30** and observed for 3 h after dosage. No effects were observed on glomerular filtration rate, effective renal plasma flow, urine flow, urinary excretion of sodium and potassium, or plasma electrolytes. While blood pressure and QTc and QRS intervals were unaffected in this assay, increased heart rate and minor behavioral changes (lethargy) were observed, consistent with data collected from anesthetized dogs. Peak plasma concentration of **30** was 2 μM during the experiment.

In summary, the T-type selective blocker **30** had no effect on QTc, QRS, and PR intervals and showed only modest decreases in heart rate and blood pressure at plasma concentrations greater than 5.6 μM, exposure where its L-type activity might begin to operate. In comparison, we observed robust CNS effects at 33 nM. These results clearly demonstrated that such a selective T-type Ca^{2+} channel blocker has great therapeutical

potential with significant safety margin for undesired cardiovascular effects.

Conclusion

Truly selective T-type calcium channel antagonists are potential therapeutic agents for treatment of a variety of neurological disorders. We identified a potent and highly selective T-type blocker (**30**) starting from HTS 1,4-substituted piperidine leads. We found that introducing 3-*S* F onto the piperidine ring increased potency on T-type channels, as well as decreasing activity on L-type and hERG channels. Compound **30** displays good pharmacokinetics in three preclinical species. Robust efficacy was seen in WAG/Rij rat absence epilepsy and harmaline-induced tremor models at plasma exposure well below the no effect level in CV dog experiments. In addition, compound **30** demonstrates a significant reversal of haloperidol induced catalepsy in a preclinical model of Parkinson's disease. These data suggest that **30** has a good margin between on- and off-target effects. Therefore, selective T-type calcium channel antagonists such as **30** hold promise for the treatment of a variety of neurological disorders without adverse cardiovascular side effects.

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Supporting Information Available: Experimental procedures and analytical data for the synthesis of compounds **6**, **8**−**14**, **17**, **19**−**21**, **23**, **25**, **30**, **32**; FLIPR assay protocol; assay protocol for voltage-clamp assay; pharmacokinetic experiment procedure; dog renal function assay protocol; and anesthetized CV dog data. This material is available free of charge via the Internet at http://pubs.acs.org.

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