Journal of Medicinal Chemistry

Article

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Aminopiperidine Sulfonamide $Ca_v2.2$ Channel Inhibitors for the Treatment of Chronic Pain

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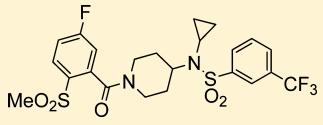
ABSTRACT: The voltage-gated calcium channel $Ca_v2.2$ (N-type calcium channel) is a critical regulator of synaptic transmission and has emerged as an attractive target for the treatment of chronic pain. We report here the discovery of sulfonamide-derived, state-dependent inhibitors of $Ca_v2.2$. In particular, **19** is an inhibitor of $Ca_v2.2$ that is selective over cardiac ion channels, with a good preclinical PK and biodistribution profile. This compound exhibits dose-dependent efficacy in preclinical models of inflammatory hyperalgesia and neuropathic allodynia and is devoid of ancillary cardiovascular or CNS pharmacology at the doses tested. Importantly, **19** exhibited no efficacy in $Ca_v2.2$ gene-deleted mice. The discovery of metabolite **26** confounds further development of members of this aminopiperidine sulfonamide series. This discovery also suggests specific structural liabilities of this class of compounds that must be addressed.



INTRODUCTION

Neuropathic pain is a chronic, debilitating state that results from injury to the peripheral or central nervous system. It can be triggered by a variety of events or conditions, including traumatic injury, diabetes, shingles, and chemotherapies. It is estimated that neuropathic pain affects 4 million people in the U.S. Despite aggressive treatment, some patients remain refractory.[1] Intrathecal administration of analgesics has proven to be effective in severe cases, providing relief by dispensing the therapeutic agent proximal to the pharmacologic target in the central nervous system.[2] N-Type calcium channels ($Ca_v2.2$) are one such target, as the expression of these channels is high in the presynaptic termini of primary afferent nociceptors in the dorsal horn of the spinal chord.[3] The $Ca_v2.2$ channels control the influx of extracellular calcium into primary afferent termini, which results in the release of the neurotransmitters glutamate, substance P, and calcitonin gene-related peptide (CGRP) into the synaptic cleft.[4] Therefore, blocking $Ca_v2.2$ may lead to decreased neurotransmitter release and suppression of pain signals to the brain sensory centers.[5]

The involvement of $Ca_v2.2$ in analgesia is substantiated by several lines of evidence. Preclinical support is provided by rodent pain models. In rat models of both neuropathic and inflammatory pain, the expression of the α subunit of $Ca_v2.2$ is up-regulated in dorsal horn.[6,7] Spinal administration of $Ca_v2.2$ blocking peptides has been shown to ameliorate painful behavior in rodent models of neuropathic and inflammatory pain.[8] Genetic support for the involvement of $Ca_v2.2$ in pain signaling is provided by three independently derived strains of $Ca_v2.2^{-/-}$ mice.[9] The $Ca_v2.2^{-/-}$ mice are healthy and can live a normal life span. However, they show significantly reduced sensitivity to spinal nerve ligation (SNL)-induced mechanical allodynia and thermal hyperalgesia.[10] Clinical validation of $Ca_v2.2$ as a nonopioid analgesic target has been established by the registration of intrathecal ziconotide, a 25 amino acid synthetic peptide based on ω-conotoxin MVIIA from marine snail *Conus magus* that is approved for the treatment of chronic intractable pain.[11] However, ziconotide is a state-*independent* $Ca_v2.2$ blocker and therefore inhibits the activity of the channel in both the hyperpolarized state (closed) and the depolarized state (open or inactivated).[12] Intrathecal administration of the peptide is associated with dizziness and sedation, and systemic administration leads to profound hemodynamic effects.[11,13]

We sought to discover state-dependent small molecule $Ca_v2.2$ antagonists that inhibit the channel preferentially under the conditions of hyperexcitability associated with chronic pain syndromes (depolarized, open, and inactivated state), while sparing low frequency signaling associated with normal nociceptive function (hyperpolarized, closed state). We hypothesize that, by inhibiting the channel in a state-dependent manner, a small molecule $Ca_v2.2$ inhibitor may achieve equal analgesia with minimal neuronal or hemodynamic side effects.[14] Additionally, a small molecule antagonist may provide the additional advantage of oral administration rather than the intrathecal administration required of the petide ziconotide.

Received: July 19, 2012
Published: October 25, 2012

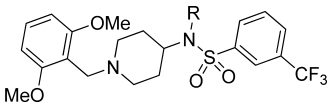
dx.doi.org/10.1021/jm301056k | *J. Med. Chem.* 2012, 55, 9847−9855

Previously we reported the discovery of indole-derived $Ca_v2.2$ inhibitors and an oxindoline-based Ca_v2 inhibitor that afford significant efficacy in rodent pain models while exhibiting minimal ancillary pharmacology.[7,15] In addition, there have been several recent reports of the discovery of a variety of chemotypes of small molecule $Ca_v2.2$ inhibitors that include varying levels of in vitro and in vivo characterization.[16,17] In the present study, we report the discovery and full characterization of a novel series of aminopiperidine sulfonamide state-dependent $Ca_v2.2$ inhibitors.[18] During the course of these investigations, additional aminopiperidine sulfonamide derivatives have independently appeared in the patent literature as $Ca_v2.2$ blockers, indicating the possible generality of this class of compounds as analgesics.[19−22]

RESULTS AND DISCUSSION

A high-throughput screen of our internal sample collection was performed using a calcium-influx assay detected with a fluorescent calcium indicator dye measured on a fluorometric imaging plate reader (FLIPR) with HEK-293 cells selectively expressing $Ca_v2.2$, as described in the Experimental Section.[23] One of the lead compounds identified from this screen was the aminopiperidine sulfonamide derivative **1** (Table 1). This compound provided an advantageous starting point for SAR optimization as a state-dependent channel inhibitor. It exhibits more potent $Ca_v2.2$ inhibition of the calcium influx signal under assay conditions in which the cells were depolarized (30 mM potassium, IC_{50} of 0.12 μM) than when cells are hyperpolarized (4 mM potassium, IC_{50} = 4.8 μM). For comparison, the state-independent $Ca_v2.2$ blocking peptide ω-conotoxin GVIA was also examined in our FLIPR assay.[5,8] This peptide exhibited similar inhibition under depolarizing conditions (30 mM potassium, IC_{50} of 0.04 μM) as under hyperpolarizing conditions (4 mM potassium, IC_{50} of 0.02 μM).

Table 1. Aminopiperidine Sulfonamide Screening Hit Modification



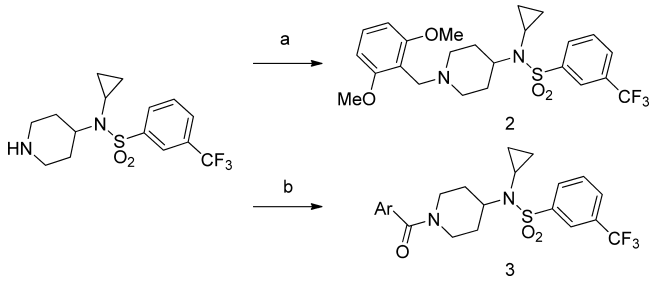
compound	R	Cav2.2 FLIPR IC_{50}, μM[3]	Cav1.2 FLIPR IC_{50}, μM[a]	hERG % inh @ 1 μM[a,b]
1	n-Pr	0.12 (0.07)	3.2 (0.7)	91 (5)
2	^{c}Pr	0.06 (0.03)	ND	70 (2)

[a]IC_{50} values and % inhibition values are averages of $n \geq 3$. Standard deviation is provided in parentheses. [b]hERG inhibition was measured by binding displacement of ^{35}S-labeled MK-0499, a known hERG blocker.

The sulfonamide **1** is not selective against ion channels that mediate cardiovascular function, particularly L-type calcium channels ($Ca_v1.2$), and hERG, which are known to regulate blood pressure and QT intervals, respectively. In addition, the compound exhibits a poor pharmacokinetic profile when dosed in rats (oral normalized AUC = 0.007 μM·h·kg/mg dose, Clp = 169 mL/min/kg, bioavailability = 4%). Initial modification of the sulfonamide alkyl substituent afforded the cyclopropyl derivative **2**, which provided improved potency in the $Ca_v2.2$ FLIPR assay. This cyclopropyl aminopiperidine sulfonamide was utilized as the core structure for further modification.

Chemistry. The synthesis of piperidine sulfonamide compounds is illustrated in Scheme 1. Both the amine derivative **2** and amide derivatives **3** may be synthesized in one step from commercially available *N*-cyclopropyl-*N*-(piperidin-4-yl)-3-(trifluoromethyl)benzenesulfonamide via a reductive amination or Bop-mediated amide coupling reaction in good yield.

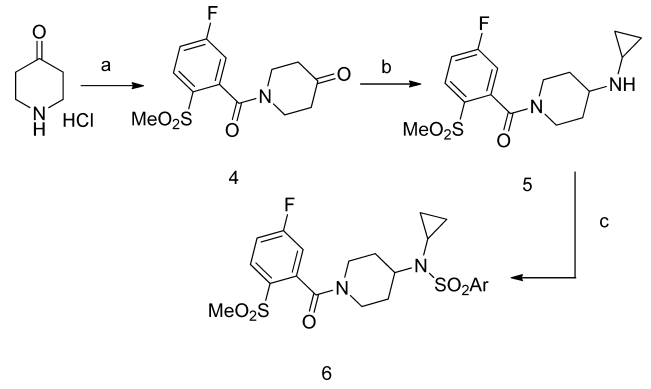
Scheme 1[a]



[a]Reagents and conditions: (a) $NaBH(OAc)_3$, AcOH, 2,6-dimethoxybenzaldehyde, THF, 25 °C. (b) $ArCO_2H$, Bop reagent, DIEA, DMF, 25 °C.

To investigate the effect of substitutions on the aryl sulfonamide, an alternative synthetic scheme was pursued that adds the sulfonamide moiety in the final step (Scheme 2).

Scheme 2[a]



[a]Reagents and conditions: (a) 5-fluoro-2-(methylsulfonyl)benzoic acid, Bop reagent, DIEA, DMF, 25 °C. (b) i. Cyclopropylamine, MeOH, 45 °C, 1 h; ii. $NaBH_4$, 25 °C, 1 h. (c) $ArSO_2Cl$, DIEA, DMAP (cat.), CH_2Cl_2, 25 °C.

Bop-mediated amide coupling reaction between piperidin-4-one hydrochloride and 5-fluoro-2-(methylsulfonyl)benzoic acid gave **4**, which underwent reductive amination with cyclopropyl amine to afford **5**. Reacting **5** with various arylsulfonyl chlorides afforded the desired sulfonamide derivatives **6**.

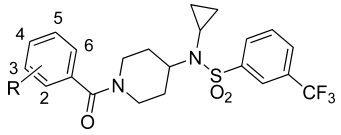
Inhibition of Ion Channels. Once synthesized, these compounds were evaluated for their ability to inhibit $Ca_v2.2$ using the FLIPR calcium-influx assay. Compounds were initially screened for % inhibition of $Ca_v2.2$ FLIPR activity at 1 μM compound concentration. Compounds showing good activity inhibiting $Ca_v2.2$ channels were titrated to determine the IC_{50} and were also counter screened against $Ca_v1.2$ using a related FLIPR assay and against the hERG K^+ channel using a binding assay that measures the displacement of ^{35}S-labeled MK-499, a well characterized hERG K^+ channel blocker.[24]

Our initial SAR effort was focused on reducing hERG binding activity while maintaining the $Ca_v2.2$ potency of this class. Although incorporation of the cyclopropyl substituent in **2** afforded improved selectivity for $Ca_v2.2$ potency over hERG, **2** retained strong potency as an hERG channel modulator.

dx.doi.org/10.1021/jm301056k | J. Med. Chem. 2012, 55, 9847−9855

It is well documented that lipophilic compounds with a basic tertiary amine tend to have high affinity for the hERG channel.[25,26] To reduce hERG binding activity, we decided to replace the basic amine with an amide substituent, and the results are summarized in Table 2. The benzamide derivative **7** was

Table 2. Effect of Benzamide Substitutions on $Ca_v2.2$ Potency and Selectivity



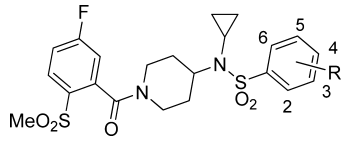
compound	R	Cav2.2 FLIPR % inh. @ 1 μM or IC_{50}, μM[a]	MK-0499% -inh @ 1 μM	MK-0499% -inh @ 10 μM[a,b]	Cav1.2 FLIPR-% inh @ 1 μM	Cav1.2 FLIPR-% inh @ 3 μM[a]
7	H	42 (3)%		ND[c]		ND
8	2-OCF_3	0.12 (0.06)	21	75		ND
9	3-OCF_3	0.37(0.15)	4	72	60	78
10	4-OCF_3	0.30 (0.08)	0	77	73	88
11	3-CO_2Me	0.38 (0.04)	2.4	66		ND
12	3-NMe_2	0.24(0.17)	0	51	40	63
13	2-$CONH_2$	15 (5)%		ND	11	17
14	3-$CONH_2$	0 (10)%		ND		ND
15	4-$CONH_2$	12 (11)%		ND	0	0
16	2-SO_2Me	0.66 (0.09)	1	67	19	39
17	3-SO_2Me	15 (25)%		ND		ND
18	4-SO_2Me	52 (5)%		ND		ND
19	2-SO_2Me-5-F	0.56 (0.24)	1.6	32	18	34

[a]IC_{50} values and % inhibition values are averages of $n \geq 3$. Standard deviation is provided in parentheses. [b]hERG inhibition was measured by binding displacement of ^{35}S-labeled MK-0499, a well characterized hERG blocker. [c]ND: not determined.

significantly less potent as a $Ca_v2.2$ inhibitor. By introducing a lipophilic moiety on the phenyl ring, regardless of electronic nature and position of the substituent, the $Ca_v2.2$ inhibitory activity was retained (**8**–**12**). However, these ring substitutions afforded little significant improvement in selectivity against hERG for **8**–**12** in comparison to **2**. In addition, selectivity against $Ca_v1.2$ was poor for the compounds in which it was measured (**9**, **10**, and **12**). The incorporation of a hydrogen bond donor substituent on the benzamide substituent afforded a reduction in $Ca_v2.2$ activity. For example, the addition of a primary amide caused complete loss of potency regardless of substitution position (**13**–**15**). The effect of the polar methylsulfone substitution varies depending on the substitution position (**16**–**18**). The *ortho*-methylsulfone substitution was well tolerated (**16**), however similar substitution in the meta or para position afforded lower $Ca_v2.2$ potency (**17**–**18**). Introduction of the polar methylsulfone group at the ortho position in **16** also enhanced selectivity against $Ca_v1.2$. Addition of a fluoro group to the 5-position of the methylsulfone-substituted phenyl ring (**19**) afforded slightly enhanced $Ca_v2.2$ potency and also showed good selectivity against hERG and $Ca_v1.2$ channels.

Because the 2-methylsulfonyl-5-fluorobenzamide substituent in **19** exhibited desirable potency and selectivity, this substituent was retained and the further optimization of substitutions on the sulfonamide phenyl ring was attempted. It appeared that a CF_3 group at the meta position of the sulfonamide phenyl ring, as in **19**, was optimal for $Ca_v2.2$ potency (Table 3). Compounds in

Table 3. Effect of Sulfonamide Substitutions on $Ca_v2.2$ potency



compound	R	Cav2.2 FLIPR IC_{50}, μM or% inh. @ 1 μM[a]
19	3-CF_3	0.56 (0.24)
20	2-CF_3	17 (7)%
21	4-CF_3	1 (13)%
22	2-F-5-CF_3	2.5 (0.1)
23	3-SO_2Me	0 (12)%
24	3-OCF_3	1.1 (0.2)
25	4-OCF_3	0 (15)%

[a]IC_{50} values and % inhibition values are averages of $n \geq 3$. Standard deviation is provided in parentheses.

which the CF_3 group was in the ortho or para position exhibited a significant loss of $Ca_v2.2$ potency (**20** and **21**). Adding a fluoro group to the ortho position resulted in a 4-fold loss of potency (**22**). The replacement of the CF_3 group with a polar methyl sulfone moiety resulted in complete loss of potency (**23**). In fact, only the OCF_3 was found to be comparable to CF_3, as this substitution resulted in only a moderate reduction of potency (**24**). However, the OCF_3 group in the para position again resulted in a significant loss of $Ca_v2.2$ potency (**25**).

Pharmacokinetic and Efficacy Profiling. The most promising candidates **8**, **16**, and **19** were evaluated further for microsomal stability by determining the percentage of remaining parent compound following incubation (1 μM) in human or rat liver microsome preparations (1 mg/mL) for 1 h, and the pharmacokinetic profile of the compounds in rats was also determined. The in vivo efficacy was evaluated using the rat complete Freund's adjuvant (CFA)-induced inflammatory pain model.[27] The results are summarized in Table 4. The trifluoromethoxy derivative **8** exhibited poor human and modest rat microsome stability with 1% and 21% of the parent compound remaining, respectively, after 1 h of microsomal incubation. The compound exhibited a modest PK profile with relatively high clearance rate and moderate bioavailability. Compounds **16** and **19** exhibited improved stability in both human and rat microsomes. These compounds had an improved rat PK profile, exhibiting a moderate clearance rate and excellent bioavailability when dosed at 1 mg/kg iv and 3 mg/kg po. The half-life is fairly short for these compounds (calculated from an iv dosing study); however, the po arm of the study provided a later t_{max} of 1.7 h, which is consistent with slow oral absorption of **19**. These compounds were evaluated in the rat CFA pain model dosed orally at 30 mg/kg. Compound **19** showed the best efficacy among the three compounds with 66% and 57% reversal of allodynia at 1 and 3 h time points, respectively, after dosing. Because of its significant in vivo efficacy, compound **19** was profiled further as a potential development candidate.

Profile of Compound ***19****. In Vitro Electrophysiology Characterization.* Compound **19** was evaluated for its ability to inhibit $hCa_v2.2$ using conventional patch clamp electrophysiology, and the results are shown in Figure 1. At a depolarized membrane potential (−40 mV), application of 3 μM of compound **19** inhibited $Ca_v2.2$ channel current by 84 ± 5% (n = 4) (Figure 1A). From a limited titration at four compound concentrations (Figure 1B), the compound achieves >50% block

dx.doi.org/10.1021/jm301056k | J. Med. Chem. 2012, 55, 9847−9855

Table 4. Microsome Stability, Rat PK Profile, and Rat CFA Efficacy of Selected Compounds

	microsome stability, % remaining after 1 h		rat pharmacokinetic profile				rat CFA pain model[e,f]			
								% reversal of allodynia hours after dosing		
compound	human	rat	F%[a]	AUC_N[b]	Clp[c]	$t_{1/2}$[d]	dose (mg/kg)	1 h	3 h	plasma exposure 3 h
8	1	21	47	0.23[g]	65	1.6	3 (iv)	23	17	ND
16	73	45	100	1.06[h]	36	0.91	30 (po)	47	45	2.4 (0.8)
19	30	40	99	0.86[h]	35	1.3	30 (po)	66	57	1.6 (1.1)

[a]Bioavailability. [b]Normalized AUC (po, μM·h·kg/mg). [c]Clearance (mL/min/kg). [d]Half-life (h). [e]Average percent reversal is calculated as (postdose − predose)/(preinjury-predose) for each rat (n = 6). [f]Plasma exposure (μM) satellite rats (n = 2, standard deviation), ND: not determined. [g]Dose 1.8 mg/kg po, 0.6 mg/kg iv. [h]Dose 3 mg/kg po, 1 mg/kg iv;.

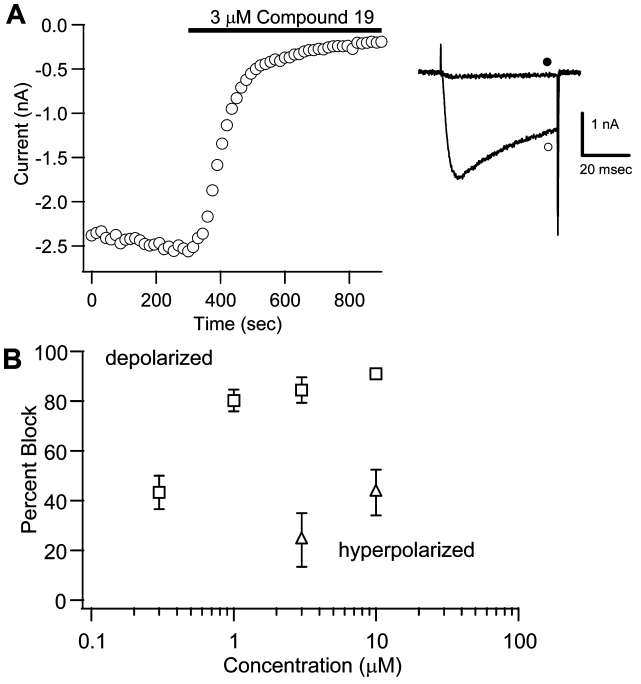


Figure 1. In vitro electrophysiology characterization of compound **19**. (A) Electrophysiological measurement of peak $hCa_v2.2$ current versus time. Compound **19** (3 μM) was applied by bath perfusion for the time indicated by the solid bar. The holding membrane potential was −40 mV. (Inset) Representative traces are shown prior to (○) and after (●) addition of **19**. (B) Plot of the percent block of Cav2.2 versus concentration of **19**. Inhibition was measured at a depolarized (−40 mV; □) and a hyperpolarized potential (−90 mV, Δ). Symbols represent the average ± SEM of two, three, four, and one measurement(s) at 0.3, 1, 3, and 10 μM, respectively, for −40 mV and four and six measurements at 3 and 10 μM, respectively, for −90 mV.

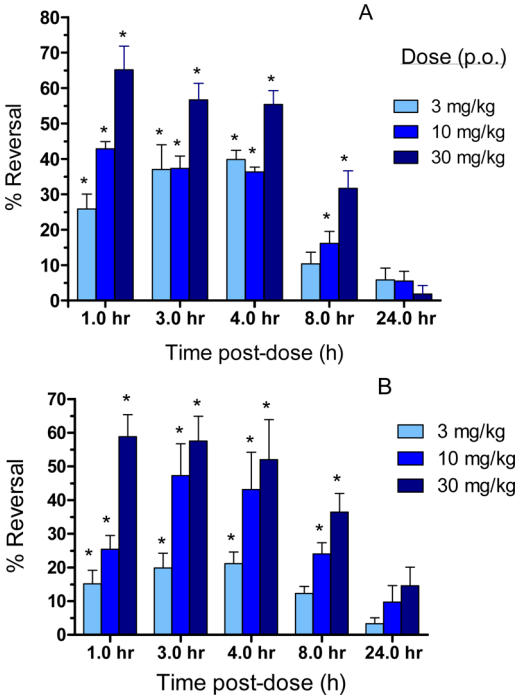


Figure 2. In vivo analgesic efficacy of compound **19** (A) in rat CFA inflammatory pain model and (B) in rat SNL neuropathic pain model. All experimental and control groups contained at least six animals per group, and data are expressed as mean + SEM. Percent reversal are calculated as (postdose − predose)/(preinjury-predose) for each rat. Significance was calculated using a two-way ANOVA followed by Bonferroni posthoc tests for multiple comparisons with vehicle values at each time point (*, $p < 0.05$).

for depolarized $Ca_v2.2$ channels at concentrations of <1 μM. To assess whether inhibition of $Ca_v2.2$ was state-dependent, **19** was applied to cells held at a hyperpolarized membrane potential (−90 mV) where channels are largely in the resting (closed) state. At −90 mV, application of 10 μM **19** inhibited $Ca_v2.2$ channels only 43 ± 9% (n = 6), indicating that **19** is a state-dependent Cav2.2 inhibitor.

In Vivo Efficacy. Compound **19** was evaluated for its antinociceptive activity in both inflammatory and neuropathic pain models. Inflammatory pain was induced by intraplantar injection of complete Freund's adjuvant (CFA) into the hind paws of rats.[27] Mechanical hyperalgesia was assessed at 1, 3, 4, 8, and 24 h following a single oral dose of 1, 3, 10, and 30 mg/kg. Compound **19** dose-dependently reversed mechanical allodynia with a maximal effect 1 h after dosing of 65% reversal for a 30 mg/kg oral dose, and 45% reversal for a 10 mg/kg dose (Figure 2A). We were unable to reproducibly dose the $Ca_v2.2$ blocking peptide ziconotide in these rat experiments, due to the need for intrathecal administration for this agent. However, the level of efficacy exhibited by **19** compares favorably to the effect found with naproxen, which was utilized as a positive control (47% reversal at 1 h following a 10 mg/kg dose, $p < 0.05$, n = 6). In separate CFA-treated rats, the plasma exposure of a 10 mg/kg oral dose was found to be 1.66 μM after 3 h. The efficacy of **19** was significantly lower at the 8 h time point and diminished at 24 h postdose in this model. A similar dose-dependent efficacy was observed in the rat spinal nerve ligation (SNL) model[28] of neuropathic pain, with maximal effect of 59% reversal of mechanical allodynia at 3 h after dosing for a 30 mg/kg dose, and 49% reversal for a 10 mg/kg dose (Figure 2B). The level of

dx.doi.org/10.1021/jm301056k | J. Med. Chem. 2012, 55, 9847−9855

efficacy of **19** in neuropathic allodynia compared favorably with the effect observed with the positive control pregabalin (58% reversal at 3 h following a 10 mg/kg dose, $p < 0.05$, $n = 6$). In separate SNL-treated rats, the plasma exposure of a 10 mg/kg oral dose was found to be 1.26 μM after 3 h.

The target-mediated efficacy of **19** was established by measuring the antinoccipetive activity of the compound in $Ca_v2.2$-deficient mice as compared to wild-type mice (Figure 3).

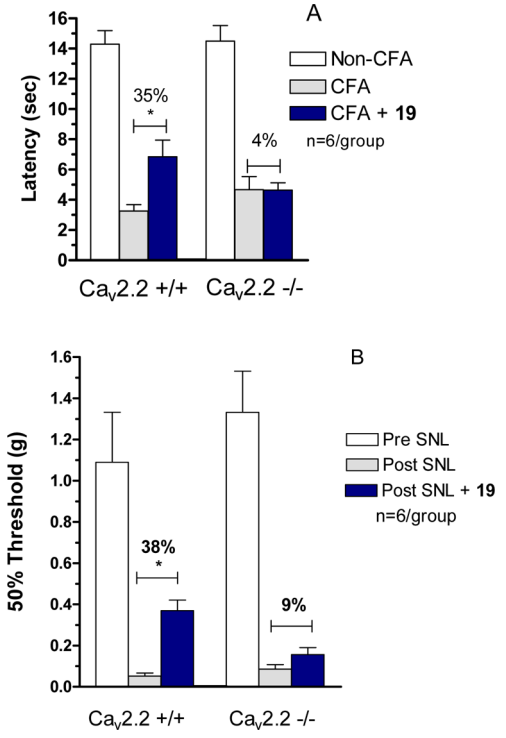


Figure 3. Efficacy of **19** (30 mg/kg p.o., 2 h.) in $Ca_v2.2^{+/+}$ and $Ca_v2.2^{-/-}$ mice . (A) CFA sensitization model, hindpaw withdraw latency to thermal stimuli. (B) SNL pain model, 50% hindpaw withdraw thresholds to stimulation with von Frey filaments. Significance was calculated using a two-way ANOVA followed by Bonferroni posthoc tests for multiple comparisons with vehicle values (*, $p < 0.05$).

The mice were made hypersensitive to heat by intraplantar injection of CFA, and the thermal threshold latency was compared to that in the absence of injury (non-CFA) (Figure 3A). While **19** showed significant efficacy reversing thermal threshold in the CFA assay with wild type mice 1 h after a 30 mg/kg oral dose, the compound exhibited no effect on $Ca_v2.2^{-/-}$ mice in the same pain model It is noteworthy that the $Ca_v2.2^{-/-}$ mice remain responsive to the noccieptive sensitization of CFA, indicating the utilization of multiple pain pathways in this model.[29] As a positive control, naproxen reversed the CFA sensitivity in both strains 1 h following a 30 mg/kg oral dose (60% reversal in −/− mice, 67% in +/+ mice, $p < 0.05$, $n = 7$), which indicates the appropriateness of this model to investigate selective $Ca_v2.2$-mediated analgesia. Sulfonamide **19** exhibited similar efficacy 2 h following a 30 mg/kg oral dose in the reversal of mechanical allodynia in the SNL assay in +/+ mice, while this activity was ablated in −/− mice (Figure 3B). In this instance, both strains of mice remained responsive to the positive control gabapenin (48% reversal in −/− mice, 71% reversal in +/+ mice 2 h following a 30 mg/kg oral dose).

In Vitro Selectivity and in Vivo Side-Effect Profile. Sulfonamide **19** was counter-screened against a panel of 166 additional targets (a collection of representative drug and known toxicity-causing targets) at Ricerca Laboratories and exhibited no significant activity (>50% inhibition) at 10 μM compound concentration in any of these assays. The compound was evaluated for deleterious CNS effect in the rat rota-rod model and showed no effect on rat motor coordination 3 h following a 100 mg/kg oral dose. The plasma and brain exposure of **19** following this experiment was 9.5 μM and 11.9 μM, respectively, indicating a broad preclinical therapeutic window for $Ca_v2.2$-mediated analgesia.

As discussed above, the state-*independent* $Ca_v2.2$ blocking peptide ziconotide exhibits profound hemodynamic effects when administered systemically, and we have observed mild to moderate preclinical cardiovascular effects with unrelated chemotypes of state-dependent Cav2.2 inhibitors.[7,15] To further evaluate any potential cardiovascular liabilities of blocking $Ca_v2.2$ channels, compound **19** was administrated with intravenous infusion in sequential rising doses at 1, 3, and 10 mg/kg (cumulative) to anesthetized, vagotomized dogs. Treatment with compound **19** did not show any effect on the evaluated CV parameters (MAP, HR, QRS, PR, and QTc) up to a maximal plasma exposure of 20.4 μM (Figure 4), which is over

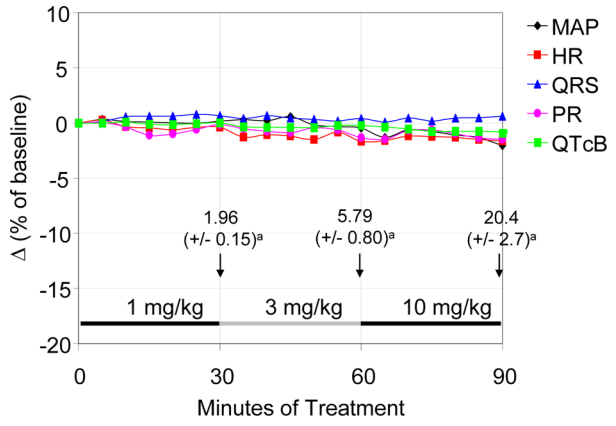


Figure 4. Cardiovascular effect of **19** administered in rising continuous infusion to anesthetized, vagotomized dogs. [a]Plasma exposure at the indicated time point (μM, $n = 3$).

10× higher than the exposure associated with significant efficacy in the rat CFA assay (Table 4).

Drug Metabolism Profile. The favorable exposure and preclinical analgesic efficacy of **19**, coupled with the lack of ancillary pharmacology, lead us to investigate the full drug metabolism profile of this compound as a potential development candidate. The compound has a low free fraction in plasma from rat (2.0%) and human (1.4%). Additionally, the compound is a modest CYP3A4 inhibitor with IC_{50} of 5.1 μM and a relatively strong PXR activator (EC_{50} of 0.43 μM, maximal activation 124% at 10 μM), which indicates the likelihood of compound-mediated CYP3A4 induction in vivo.[30] By evaluating the in vitro metabolism of **19** in the presence of selective CYP inhibitors, we found that CYP3A4 was the primary CYP isoform responsible for the metabolism of the compound. These findings raised the concern for potential drug−drug interactions and made prediction of a human PK profile more difficult. Furthermore, metabolite identification studies revealed formation of 3-trifluoromethylbenzenesulfonamide (**26**) as a major metabolite (Figure 5).

dx.doi.org/10.1021/jm301056k | J. Med. Chem. 2012, 55, 9847−9855

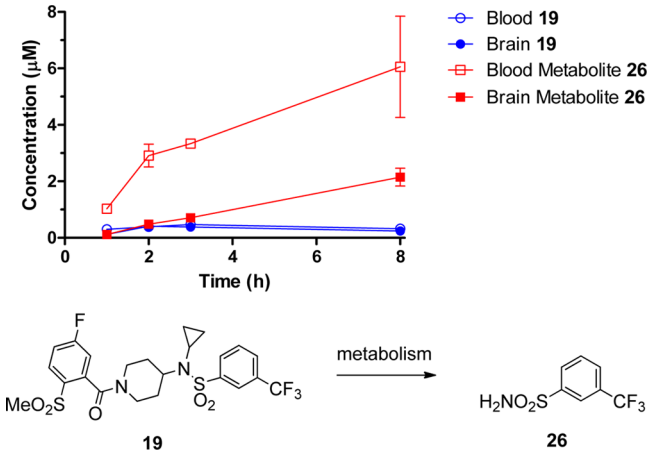


Figure 5. Concentration of parent **19** and metabolite **26** in whole blood and brain following 10 mg/kg oral dose of **19** in rats.

Although **26** formed at low concentration during in vitro incubation with both human and rat liver microsomes, it was identified as a significant circulating species in vivo. When male Sprague−Dawley rats are administered an oral dose of **19** at 10 mg/kg, the concentration of metabolite **26** in whole blood and brain is substantially higher than parent compound **19** (Figure 5). At 8 h postdose, the concentration of compound **19** in whole blood was only 0.31 μM, while the concentration of the metabolite was 6.1 μM. The metabolite **26** clears slowly in whole blood (clearance rate: 0.31 mL/min/kg in rat), and the half-life could not be accurately measured in 24 h. This metabolite is efficacious in both the CFA and SNL pain models,[31] even though it does not appreciably block $Ca_v2.2$ channels in vitro at concentrations up to 10 μM. The antinociceptive mechanism of **26** is currently unknown. In fact, an in vitro screen of **26** against a collection of 48 known and proprietary putative pain targets revealed no activity (>50% inhibition or activation) of these targets at 10 μM compound concentration. The abundance of the metabolite in both the CNS and peripheral circulation contributes to an unknown effect to the preclinical efficacy ascribed to **19**. The presence of an efficacious metabolite of undetermined mechanism would introduce additional complexity and risk to the development of **19** or closely related sulfonamide derivatives. Due to these drug metabolism related issues, development effort on compound **19** was terminated.

CONCLUSIONS

We have identified the aminopiperidine sulfonamide derivative **1** as a state-dependent inhibitor of $Ca_v2.2$. Incorporation of the benzamide substituent significantly improved the off-target profile of this class of compounds, The lead class was optimized to provide **19**, a potent $Ca_v2.2$ inhibitor with significant efficacy in preclinical assays of both neuropathic and inflammatory pain, and a clean profile in ancillary CNS and CV evaluation. However the compound has several drug metabolism related issues including inhibition of CYP3A4 enzyme, activation of PXR, and the significant formation of the circulating metabolite **26** in vivo. The metabolic liability of the exocyclic tertiary sulfonamide will have to be addressed in order for members of the lead class outlined herein to be developed as candidates for analgesia, and overcoming this liability represents the focus of our forthcoming work in this series of compounds.

EXPERIMENTAL SECTION

Experimental procedures for fluorescent $Ca_v1.2$ and $Ca_v2.2$ assays, electrophysiology characterization of compound **19**, rat CNS (rota-rod) and CV dog studies, and in vivo pharmacology efficacy studies were similar to previously published work.[7]

$Ca_v2.2$ FLIPR Assay. A fluorescent assay was used to measure compound-mediated inhibition of calcium influx with a calcium-sensitive fluorescent dye on a FLIPR Tetra instrument (Molecular Devices, Sunnyvale, CA). The assay is similar to that described previously,[23] except that a different $Ca_v2.2$ cell line (labeled CBK) was used in the present work. CBK cells express three calcium channel subunits ($\alpha_{1B\text{-}1}$, $\alpha_2\text{-}\delta$, and β_{3a}) along with an inwardly rectifying potassium channel ($Kir_{2.3}$) that enabled the cell membrane potential to be controlled by bath potassium concentration. Incubation of CBK cells in elevated potassium solution leads to depolarization of cell membrane potential. Exposing CBK cells to modestly elevated concentrations (30 mM) of potassium caused partial inactivation of the $Ca_v2.2$ channel population. In comparison, incubating cells in a bath solution containing 4 mM potassium maintained a negative membrane potential (−90 mV), at which voltage most $Ca_v2.2$ channels are in resting conformations. After compound incubation in 30 mM potassium, to evaluate blocking of open/inactivated channels, channel opening was initiated with 1:1 (by volume) addition of 140 mM potassium solution. All compounds were tested in quadruplicate, and average ± SD values were calculated for 10 point titrations (0.001−30 μM).

Chemistry. All procedures were carried out under a nitrogen atmosphere using commercially available solvents that were used without further purification. Starting materials were obtained from commercial sources and were used without further purification. Normal phase column chromatography was carried out using prepacked silica gel cartridges on a Combiflash Rf separation system by Teledyne ISCO. Reverse phase HPLC purification was carried out using a YMC C18 column on a Gilson HPLC system utilizing a gradient of 0.1% TFA in water/acetonitrile as the eluent. NMR spectra were recorded using Varian Inova500 spectrometers. The reported compounds are of ≥95% purity, unless otherwise noted. Purity analysis was carried out by LC-Mass analysis using an Agilent 1100 system fitted with Waters Micromass.

***N*-Cyclopropyl-*N*-[1-(2,6-dimethoxybenzyl)piperidin-4-yl]-3-(trifluoromethyl)benzenesulfonamide (2).** To a vial were added *N*-cyclopropyl-*N*-(piperidin-4-yl)-3-(trifluoromethyl)benzenesulfonamide (50 mg, 0.144 mmol), acetic acid (8.2 μL, 0.144 mmol), 2,6-dimethoxybenzaldehyde (28.6 mg, 0.172 mmol), sodium triacetoxyborohydride (91 mg, 0.43 mmol), and 2 mL of THF. The resulting reaction mixture was stirred at room temperature for 2 h. It was acidified with TFA, diluted with DMSO and water, and purified on Gilson HPLC using a reverse phase column to give the desired product (TFA salt) as a white solid (80 mg, 91%). ^{1}H NMR ($CDCl_3$): δ: 8.11 (s, 1H), 8.05 (d, J = 8.1 Hz, 1H), 7.90 (d, J = 7.6 Hz, 1H), 7.71 (t, J = 7.9 Hz, 1H), 7.41 (t, J = 8.4 Hz, 1H), 6.62 (d, J = 8.4 Hz, 2H), 4.29 (s, 2H), 4.1 (m, 1H), 3.86 (s, 6H), 3.65 (m, 2H), 2.75 (m, 2H), 2.6 (m, 2H), 1.7 (m, 3H), 0.9 (m, 2H) 0.8 (m, 2H). [M + H$^+$]: m/z 499.

1-{[5-Fluoro-2-(methylsulfonyl)phenyl]carbonyl}piperidin-4-one (4). To a 250 mL round-bottom flask were added piperidin-4-one hydrochloride (0.5 g, 3.69 mmol), 5-fluoro-2-(methylsulfonyl)benzoic acid (0.96 g, 4.43 mmol), BOP reagent (1.9 g, 4.43 mmol), *N*,*N*-diisopropylethylamine (1.92 mL, 11.07 mmol), and THF (50 mL). The resulting solution was stirred at ambient temperature for 1 h. LC-MS indicated complete conversion to product. The reaction was quenched by the addition of 50 mL of 1 N HCl solution, and the product was extracted with EtOAc. Organics were dried over sodium sulfate, filtered, and concentrated. The crude product was purified by flash chromatography with EtOAc/hexane 1:1 as the eluent to afford compound **4** (0.95 g, 86%) as white solid. ^{1}H NMR ($CDCl_3$): δ: 8.17 (m, 1H), 7.35 (t, J = 7.8 Hz, 1H), 7.16 (d, J = 7.9 Hz, 1H), 4.62 (m, 1H), 3.7 (m, 1H), 3.56 (m, 2H), 4.7 (m, 1H), 3.3 (s, 3H), 2.88 (m, 2H), 2.55 (m, 1H), 2.32 (m, 1H). [M + H$^+$]: m/z 300.

[4-(Cyclopropylamino)piperidin-1-yl][5-fluoro-2-(methylsulfonyl)phenyl]methanone (5). Compound **4** (0.60 g, 2 mmol) and cyclopropylamine (0.137 g, 2.4 mmol) were dissolved in

MeOH (5 mL) in a 100 mL round-bottom flask. The solution was heated to 45 °C for 2 h. The solution was cooled, to this solution was added $NaBH_4$ (0.3 g, 8 mmol) in portions at ambient temperature, and resulting reaction mixture was stirred overnight. The volatiles were removed by rotary evaporation, and the residue was redissolved in EtOAc. The solution was washed with water, dried over sodium sulfate, filtered, and concentrated to afford 0.6 g compound **5** as white solid. It was used for the next step without further purification. ^{1}H NMR ($CDCl_3$): δ: 8.13 (m, 1H), 7.22 (t, J = 7.8 Hz, 1H), 7.05 (m, 1H), 4.58 (m, 1H), 4.42 (m, 1H), 3.75 (m, 1H), 3.42 (m, 1H), 3.25 (s, 3H), 3.15 (m, 2H), 3.0 (m, 1H), 2.85 (m, 2H), 2.15 (m, 2H), 1.95 (m, 1H), 1.45 (m, 2H), [M + H$^+$]: m/z 341.

***N*-Cyclopropyl-*N*-[1-(phenylcarbonyl)piperidin-4-yl]-3-(trifluoromethyl)benzenesulfonamide (7).** Compound 7 was synthesized with the same procedure as that used for compound **19**. ^{1}H NMR ($CDCl_3$): The compound exists as a pair of equal amount rotamers at ambient temperature. δ: 8.18 (s, 1H), 8.08 (d, J = 8.0 Hz, 1H), 7.9 (d, J = 8.0 Hz, 1H), 7.7 (t, J = 8.2 Hz, 1H), 7.42 (m, 5H), 4.8 (b, 1H), 4.18 (m, 1H), 3.8 (b, 1H), 3.2 (m, 1H), 2.8 (m, 1H), 2.0 (m, 1H), 1.8 (m, 1H), 1.7 (m, 1H), 1.0 (m, 2H), 0.8 (m, 2H). [M + H$^+$]: m/z 453.

***N*-Cyclopropyl-*N*-(1-{[2-(trifluoromethoxy)phenyl]carbonyl}piperidin-4-yl)-3-(trifluoromethyl)benzenesulfonamide (8).** Compound **8** was synthesized according to the same procedure as that used for compound **19**. ^{1}H NMR (CD_3OD): δ: 8.18 (d, J = 7.8 Hz, 1H), 8.15 (s, 1H), 7.99 (d, J = 8.0 Hz, 1H), 7.83 (t, J = 8.0 Hz, 1H), 7.6 (m, 1H), 7.4 (m, 3H), 4.7 (m, 1H), 4.2 (m, 1H), 3.4 (m, 1H), 3.2 (m, 1H), 2.8 (m, 1H), 3.5 (m, 1H), 1.5−2.1 (m, 5H), 0.8−1.0 (m, 4H). [M + H$^+$]: m/z 537.

***N*-Cyclopropyl-*N*-(1-{[3-(trifluoromethoxy)phenyl]carbonyl}piperidin-4-yl)-3-(trifluoromethyl)benzenesulfonamide (9).** Compound **9** was synthesized with the same procedure as that used for compound **19**. ^{1}H NMR ($CDCl_3$): δ: 8.18 (s, 1H), 8.10 (d, J = 8.0 Hz, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.72 (t, J = 7.7 Hz, 1H), 7.49 (t, J = 8.0 Hz, 1H), 7.35−7.25 (m, 3H), 4.8 (b, 1H), 4.2 (m, 1H), 3.8 (b, 1H), 3.1 (b, 1H), 2.8 (b, 1H), 2.0 (m, 1H), 1.85 (m, 1H), 1.75 (m, 4H), 1.0 (b, 2H), 0.85 (m, 2H).[M + H$^+$]: m/z 537.

***N*-Cyclopropyl-*N*-(1-{[4-(trifluoromethoxy)phenyl]carbonyl}piperidin-4-yl)-3-(trifluoromethyl)benzenesulfonamide (10).** Compound **10** was synthesized with the same procedure as that used for compound **19**. ^{1}H NMR ($CDCl_3$): δ: 8.84 (s, 1H), 8.2 (s, 1H), 8.1 (t, J = 8.0 Hz, 2H), 7.9 (d, J = 8.4 Hz, 1H), 7.8 (d, J = 8.4 Hz, 1H), 7.7 (t, J = 8.4 Hz, 1H), 4.85 (b, 1H), 4.2 (m, 1H), 4.0 (b, 1H), 3.15(b, 1H), 3.2 (b, 1H), 2.8 (b, 1H), 2.0 (m, 2H), 1.8 (b, 1H), 1.65 (b, 1H), 1.0 (m, 2H), 0.8 (m, 2H). [M + H$^+$]: m/z 537.

Methyl 3-{[4-(Cyclopropyl{[3-(trifluoromethyl)phenyl]sulfonyl}amino)piperidin-1-yl]carbonyl}benzoate (11). Compound **11** was synthesized with the same procedure as that used for compound **19**. ^{1}H NMR ($CDCl_3$): δ: 8.18 (s, 1H), 8.1 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 8.0 Hz, 1H), 8.05 (s, 1H), 7.85 (d, J = 8.4 Hz, 1H), 7.7 (t, J = 8.2 Hz, 1H), 7.6 (d, J = 8.4 Hz, 1H), 7.5 (t, J = 8.5 Hz, 1H), 5.2 (b, 1H), 4.8 (b, 1H), 4.18 (m, 1H), 3.92 (s, 3H), 3.8 (b, 1H), 3.15(b, 1H), 2.8 (b, 1H), 2.0 (m, 1H), 1.8 (b, 1H), 1.6 (b, 1H), 1.0 (m, 2H), 0.8 (m, 2H). [M + H$^+$]: m/z 511.

***N*-Cyclopropyl-*N*-(1-{[3-(dimethylamino)phenyl]carbonyl}piperidin-4-yl)-3-(trifluoromethyl)benzenesulfonamide (12).** Compound **12** was synthesized with the same procedure as that used for compound **19**. ^{1}H NMR ($CDCl_3$): The compound exists as a pair of equal amount rotamers at ambient temperature. δ: 8.18 (s, 1H), 8.08 (d, J = 8.0 Hz, 1H), 7.9 (d, J = 8.0 Hz, 1H), 7.7 (t, J = 8.2 Hz, 1H), 7.4 (t, J = 8.2 Hz, 1H), 7.1 (m, 2H), 6.9 (d, J = 8.0 Hz, 1H), 4.8 (b, 1H), 4.18 (m, 1H), 3.8 (b, 1H), 3.2 (m, 1H), 3.1 (s, 6H), 2.8 (m, 1H), 2.0 (m, 1H), 1.8 (m, 1H), 1.7 (m, 1H), 1.6 (b, 1H), 1.0 (m, 2H), 0.8 (m, 2H). [M + H$^+$]: m/z 496.

2-{[4-(Cyclopropyl{[3-(trifluoromethyl)phenyl]sulfonyl}amino)piperidin-1-yl]carbonyl}benzamide (13). Compound **13** was synthesized with the same procedure as that used for compound **19**. ^{1}H NMR ($CDCl_3$): The compound exists as a pair of equal amount rotamers at ambient temperature. δ: 8.18 (s, 1H), 8.08 (d, J = 8.0 Hz, 1H), 7.9 (d, J = 8.0 Hz, 1H), 7.8 (d, J = 8.2 Hz, 1H), 7.78 (t, J = 8.2 Hz, 1H), 7.58 (t, J = 8.0 Hz, 1H), 7.5 (t, J = 8.0 Hz, 1H), 7.3(m, 1H), 6.2 (b, 1H), 4.85 (b, 1H), 4.18 (b, 1H), 3.45 (m, 1H), 3.15 (m, 1H), 2.8 (m, 1H), 1.8 (m, 1H), 1.5 (m, 1H), 1.0 (m, 2H), 0.8 (m, 2H). [M + H$^+$]: m/z 496.

3-{[4-(Cyclopropyl{[3-(trifluoromethyl)phenyl]sulfonyl}amino)piperidin-1-yl]carbonyl}benzamide (14). Compound **14** was synthesized with the same procedure as that used for compound **19**. ^{1}H NMR (CD_3OD): δ: 8.2 (d, J = 8.0 Hz, 1H), 8.18 (s, 1H), 8.0 (d, J = 8.1 Hz, 1H), 7.86 (m, 3H), 7.5 (d, J = 8.4 Hz, 2H), 4.7 (b, 1H), 4.2 (m, 1H), 3.7 (b, 1H), 3.2 (b, 1H), 2.85 (b, 1H), 2.2 (m, 1H), 2.0 (b, 2H), 1.75 (b, 1H), 1.6 (b, 1H), 1.0 (m, 2H), 0.8 (m, 2H). [M + H$^+$]: m/z 496.

4-{[4-(Cyclopropyl{[3-(trifluoromethyl)phenyl]sulfonyl}amino)piperidin-1-yl]carbonyl}benzamide (15). Compound **15** was synthesized with the same procedure as that used for compound **19**. ^{1}H NMR ($CDCl_3$): δ: 8.18 (s, 1H), 8.08 (d, J = 8.0 Hz, 1H), 7.9 (m, 1H), 7.88 (d, J = 8.5 Hz, 2H), 7.78 (t, J = 8.2 Hz, 1H), 7.5 (d, J = 8.0 Hz, 2H), 6.1(b, 1H), 4.2 (m, 1H), 3.78 (b, 1H), 3.1 (b, 1H), 2.8 (b, 1H), 2.0 (m, 1H), 1.0 (m, 2H), 0.8 (m, 2H). [M + H$^+$]: m/z 496.

***N*-Cyclopropyl-*N*-(1-{[2-(methylsulfonyl)phenyl]carbonyl}piperidin-4-yl)-3-(trifluoromethyl)benzenesulfonamide (16).** Compound **16** was synthesized with the same procedure as that used for compound **19**. ^{1}H NMR ($CDCl_3$): The compound exists as a pair of rotamers at ambient temperature. δ: 8.18 (s, 1H), 8.08 (m, 1H), 8.15 (m, 1H), 7.7 (m, 2H), 7.6 (m, 1H), 7.38 (d, J = 6.5 Hz, 1H), 4.83 (b, 1H, major rotamor), 4.75 (b, 1H, minor rotamor), 4.2 (m, 1H, major rotamer), 4.15 (m, 1H, minor rotamer), 3.45 (b, 1H, major rotamer), 3.35 (m, 1H, minor rotamer), 3.25 (s, 3H, major rotamor), 3.2 (s, 3H, minor rotamer), 3.1 (m, 2H, major rotamer), 2.8 (m, 2H, minor rotamer), 2.3 (m, 1H, major rotamor), 2.18 (m, 1H, minor rotamor), 2.1 (m, 1H), 1.9 (m, 1H), 1.8 (m, 1H), 1.65 (b, major rotamor), 1.6 (b, 1H, minor rotamor), 1.35 (b, 1H), 1.0 (m, 2H), 0.8 (m, 2H). [M + H$^+$]: m/z 531.

***N*-Cyclopropyl-*N*-(1-{[3-(methylsulfonyl)phenyl]carbonyl}piperidin-4-yl)-3-(trifluoromethyl)benzenesulfonamide (17).** Compound **17** was synthesized with the same procedure as that used for compound **19**. ^{1}H NMR ($CDCl_3$): δ: 8.18 (s, 1H), 8.10 (d, J = 7.8 Hz, 1H), 8.02 (d, J = 7.4 Hz, 1H), 8.0 (s, 1H), 7.87 (d, J = 7.7 Hz, 1H), 7.6−7.8 (m, 3H), 4.8 (b, 1H), 4.2 (m, 1H), 3.65 (b, 1H), 3.15 (b, 1H), 3.1 (s, 3H), 2.8 (b, 1H), 2.0 (m, 1H), 1.85 (m, 1H), 1.65 (m, 1H), 1.0 (b, 2H), 0.85 (m, 2H).[M + H$^+$]: m/z 531.

***N*-Cyclopropyl-*N*-(1-{[4-(methylsulfonyl)phenyl]carbonyl}piperidin-4-yl)-3-(trifluoromethyl)benzenesulfonamide (18).** Compound **18** was synthesized with the same procedure as that used for compound **19**. ^{1}H NMR ($CDCl_3$): δ: 8.18 (s, 1H), 8.10 (d, J = 8.0 Hz, 1H), 8.02 (d, J = 8.3 Hz, 2H), 7.89 (d, J = 7.8 Hz, 1H), 7.73 (t, J = 7.8 Hz, 1H), 7.6 (d, J = 8.3 Hz, 2H), 4.8 (b, 1H), 4.2 (m, 1H), 3.65 (b, 1H), 3.15 (b, 1H), 3.1 (s, 3H), 2.8 (b, 1H), 2.0 (m, 1H), 1.85 (m, 1H), 1.65 (m, 1H), 1.0 (b, 2H), 0.85 (m, 2H).[M + H$^+$]: m/z 531.

***N*-Cyclopropyl-*N*-(1-{[5-fluoro-2-(methylsulfonyl)phenyl]carbonyl}piperidin-4-yl)-3-(trifluoromethyl)-benzenesulfonamide (19).** To a 100 mL round-bottom flask were added 5-fluoro-2-(methylsulfonyl)benzoic acid (0.702 g, 3.22 mmol), *N*-cyclopropyl-*N*-(piperidin-4-yl)-3-(trifluoromethyl)benzenesulfonamide (1.12 g, 3.22 mmol), Bop reagent (1.57 g, 3.54 mmol), DIEA (1.68 mL, 9.66 mmol), and 10 mL of DMF. The resulting reaction mixture was stirred at ambient temperature for 1 h. It was diluted with 60 mL of EtOAc and washed sequentially with 60 mL of 1 N HCl, 30 mL of 5% KOH, and 20 mL of brine. Organics were dried over Na_2SO_4, filtered, and concentrated. The residue was purified on prepacked silica gel column eluted with 2:3 ethyl acetate/hexane to give the desired product as a fluffy white solid after lyophilization (1.5 g, 85%). ^{1}H NMR ($CDCl_3$): The compound exists as a pair of rotamers at rt (ratio: 2.4:1). The major rotamer: δ: 8.16 (s, 1H), 8.1 (m, 2H), 7.86 (d, J = 7.9 Hz, 1H), 7.7 (d, J = 8.1 Hz, 1H), 7.3 (m, 1H), 7.07 (dd, J = 2.5, 7.9 Hz, 1H), 4.8 (m, 1H), 4.2 (m, 1H), 3.5 (m, 1H), 3.26 (s, 3H, major rotamer), 3.1 (m, 1H), 2.8 (m, 1H), 2.25 (m, 1H), 2.15 (m, 1H), 1.95 (m, 1H), 1.65 (m, 1H), 1.35 (m, 1H), 1.0 (m, 2H), 0.8 (m, 2H); The minor rotamer: many peaks overlap with the major rotamer. Only peaks that are clearly separated from the major rotamer are listed: δ: 4.7 (m, 1H),

4.05 (m, 1H), 3.35 (m, 1H), 2.05 (m, 2H), 1.6 (m, 1H); [M + H$^+$]: *m*/*z* 549. HRMS calcd for $C_{23}H_{25}F_4N_2O_5S_2$ (MH$^+$) 549.1136, found 549.1149.

***N*-Cyclopropyl-*N*-(1-{[5-fluoro-2-(methylsulfonyl)phenyl]carbonyl}piperidin-4-yl)-2-(trifluoromethyl)benzenesulfonamide (20).** Compound **20** was synthesized with the same procedure as that used for compound **21**. ^{1}H NMR ($CDCl_3$): δ: 8.32 (m, 1H), 8.15 (m, 1H), 7.85 (m, 1H), 7.75 (m, 2H), 7.3(m, 1H), 7.05 (d, *J* = 8.0 Hz, 1H), 4.85 (b, major rotamer, 1H), 4.75 (b, minor rotamer, 1H), 4.4 (m, major rotamer, 1H), 3.5 (b, major rotamer, 1H), 3.4(b, 1H, minor rotamer), 3.25 (s, 3H, major rotamer), 3.2 (s, 3H, minor rotamer), 3.2 (m, 1H), 2.85 (m, 1H), 2.4 (m, 1H), 2.25 (m, 1H), 1.85 (b, 1H), 1.5 (s, 3H), 0.7 (m, 2H), 0.5 (m, 2H). [M + H$^+$]: *m*/*z* 549.

***N*-Cyclopropyl-*N*-(1-{[5-fluoro-2-(methylsulfonyl)phenyl]carbonyl}piperidin-4-yl)-4-(trifluoromethyl)benzenesulfonamide (21).** To a 25 mL round-bottom flask were added compound **4** (167 mg, 0.491 mmol), 4-(trifluoromethyl)-benzene-1-sulfonyl chloride (100 mg, 0.409 mmol), DIEA (0.107 mL, 0.613 mmol), DMAP (5 mg, 0.041 mmol), and 5 mL of THF. The resulting reaction mixture was stirred at ambient temperature for 10 min and heated at 50 °C for an additional 2 min. Analysis by LC-MS indicated a complete conversion. The mixture was acidified with TFA and purified on Gilson HPLC using a reverse phase column to give the desired product as a fluffy white solid after lyophilization (120 mg, 53%). ^{1}H NMR ($CDCl_3$): The compound exists as a pair of rotamers at ambient temperature. δ: 8.1 (m, 1H), 8.0 (d, *J* = 8.3 Hz, 2H), 7.8 (t, *J* = 7.2 Hz, 2H), 7.25 (m, 1H), 7.05 (d, *J* = 8.3 Hz, 1H), 4.8 (b, 1H, major rotamor), 4.7 (b, 1H, minor rotamor), 4.2 (m, 1H, major rotamer), 4.05 (m, 1H, minor rotamer), 3.45 (b, 1H, major rotamer), 3.35 (m, 1H, minor rotamer), 3.25 (s, 3H, major rotamor), 3.2 (s, 3H, minor rotamer), 3.15 (m, 2H, major rotamer), 2.8 (m, 2H, major rotamer), 2.3 (m, 1H, major rotamor), 2.2 (m, 1H, minor rotamor), 2.0 (m, 1H), 1.85 (m, 1H), 1.7 (b, 1H, major rotamor), 1.6 (b, minor rotamor), 1.4 (b, 1H), 1.0 (m, 2H), 0.8 (m, 2H). [M + H$^+$]: *m*/*z* 549.

***N*-Cyclopropyl-2-fluoro-*N*-(1-{[5-fluoro-2-(methylsulfonyl)-phenyl]carbonyl}piperidin-4-yl)-5-(trifluoromethyl)-benzenesulfonamide (22).** Compound **22** was synthesized with the same procedure as that used for compound **21**. ^{1}H NMR ($CDCl_3$): δ: 8.25 (m, 1H), 8.18 (m, 1H), 7.85 (m, 1H), 7.38 (m, 1H), 7.3 (m, 1H), 7.05 (d, *J* = 8.0 Hz, 1H), 4.85 (b, major rotamer, 1H), 4.75 (b, minor rotamer, 1H), 4.4 (m, major rotamer, 1H), 4.2 (m, minor rotamer), 3.5 (b, major rotamer, 1H), 3.4(b, 1H, minor rotamer), 3.25 (s, 3H, major rotamer), 3.2 (s, 3H, minor rotamer), 3.2 (m, 1H), 2.85 (m, 1H), 2.4 (m, 1H), 2.25 (m, 1H), 1.85 (b, 1H), 1.6 (m, 1H), 0.85 (m, 2H), 0.8 (m, 2H). [M + H$^+$]: *m*/*z* 567.

***N*-Cyclopropyl-*N*-(1-{[5-fluoro-2-(methylsulfonyl)phenyl]carbonyl}piperidin-4-yl)-3-(methylsulfonyl)-benzenesulfonamide (23).** Compound **23** was synthesized with the same procedure as that used for compound **21**. ^{1}H NMR ($CDCl_3$): The compound exists as a pair of rotamers at ambient temperature. δ: 8.45 (s, 1H), 8.2 (d, *J* = 8.3 Hz, 1H), 8.15 (m, 1H), 7.82 (d, *J* = 8 Hz, 1H), 7.79 (d, *J* = 8 Hz, 1H), 7.25 (m, 1H), 7.05 (d, *J* = 8.3 Hz, 1H), 4.8 (b, 1H, major rotamor), 4.7 (b, 1H, minor rotamor), 4.2 (m, 1H, major rotamer), 4.1 (m, 1H, minor rotamer), 3.45 (b, 1H, major rotamer), 3.35 (m, 1H, minor rotamer), 3.25 (s, 3H, major rotamor), 3.2 (s, 3H, minor rotamer), 3.18 (s, 3H, major rotamer), 3.15 (s, 3H, minor rotamer), 2.8 (m, 2H, major rotamer), 2.3 (m, 1H, major rotamor), 2.2 (m, 1H, minor rotamor), 1.9 (m, 1H), 1.85 (m, 1H), 1.85 (b, 1H, major rotamor), 1.65 (b, major rotamor), 1.4 (b, 1H), 1.0 (m, 2H), 0.8 (m, 2H). [M + H$^+$]: *m*/*z* 559.

***N*-Cyclopropyl-N-(1-(5-fluoro-2-(methylsulfonyl)benzoyl)-piperidin-4-yl)-3-(trifluoromethoxy)benzenesulfonamide (24).** Compound **24** was synthesized with the same procedure as that used for compound **21**. ^{1}H NMR ($CDCl_3$): The compound exists as a pair of rotamers at 2.2:1 ratio at ambient temperature. δ: 8.12 (m, 1H), 7.84 (d, *J* = 7.5 Hz, 1H), 7.77 (s, 1H), 7.61 (t, *J* = 8.1 Hz 1H), 7.48 (m, 1H), 7.25 (m, 1H), 7.06 (m, 1H), 4.82 (d, *J* = 11.2, 1H, major rotamer), 4.7 (m, 1H, minor rotamer), 4.2 (m, 1H, major rotamer), 4.05 (m, 1H, minor rotamer), 3.46 (d, *J* = 8.5 Hz, 1H, major rotamer), 3.35 (m, 1H, minor rotamer), 3.26 (s, 3H, major rotamor), 3.22 (s, 3H, minor rotamer), 3.1 (m, 1H), 2.8 (m, 1H), 1.8 - 2.3 (m, 3H), 1.66 (d, *J* = 13.2, 1H, major rotamor), 1.60 (m, 1H, minor rotamor), 1.37 (d, *J* = 12.4 Hz, 1H, major rotamor), 1.0 (m, 2H), 0.8 (m, 2H). [M + H$^+$]: *m*/*z* 565.

***N*-Cyclopropyl-N-(1-(5-fluoro-2-(methylsulfonyl)benzoyl)-piperidin-4-yl)-4-(trifluoromethoxy)benzenesulfonamide (25).** Compound **25** was synthesized with the same procedure as that used for compound **21**. ^{1}H NMR ($CDCl_3$): The compound exists as a pair of rotamers at RT. δ: 8.16 (m, 1H), 7.93 (m, 2H), 7.38 (m, 2H), 7.4 (t, *J* = 8.0 Hz 2H), 7.25 (m, 1H), 7.1 (m, 1H), 4.82 (m, 1H, major rotamer), 4.7 (m, 1H, minor rotamer), 4.2 (m, 1H, major rotamer), 4.05 (m, 1H, minor rotamer), 3.45 (m, 1H, major rotamer), 3.35 (m, 1H, minor rotamer), 3.25 (s, 3H, major rotamor), 3.2 (s, 3H, minor rotamer), 3.15 (m, 2H, major rotamer), 2.8 (m, 2H, major rotamer), 2.3 (m, 1H, major rotamor), 2.2 (m, 1H, minor rotamor), 2.0 (m, 1H), 1.85 (m, 1H), 1.7 (m, 1H, major rotamor), 1.6 (m, minor rotamor), 1.4 (m, 1H), 1.0 (m, 2H), 0.8 (m, 2H). [M + H$^+$]: *m*/*z* 565.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

AcOH, acetic acid; AUC, area under the pharmacokinetic exposure curve; b, broad; Bop reagent, Bbenzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; Ca_v, voltage-gated calcium channel; CFA, complete Freund's adjuvant; CLp, plasma pharmacokinetic clearance; CNS, central nervous system; CV, cardiovascular; CYP, cytochrome P450 enzymes; d, doublet; DIEA, diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EtOAc, ethyl acetate; HEK cells, human embrionic kidney; hERG, human ether-a-go-go-related gene; HPLC, high performance liquid chromatography; HR, heart rate; LC-MS, liquid-chromatography−mass spectroscopy; m, multiplet; MAP, mean arterial pressure; MeOH, methanol; NMR, nuclear magnetic resonance; PK, pharmacokinetic; PXR, pregnane X receptor; s, singlet; SAR, structure−activity relationship; SNL, spinal nerve ligation; t, triplet; THF, tetrahydrofuran; TFA, trifluoroacetic acid

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