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# Improved $Ca_v2.2$ Channel Inhibitors through a *gem*-Dimethylsulfone Bioisostere Replacement of a Labile Sulfonamide

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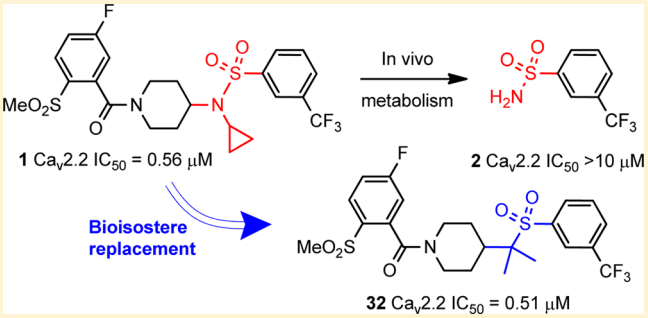
Supporting Information

**ABSTRACT:** We report the investigation of sulfonamide-derived $Ca_v2.2$ inhibitors to address drug-metabolism liabilities with this lead class of analgesics. Modification of the benzamide substituent provided improvements in both potency and selectivity. However, we discovered that formation of the persistent 3-(trifluoromethyl)-benzenesulfonamide metabolite was an endemic problem in the sulfonamide series and that the replacement of the center aminopiperidine scaffold failed to prevent this metabolic pathway. This issue was eventually addressed by application of a bioisostere strategy. The new *gem*-dimethyl sulfone series retained $Ca_v2.2$ potency without the liability of the circulating sulfonamide metabolite.




**KEYWORDS:** *$Ca_v2.2$, N-type calcium channel, pain, sulfonamide, bioisostere, sulfone*

N-Type calcium channels ($Ca_v2.2$) are expressed in the presynaptic termini of primary afferent nociceptors in the spinal cord and are key components of the pain transmission pathway. We have previously reported the discovery of a series of aminopiperidine sulfonamide state-dependent $Ca_v2.2$ channel inhibitors with potential utility for the treatment of chronic pain. Members of this class are orally bioavailable and efficacious in preclinical pain models.[1] In particular, **1** (Figure 1) is selective for inhibition of the $Ca_v2.2$ channel over the

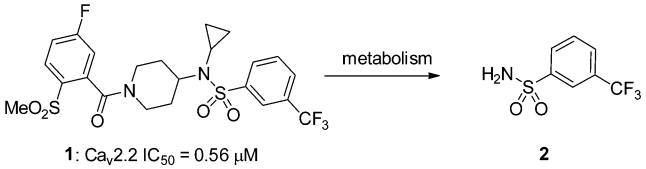



**Figure 1.** In vivo metabolism of **1** to afford the primary sulfonamide **2**.

cardiovascular ion channels hERG and $Ca_v1.2$, with a decent preclinical PK and biodistribution profile. The compound exhibits dose-dependent efficacy in preclinical models of inflammatory hyperalgesia and neuropathic allodynia, but it has no efficacy in $Ca_v2.2$ gene-deleted mice. The compound is also devoid of ancillary preclinical cardiovascular or CNS pharmacology. While **1** may be used as a preclinical tool to evaluate state-dependent inhibition of $Ca_v2.2$ channels, it has several drug metabolism related issues that confound further development of the compound. These include moderate CYP3A4 inhibition ($IC_{50}$ = 5.1 $\mu$M), PXR activation ($EC_{50}$ = 0.43 $\mu$M), and the formation of the persistent circulating metabolite **2**. The sulfonamide metabolite **2** exhibited preclinical antinociceptive activity in its own right, although it was devoid of significant $Ca_v2.2$ activity. In order to further improve this lead class of $Ca_v2.2$ inhibitors and address the drug metabolism issues associated with this series, SAR optimization studies were carried out on the amide substituent and the aminopiperidine core, and a bioisostere replacement strategy was pursued for the sulfonamide moiety.

The first substituent to be investigated for further optimization was the benzoic amide moiety (Table 1). The synthesis of piperidine sulfonamide compounds listed in Table 1 was straightforward as described previously.[1] This synthetic route allowed rapid SAR studies modifying substitutions on the benzamide phenyl ring. In some instances, the desired benzoic acid partner had to be synthesized. For example, the intermediate 4-(methylsulfonyl)-2-trifluoromethoxy-benzoic

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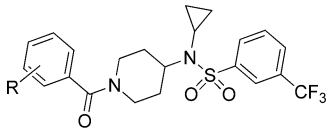
**Table 1. Effect of Benzamide Substitutions on $Ca_v2.2$ Inhibitory Potency and Selectivity**



| compd | R | $Ca_v2.2$ FLIPR $IC_{50}$, $\mu$M or % inh @ 1 $\mu$M[a] | MK-499 % inh @ 10 $\mu$M[b] | CYP 3A4% inh @ 10 $\mu$M | PXR $EC_{50}$ ($\mu$M) |
|---|---|---|---|---|---|
| **1** | 2-$SO_2$Me-5-F | 0.56 | 32% | 75% | 0.42 |
| **3** | 2-$SO_2$Me-5-CN | 0.67 | 41% | 59% | 5.0 |
| **4** | 2-$SO_2$Me-4-CN | 0.45 | 66% | 86% | 8.4 |
| **5** | 2-$SO_2$Me-4-$CF_3$ | 0.027 | 43% | 78% | 4.3 |
| **6** | 3-$SO_2$Me | 15% | ND[c] | ND | ND |
| **7** | 4-$SO_2$Me | 52% | ND | ND | ND |
| **8** | 2-Cl-5-$SO_2$Me | 0.34 | 49% | 69% | 2.7 |
| **9** | 2-F-4-$SO_2$Me | 0.35 | 78% | ND | 6.8 |
| **10** | 2-Cl-4-$SO_2$Me | 0.29 | 32% | 78% | 10.8 |
| **11** | 2-$CF_3$-4-$SO_2$Me | 0.021 | 58% | 75% | 2.8 |
| **12** | 2-$OCF_3$-4-$SO_2$Me | 0.048 | 63% | 16% | 1.7 |
| **13** | 4-$CONH_2$ | 12% | ND | ND | ND |
| **14** | 2-$CF_3$-4-$CONH_2$ | 0.76 | 32% | 61% | 1.9 |
| **15** | 2-$OCF_3$-4-$CONH_2$ | 0.39 | 41% | 60% | >10 |

[a]$IC_{50}$ values and % inhibition values are averages of $n \geq 3$. [b]hERG inhibition was measured by binding displacement of [$^{35}$S]MK-499. [c]ND: not determined.

acid used for **12** was prepared from commercially available 4-bromo-1-iodo-2-trifluoromethoxy-benzene as indicated in the Supporting Information.

The resulting compounds were evaluated for their ability to inhibit $Ca_v2.2$ channels using the FLIPR calcium-influx assay that has been described previously.[2] Compounds were screened for % inhibition of $Ca_v2.2$ FLIPR activity at 1 $\mu$M concentration. Compounds showing good activity inhibiting $Ca_v2.2$ channels were titrated to determine their $IC_{50}$ and were also counter-screened against the related L-type calcium channel $Ca_v1.2$ using a related FLIPR assay. Potent compounds were also screened against the hERG $K^+$ channel using a binding assay that measures the displacement of [$^{35}$S]MK-499, a well characterized hERG $K^+$ channel blocker.[3] Additionally, the active $Ca_v2.2$ inhibitors were profiled for inhibition of CYP3A4 and activation of PXR, which may indicate the likelihood of the compound to induce CYP3A4 expression in vivo.[4] The resulting data are summarized in Table 1.

Compound **1** is a relatively potent PXR activator. Thus, reducing PXR activity while improving $Ca_v2.2$ inhibition was the focus of our initial SAR optimization. The addition of polar substituents to specific positions of the molecule (often at the end region of the molecule) has been shown to reduce PXR potency in other instances.[5] We decided to apply this strategy to compound **1** by adding more polar functional groups at the meta or para position of the benzamide phenyl ring while retaining the methylsulfone group at ortho position. Replacing the fluoro group in compound **1** with a cyano group (**3**) had a minimal impact on $Ca_v2.2$ inhibitory potency and resulted in over 10-fold reduction in PXR potency. The cyano group in the para position exhibited further reduced PXR activity, with a slight gain in $Ca_v2.2$ potency (**4**). Interestingly, placing a lipophilic $CF_3$ group at the para position as in **5** led to a 10-fold reduction in PXR activity as compared to **1**. Compound **5** also exhibited a 20-fold increase in $Ca_v2.2$ potency over **1**. A substantial loss in potency was observed for compounds with the sulfone in the meta (**6**) or para (**7**) positions. However, in each instance the potency was restored by the addition of an ortho substituent (**8**–**12**). Among ortho substituents, the lipophilic trifluoromethy and trifluoromethoxy substituents (**11** and **12**) provided a greater enhancement of $Ca_v2.2$ inhibitory potency than the halogen substituents (**8**, **9**, or **10**). PXR activity was reduced when the methylsulfone group was introduced at the para or meta position, and the para substitution has a stronger effect (**8** vs **10**). Substitution with other polar functional groups was also explored. With a primary amide at the para position, compound **13** was significantly less potent as a $Ca_v2.2$ inhibitor. However, again in this instance, the addition of a $CF_3$ or $OCF_3$ group at the ortho position restored $Ca_v2.2$ inhibitory potency (**14** and **15**). Many of the compounds listed in Table 1 were reasonably devoid of MK-499 binding activity. However, CYP3A4 inhibition was substantially reduced only for compound **12**.

In a parallel effort, the SAR investigation on the center aminopiperidine portion of the molecule was undertaken. This region of the molecule was found to be surprisingly tolerant of modification while maintaining $Ca_v2.2$ inhibitory potency, as illustrated in Figure 2. The addition of an *ipso*-methyl group at

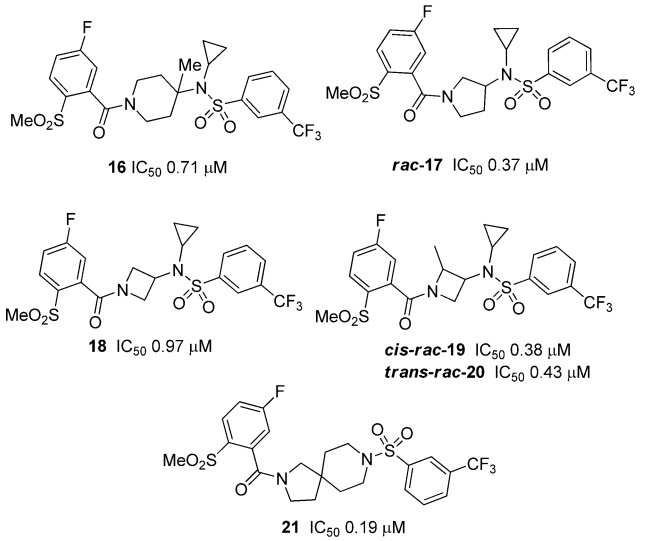



**Figure 2.** Summary of the center scaffold modifications and SAR depenence on $Ca_v2.2$ potency. $IC_{50}$ values are averages of $n > 3$.

the 4-position of the aminopiperidine ring (**16**) resulted in only a slight loss of potency as compared to **1**. The analogous 3-aminopyrrolidine (**17**) exhibited a small improvement in potency, even as a racemic mixture. Although with azetidine as the center scaffold compound **18** is less potent in comparison to its piperidine analogue **1**, the addition of the 2-methyl group resulted in a significant increase in potency (**19** and **20**). In these compounds, the relative stereochemistry has little effect on potency as both cis- and trans-diastereomers have similar activity (***cis-rac*-19** and ***trans-rac*-20**). With 2-azaspiro[4.5]decane as the center scaffold, compound **21** was 3-fold more potent than compound **1** as a $Ca_v2.2$ inhibitor. From this investigation of aminopiperidine replacement moieties, we concluded that the center substituent of this lead class provided limited influence on the potency of the compounds as $Ca_v2.2$ inhibitors but rather was acting as a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scaffold for the presentation of the distal pharmacophores of the molecules. The compounds in Figure 2 were synthesized according to the similar procedure used for the synthesis of the compounds in Table 1. The synthesis of the 4-methylpiperidine intermediate for the synthesis of **16** is described in the Supporting Information.

In an earlier report on this lead class,[1] we discovered that a critical issue of compound **1** was formation of the long lasting sulfonamide metabolite **2** both in vitro and in vivo (Figure 1). One possible mechanism for formation of metabolite **2** is through a two-step metabolism sequence involving hydroxylation of the $\alpha$-carbon of the sulfonamide group, followed by hydrolysis of hemiamino ketal. Several strategies were pursued to inhibit the formation of metabolite **2** based on this hypothesis. These strategies included replacing the piperidine with more oxidation resisting azetidine,[6] making $\alpha$-carbon quaternary to prevent hydroxylation, and using the spiro ring system to provide extra stability. A selection of structurally diverse compounds that share common 3-$CF_3$-benzene sulfonamide fragment are illustrated in Figure 3. These

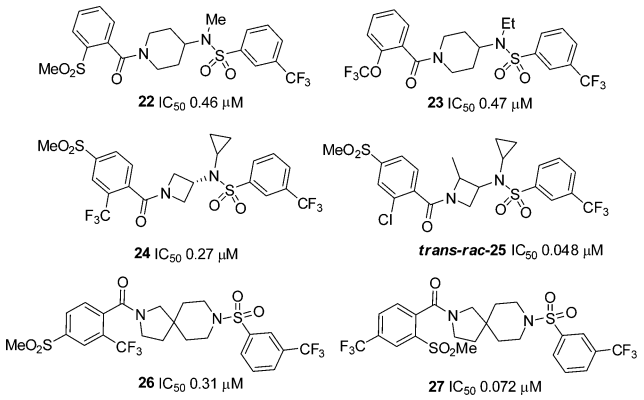


**Figure 3.** Compounds tested for microsomal stability and metabolite 2 formation. $Ca_v2.2$ $IC_{50}$ values are averages of $n \geq 3$.

compounds were incubated with human or rat liver microsomes and analyzed for formation of metabolite **2**. To our surprise, for all the compounds tested in this assay (**16** and **22**–**27**), the formation of metabolite **2** occurred at a greater rate than that of compound **1** (Table 2).

To determine if metabolite **2** is formed in vivo as well, compound ***trans-rac*-25** and **27** were evaluated in rat

**Table 2. Formation of Metabolite 2 in Microsome Incubation Studies**[a]

| | % of parent remaining | | conc of **2** ($\mu$M) | |
|---|---|---|---|---|
| compd | rat | human | rat | human |
| **1** | 40 | 30 | 0.01 | 0.03 |
| **16** | ND[b] | ND | 0.06 | 0.04 |
| **22** | 18 | 26 | 0.08 | 0.95 |
| **23** | 3.4 | 30 | 0.08 | 1.16 |
| **24** | 72 | 78 | 0.04 | 0.45 |
| **25** | 4.7 | 5.3 | 0.11 | 0.83 |
| **26** | 3.8 | 4.2 | 0.18 | 5.13 |
| **27** | 12 | 8.5 | ND | ND |

[a]Compounds were incubated at 10 $\mu$M with 1 mM NADPH and 1 mg/mL rat or human microsomal protein for 1 h. [b]ND: not determined.

pharmacokinetics studies. Indeed, **25** and **27** gave very high concentrations of metabolite **2**, as illustrated in Figure 4. While

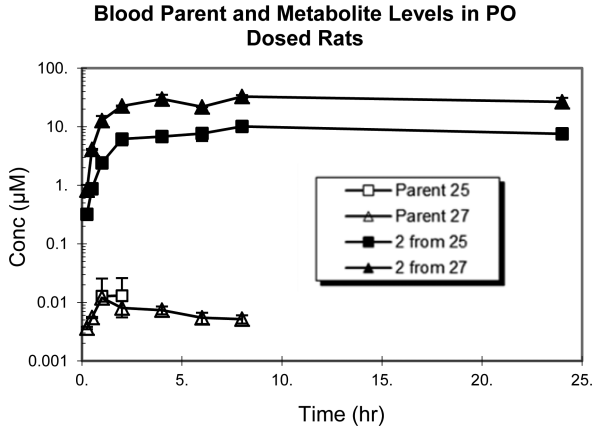


**Figure 4.** Blood levels of parent **25** (□), parent **27** (△), and metabolite **2** in rats after 3 mg/kg oral dose of **25** (■) and **27** (▲). Values are averages for $n$ = 2 rats (±SD).

the normalized AUC for the parent compound **25** in whole blood was only 0.017 $\mu$M·hr·kg/mg after a 3 mg/kg oral dose, the normalized AUC for metabolite **2** in blood was 64 $\mu$M·hr·kg/mg. Similar results were obtained with **27**. The normalized AUC for the parent and metabolite was 0.022 $\mu$M·hr·kg/mg and 218 $\mu$M·hr·kg/mg, respectively, following 3 mg/kg oral dose of **27**. At this point, we were convinced that formation of the primary sulfonamide metabolite **2** is endemic to the sulfonamide substituent and that alternative strategies such as replacing the sulfonamide moiety with a bioisostere would be required.

We thus shifted our focus to the identification of sulfonamide replacement bioisosteres, and several of these efforts are presented in Table 3. Although amide is a common bioisostere to sulfonamide, replacing sulfonamide in **12** with the corresponding amide resulted in complete loss of potency (**28**). Similarly, replacement of the sulfonamide moiety with amine, ether, alcohol, and ketone substituents also afforded compounds lacking significant inhibitory potency for the $Ca_v2.2$ channel (data not shown). The sulfone group, however, proved to be a good replacement for the sulfonamide substituent. Although sulfone compound **29** was 8-fold less potent than the analogous sulfonamide **12**, the sulfone remained a moderately potent $Ca_v2.2$ inhibitor with an $IC_{50}$ of 0.36 $\mu$M. The addition of one methyl group to the $\alpha$-position of the sulfone resulted in a 4-fold improvement in potency for one enantiomer (**30** ***en*-1**) and no change for the other (**30** ***en*-2**). The *gem*-dimethyl analogue **31** retained the potency of the most active monomethyl enantiomer of **30** and was four times more potent than the des-methyl analogue **29**. The sulfone analogue bearing the analogous benzamide substitution as compound **1** was synthesized. Compound **32** is equally potent to **1** with $Ca_v2.2$ $IC_{50}$ of 0.51 $\mu$M (Table 3). By comparison of the sulfonamide analogues (**1** and **12**) to their corresponding *gem*-dimethyl sulfone derivatives (**31** and **32**), we concluded that the *gem*-dimethyl sulfone was an adequate bioisostere for further investigation. The synthesis of the critical *gem*-dimethylsufone piperidine intermediate is described in the Supporting Information.

Compounds **31** and **32** were further profiled, and the results are summarized in Table 4. Both compounds maintain excellent


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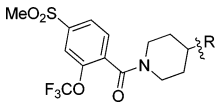
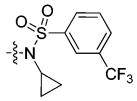
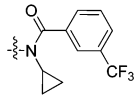
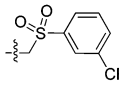
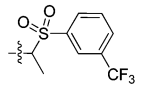
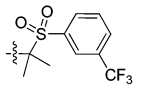
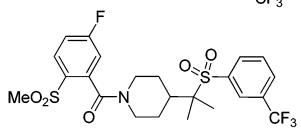
**Table 3. $Ca_v2.2$ Inhibitory Potency of Sulfone Compounds**



| Compound | R | Cav2.2 FLIPR $IC_{50}$ μM or % inh.[a] |
|---|---|---|
| 12 |  | 0.048 |
| 28 |  | 48% inh. @10μM |
| 29 |  | 0.36 |
| 30<br>*en*-1<br>*en*-2 |  | 0.088<br>0.37 |
| 31 |  | 0.092 |
| 32 |  | 0.51 |

[a]$IC_{50}$ values and % inhibition values are averages of $n \geq 3$.

**Table 4. Metabolism Profiles of 31 and 32**

| | | microsomes %[a] | | |
|---|---|---|---|---|
| compd | PXR activation $EC_{50}$ | human | rat | hPgp BA/AB ratio |
| **31** | >30 μM | 62 | 73 | 4.2 |
| **32** | 2.5 μM | 64 | 68 | 1.8 |

[a]Compounds were incubated at 10 μM with 1 mM NADPH and 1 mg/mL rat or human microsomal protein for 1 h.

selectivity against $Ca_v1.2$ and hERG channels ($IC_{50}$ > 10 μM), and both are weak CYP3A4 inhibitors with $IC_{50}$ values of about 10 μM. Although **32** showed significant improvement in PXR activation over **1**, it is still a moderate PXR activator with an $EC_{50}$ of 2.5 μM, while **31** is devoid of significant PXR activity. It is critical that the $Ca_v2.2$ channel blockers can access the CNS and are not substrates for the Pgp efflux mechanism since the $Ca_v2.2$ channel is localized in the synapse of spinal cord neurons.[7] Thus, compounds **31** and **32** were also tested to determine if they were Pgp substrates. With the same substitution groups on the benzamide as compound **1**, **32** was found not to be a Pgp substrate (human BA/AB ratio: 1.8).[8] However, **31** is a Pgp substrate with measured human BA/AB ratio of 4.2. Both compounds are highly permeable, with Papp of 35 × $10^{-6}$ cm/sec for each compound. Both compounds exhibit good metabolic stability with percentage of remaining parent compounds ranging from 62% to 73% after incubation with human or rat liver microsomes (1 mg/mL protein and 1 μM substrate) for 1 h.

Compounds **31** and **32** were evaluated for their pharmacokinetic and efficacy properties in rats, and the results are shown in Table 5, along with corresponding data for **1** and the

**Table 5. Rat PK Profile and Rat CFA Efficacy of 1, 2, 31, and 32**

| | | | | rat CFA pain model[e] | | |
|---|---|---|---|---|---|---|
| | rat PK profile[a] | | | % reversal of allodynia | | |
| compd | $AUC_N$[b] | CL[c] | F%[d] | dose[f] | 1 h | 3 h |
| **1** | 0.86 | 35 | 99% | 10 | 43% | 38% |
| | | | | 30 | 66% | 57% |
| **2** | 111 | 0.31 | 46% | 30 | 53% | 44% |
| **31** | 0.97 | 27 | 95% | 10 | 21% | 30% |
| **32** | 0.27 | 29 | 24% | 10 | 36% | 49% |
| | | | | 30 | 57% | 54% |

[a]Dose 3 mg/kg PO, 1 mg/kg IV; PK parameters based on whole blood concentrations. [b]Normalized AUC (PO, μM·h·kg/mg). [c]Clearance (mL/min/kg). [d]Bioavailability. [e]Average percent reversal are calculated as (postdose - prdose)/(preinjury predose) for each rat ($n$ = 6). [f]Oral dose in mg/kg.

metabolite **2**.[1] Sulfone **31** has good rat PK properties with moderate clearance and half-life (Cl = 27 mL/min/kg; $t_{1/2}$ = 2.3 h) and excellent bioavailability ($F$ = 95%). Compound **32** has similar moderate clearance (Cl = 29 mL/min/kg) but lower bioavailability ($F$ = 24%). Compounds **31** and **32** were evaluated for antinociceptive activity in a rat inflammatory pain model, and the results are shown alongside the previously reported results for **1** and the corresponding metabolite **2** (when dosed orally as **2**).[1] Inflammatory pain was induced by intraplantar injection of complete Freund's adjuvant (CFA) into the hind paws of rats.[9] Mechanical allodynia was assessed at 1 and 3 h post a single oral dose. Although **1** exhibits significant efficacy in this model, it is impossible to assess the contribution from the efficacious metabolite **2**, which is devoid of significant $Ca_v2.2$ activity and acts via an undetermined mechanism.[1] Compound **31** exhibited modest efficacy (21% and 30%, respectively) following a 10 mg/kg dose. Compound **32** showed robust activity following a 10 mg/kg oral dose, with 36% and 49% reversal of mechanical allodynia at 1 and 3 h time points, respectively. This activity increased to 57% and 54% reversal at 1 and 3 h, respectively, following a 30 mg/kg oral dose. Therefore, compound **32** achieved similar efficacy to **1** in the rat CFA model without the confounding contribution of metabolite **2**.

It is interesting that despite the 6-fold greater potency for compound **31** over **32**, and higher oral exposure for **31** in rat PK studies, **31** was considerably less efficacious in rat CFA model. Although the full biodistribution for these compounds into the CSF and spinal cord is not available, we infer that biodistribution differences may contribute to the discrepancy in efficacy based on in vitro studies. Sulfone **31** is a more robust Pgp substrate than **32**, which may lead to lower CNS exposure and thus lower efficacy. In addition, **31** was determined to have a lower unbound fraction in 100% rat plasma (1.3%) than **32** (6.8%), which may also contribute to lower unbound exposure of **31** for biodistribution into the CNS.

Although neither **31** nor **32** were suitable to be considered as a development candidate due to the issues of Pgp substrate and low in vivo efficacy (for **31**) and PXR activity (for **32**), these compounds demonstrated significant metabolic advantage over sulfonamide series and their potential utility as potent and selective $Ca_v2.2$ inhibitors. With these modifications, we have demonstrated that the combination of hydrophilic substitutions such as methylsulfone and lipophilic substitutions of $CF_3$ and $OCF_3$ on the benzamide phenyl ring resulted in significant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improvement of $Ca_v2.2$ potency, as well as mitigating PXR activation. In the sulfonamide series, formation of the primary sulfonamide metabolite **2** in vivo was endemic to the lead class. This issue was eventually addressed successfully by employing a bioisostere replacement strategy. The corresponding *gem*-dimethyl sulfone compounds exhibited similar $Ca_v2.2$ in vitro potency and efficacy to their sulfonamide analogues, but without the confounding liability of long lasting and efficacious sulfonamide metabolite formation. Ongoing work to further optimize the overall profile and enhance in vivo efficacy in this new series will be published in the due course.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Details for the $Ca_v2.2$ FLIPR assay, and the synthesis and characterization of compounds **12**, **31**, and **32**. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

## ■ ABBREVIATIONS

AUC, area under the pharmacokinetic exposure curve; $Ca_v$, voltage-gated calcium channel; CFA, complete Freund's adjuvant; CL, pharmacokinetic clearance; CNS, central nervous system; conc, concentration; CSF, cerebrospinal fluid; CYP, cytochrome P450 enzymes; FLIPR, fluorometric imaging plate reader; hERG, human ether-a-go-go-related gene; IV, intravenous; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; Pgp, P-glycoprotein transporter; PK, pharmacokinetic; PO, per os or by mouth; PXR, pregnane X receptor; SAR, structure−activity relationship

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