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# Benzazepinone $Na_v1.7$ blockers: Potential treatments for neuropathic pain

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**Abstract**—A series of benzazepinones were synthesized and evaluated as $hNa_v1.7$ sodium channel blockers. Several compounds from this series displayed good oral bioavailability and exposure and were efficacious in a rat model of neuropathic pain.


Neuropathic pain is a chronic, debilitating pain state that results from injury to the peripheral or central nervous system. It is estimated to affect 4 million people in the US, and can be triggered by a variety of events or conditions, including diabetes, shingles and chemotherapy.[1] Because few effective therapies exist, patients suffering neuropathic pain are often prescribed anticonvulsants or topical anesthetics as treatment. Optimized for other indications, these agents typically offer only modest pain relief, and frequently elicit dose-limiting CNS-based side effects.

Neuropathic pain signaling begins with the aberrant firing of action potential bursts in damaged axons. The initiation and propagation of these action potentials typically require the opening of voltage-gated sodium channels ($Na_v1.x$). Because they can inhibit action potential firing, $Na_v1$ blockers have been investigated as treatments for neuropathic pain.[2–4] Weak blockers such as lidocaine, carbamazepine and ralfinamide have shown preclinical and/or clinical efficacy in the treatment of neuropathic pain, thereby providing validation for this approach.[5–8]

Recent data from human genetic studies have implicated $hNa_v1.7$, a subtype located primarily in the PNS, as a key constituent in pain signaling. Individuals with gain of function mutations in *SCN9A*, the gene that encodes $hNa_v1.7$, experience bouts of intense pain that are either evoked by mild stimuli or spontaneous in nature.[9,10] As such, their symptoms resemble those presented by neuropathic pain patients. In contrast, individuals with loss of function mutations in *SCN9A*—human $Na_v1.7$ knockouts—are viable, healthy, and normal in seemingly every regard, save one: they have a complete inability to sense pain.[11,12] Collectively, these studies provide compelling genetic validation for $hNa_v1.7$ as an important pain target.

Our goal is to develop $hNa_v1.7$ blockers as treatments for neuropathic pain. Toward that end, we recently reported the discovery of a structurally novel class of benzazepinone $hNa_v1.7$ blockers.[13] An exemplar of this class, compound **1**, displayed potent, state-dependent block of $hNa_v1.7$ in vitro, blocked spontaneous

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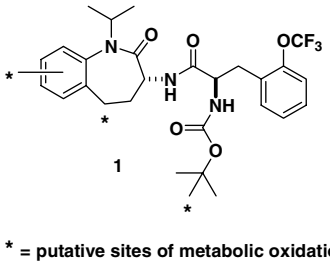
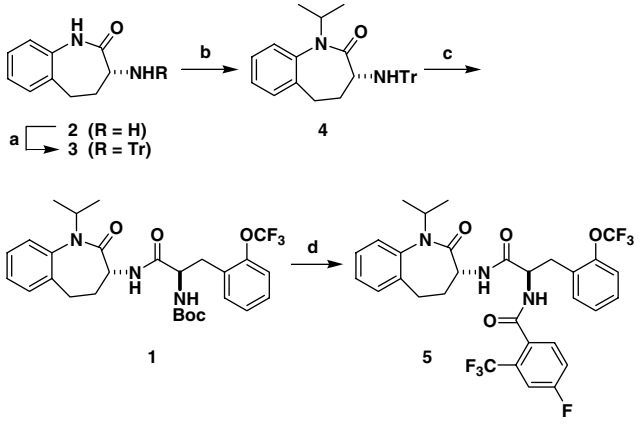



**Figure 1.** Benzazepinone $Na_v1.7$ sodium channel blocker **1**.

neuronal firing in vivo, and was orally efficacious in a rat model of neuropathic pain (Fig. 1). Because **1** exhibited modest pharmacokinetics (PK) in rat and dog, subsequent work in this series has focused specifically on improving PK. These efforts, described herein, have led to the discovery of compounds that display improved oral bioavailability and exposure as well as increased efficacy in a rat model of neuropathic pain.

The oral exposure of **1** was modest in rat and dog (rat: PO $AUC_N$ = 0.31 μM h/mpk; dog: PO $AUC_N$ = 0.27 μM h/mpk) and was limited by relatively high rates of oxidative metabolism and clearance (rat: $Cl_p$ = 24 mL/min/kg; dog: $Cl_p$ = 15 mL/min/kg). We thus sought to increase exposure by improving metabolic stability and reducing clearance. To determine its primary sites of metabolic oxidation, we incubated **1** in the presence of rat liver microsomes. Mass spectral analysis of the major metabolites revealed oxidation at the *N*-Boc *tert*-butyl group, at one or more sites on the benzazepinone phenyl ring (Fig. 1, C6–C9) and/or at the benzazepinone benzylic position (C5). Blocking or deactivating those sites became the main focus of our chemistry efforts, described below.

Analogs of **1** wherein the *N*-Boc group had been replaced were synthesized as shown in Scheme 1. The requisite starting material, (*R*)-3-amino-2,3,4,5-tetrahydro-1*H*-[1]-benzazepin-2-one **2**, was prepared according to the procedure of Armstrong and coworkers, then tritylated to yield compound **3**.[14] Treatment of **3** with sodium hydride and 2-iodopropane effected lactam alkylation to give *N*-isopropyl derivative **4**. Acid-catalyzed detritylation of **4** then furnished an amine that was coupled with *N*-Boc-D-2-$OCF_3$-phenylalanine to yield **1**.[15] Finally, exposure of **1** to standard conditions for *N*-Boc deprotection (TFA, $CH_2Cl_2$) gave an amine salt that could be coupled with 4-fluoro-2-(trifluoromethyl)benzoic acid to afford **5**, or with other commercially available carboxylic acids to provide products **6**–**19** (Table 1).

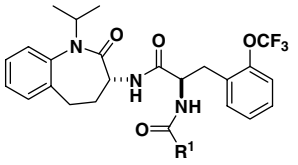



**Scheme 1.** Reagents and conditions: (a) TrCl, $Et_3N$, DMF (60%); (b) NaH, DMF, 2-iodopropane, 0–60 °C (72%); (c) HCl, MeOH; *N*-Boc-D-2-$OCF_3$Phe, EDC, HOBt, *i*-$Pr_2NEt$, THF; (d) TFA, $CH_2Cl_2$; 4-F-2-$CF_3$-benzoic acid, EDC, HOBt, *i*-$Pr_2NEt$, THF.

Once synthesized, compounds were then assayed for their ability to block $hNa_v1.7$. The extent of channel block was determined in a functional, membrane potential-based assay that measures the fluorescence resonance energy transfer (FRET) between two membrane-associated dyes. Specific details of the experimental protocols employed have recently been described.[16] Target compounds were also screened against other ion channels that are known to impact cardiac function. Because block of hERG $K^+$ channels has been associated with potentially lethal ventricular arrhythmias, compounds were tested in a binding assay that measures displacement of $^{35}S$-labelled MK-0499, a known hERG blocker.[17]

Prior work had shown that $hNa_v1.7$ block was optimized when the side chain incorporated a secondary amide or carbamate. Knowing that, we synthesized a series of analogs wherein the *N*-Boc group of **1** was replaced by a variety of secondary amides. In the alkylamide series, analogs with sterically smaller $R^1$ groups (Table 1, compounds **6**–**7**) displayed weak $hNa_v1.7$ block, while those with bulkier $R^1$ groups (compounds **8**–**10**) proved more potent. In the arylamide series, the simple benzamide derivative **11** exhibited best-in-class potency, but suffered from high activity in the MK-0499 counterscreen. Several substituted benzamides (compounds **12**–**15**), albeit less potent, were consider-

**Table 1.** Effect of the $R^1$ group on $hNa_v1.7$ potency




| Compound | $R^1$ | $hNa_v1.7$ ($IC_{50}$, nM) | MK-0499 (% inh at 10 μM) |
|---|---|---|---|
| **6** | $CH_3$ | >1000 | 9 |
| **7** | $CF_3$ | 868 | 40 |
| **8** | $C(CH_3)_3$ | 131 | 18 |
| **9** | $C(CF_3)_2CH_3$ | 203 | 10 |
| **10** | (*c*-Pr)$CH_3$ | 172 | 39 |
| **11** | Ph | 22 | 83 |
| **12** | 4-F-Ph | 94 | 26 |
| **13** | 4-$CF_3$-Ph | 175 | 43 |
| **14** | 2-$CF_3$-Ph | 98 | 0 |
| **15** | 2-$CF_3$-4-F-Ph | 128 | 31 |
| **16** | 2-Pyridyl | 177 | 61 |
| **17** | 4-Pyridyl | 453 | 44 |
| **18** | 2-Pyrimidinyl | >1000 | 15 |
| **19** | 5-Pyrimidinyl | >1000 | |

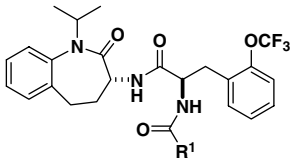
ably cleaner in that counterscreen. Finally, a wide variety of 5- and 6-membered heteroarylamides were prepared. Of the selected examples listed in Table 1, only 2-pyridyl analog **16** exhibited potent $hNa_v1.7$ block.

Several of the more potent secondary amides displayed improved rat PK profiles relative to *N*-Boc derivative **1** (Table 2).[18] Alkylamides **9** and **10**, for instance, showed increases in oral bioavailability and exposure ($AUC_N$). In the arylamide series, while the simple benzamide **11** exhibited a modest profile, substituted benzamide **15** and 2-pyridyl derivative **16** both improved upon the oral bioavailability (*F*%) and exposure of **1**. Along with **9** and **10**, these amides were deemed useful *N*-Boc replacements, and were incorporated into subsequent designs.

With a set of *N*-Boc replacements in hand, we turned our attention toward blocking metabolic oxidation of the benzazepinone core. As noted above, this oxidation was thought to occur at the benzazepinone benzylic position, and/or at various unspecified sites on the lefthand phenyl ring. Analogs wherein those positions were blocked or otherwise deactivated were synthesized as shown in Schemes 2 and 3.

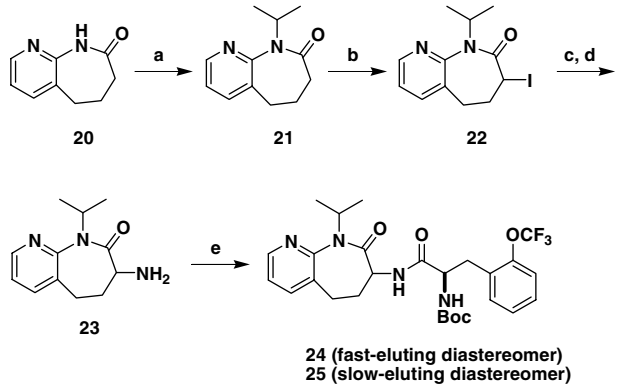
9-Aza-benzazepinones were prepared from known intermediate **20** (Scheme 2).[19] Upon treatment with sodium hydride and 2-iodopropane, **20** underwent alkylation to yield *N*-isopropyl derivative **21**. Exposure of that species to tetramethylethylenediamine (TMEDA), iodotrimethylsilane (TMSI) and iodine then effected iodination alpha to the lactam carbonyl to give **22**. Nucleophilic displacement of iodide with azide and subsequent hydrogenation furnished racemic amine **23**. That amine was then coupled with *N*-Boc-D-2-$OCF_3$-Phe under standard conditions (BOP, *i*-$Pr_2NEt$) to give two diastereomeric products, **24** and **25**, that were separable via flash chromatography on silica gel. For these compounds, the absolute stereochemistry of the stereocenter contained within the lactam (C7) was not determined.

**Table 2.** Rat pharmacokinetic data for secondary amides

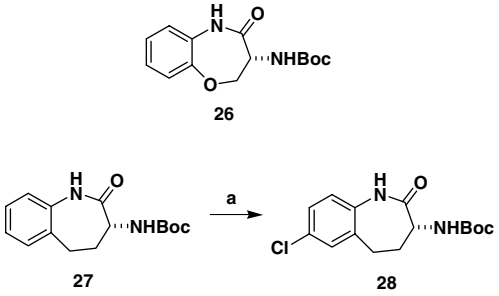


| Compound | $R^1$ | *F* (%) | $AUC_N$ [a] | $C_{max}$ [b] | $Cl_p$ [c] | $t_{1/2}$ [d] |
|---|---|---|---|---|---|---|
| **1** | $OC(CH_3)_3$ | 24 | 0.31 | 0.30 | 24 | 2.3 |
| **8** | $C(CH_3)_3$ | 16 | 0.16 | 0.14 | 31 | 1.8 |
| **9** | $C(CF_3)_2CH_3$ | 42 | 0.50 | 0.33 | 23 | 2.5 |
| **10** | (*c*-Pr)$CH_3$ | 35 | 0.65 | 0.67 | 20 | 1.4 |
| **11** | Ph | 18 | 0.13 | 0.15 | 43 | 1.5 |
| **12** | 4-F-Ph | 50 | 0.23 | 0.24 | 66 | 2.1 |
| **15** | 2-$CF_3$-4-F-Ph | 86 | 0.45 | 0.43 | 51 | 2.6 |
| **16** | 2-Pyridyl | 60 | 0.42 | 0.55 | 43 | 1.2 |

[a] (po, μM h/mpk).
[b] (μM).
[c] (mL/min/kg).
[d] (h).




**Scheme 2.** Reagents and conditions: (a) NaH, DMF, 2-iodopropane, 0–60 °C (75%); (b) TMSI, TMEDA, $I_2$, THF, −15 °C (66%); (c) $NaN_3$, DMF, rt (87%); (d) $H_2$ (1 atm), 10% Pd/C, EtOH; (e) *N*-Boc-D-2-$OCF_3$Phe, BOP, *i*-$Pr_2NEt$, $CH_2Cl_2$.



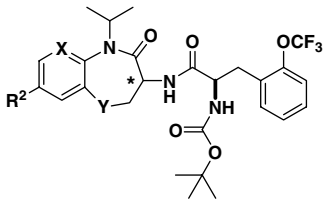

**Scheme 3.** Reagents and conditions: (a) NCS, DMF, 0 °C to rt (96%).

Benzazepinones substituted at the C5 or C7 positions were prepared from intermediates shown in Scheme 3. ((*R*)-8-Oxo-6,7,8,9-tetrahydro-5-oxa-9-aza-benzocyclohepten-7-yl)- carbamic acid *tert*-butyl ester **26** was synthesized via the procedure of DeVita and coworkers.[20] Once in hand, it was then processed via the reaction sequence shown in Scheme 1 for the conversion of **3**–**1** to yield 5-oxo analog **29** (Table 3). 7-Chlorobenzazepinones were prepared from known ((*R*)-2-oxo-2,3,4,5-tetrahydro-1*H*-benzo[*b*]azepin-3-yl)- carbamic acid *tert*-butyl ester **27**.[21] Treatment of **27** with *N*-chlorosuccinimide (NCS) in dimethylformamide (DMF) resulted in regioselective chlorination to yield **28**. That intermediate was then subjected to the reaction sequence shown in Scheme 1 for the conversion of **3**–**5** to yield 7-chlorobenzazepinone products **30**–**37** (Tables 3 and 5).

Using the methods outlined in Schemes 2 and 3, we synthesized a set of compounds wherein the C5–C9 positions were blocked or deactivated. As shown in Table 3, replacement of the C5 benzylic methylene unit with oxygen afforded a compound (**29**) that was equipotent to **1**. Conversion from the benzazepinone core of **1** to the 9-aza-benzazepinone of **24** and **25** was also well tolerated, with each diastereomer exhibiting potent $hNa_v1.7$ block. Finally, chlorination at C7 was permitted as evidenced by compound **30**.

Compounds **24**, **25**, **29**, and **30** were submitted for rat PK determination. Though the C5 benzylic methylene

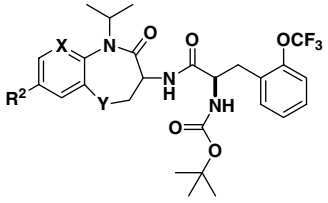
**Table 3.** Effect of benzazepinone substitution on $hNa_v1.7$ potency



| Compound | X | Y | * | $R^2$ | $hNa_v1.7$ ($IC_{50}$, nM) | MK-0499 (% inh at 10 μM) |
|---|---|---|---|---|---|---|
| **1** | C | $CH_2$ | R | H | 35 | 17 |
| **29** | C | O | R | H | 44 | 17 |
| **24** | N | $CH_2$ | Fast[a] | H | 80 | 7 |
| **25** | N | $CH_2$ | Slow[b] | H | 79 | 18 |
| **30** | C | $CH_2$ | R | Cl | 108 | 32 |

[a] Fast-eluting diastereomer.
[b] Slow-eluting diastereomer.

**Table 4.** Rat pharmacokinetic data for selected compounds



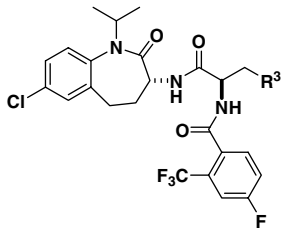
| Compound | X | Y | $R^2$ | *F* (%) | $AUC_N$[a] | $Cl_p$[b] | $t_{1/2}$[c] |
|---|---|---|---|---|---|---|---|
| **1** | C | $CH_2$ | H | 24 | 0.31 | 24 | 2.3 |
| **29** | C | O | H | 25 | 0.19 | 40 | 2.0 |
| **24** | N | $CH_2$ | H | 4 | 0.03 | 46 | 1.9 |
| **25** | N | $CH_2$ | H | 27 | 0.35 | 24 | 1.4 |
| **30** | C | $CH_2$ | Cl | 31 | 0.71 | 13 | 3.0 |

[a] (po, μM h/mpk).
[b] (mL/min/kg).
[c] (h).

of **1** was considered a potential site of metabolic oxidation, block of that site as in **29** actually yielded a compound with higher clearance and lower oral exposure (Table 4). Metabolic oxidation was also thought to occur at one or more sites on the benzazepinone phenyl ring. To mute that metabolism, the phenyl ring was replaced with a more electron-poor pyridine as in **24** and **25**. In accordance with prior results in this series, the two diastereomers exhibited markedly different pharmacokinetic profiles. Neither one, though, showed any improvement relative to **1**. In the end, it was block of a specific position—C7—that led to significant PK improvement. When compared with **1**, the 7-chloro derivative **30** displayed reduced clearance, increased oral exposure and a longer half-life, thus implicating C7 as a likely site of oxidative metabolism.

At this point, we had identified several PK-enhancing changes to the structure of **1** and were ready to combine them. We thus synthesized a set of 7-chlorobenzazepinones that incorporated the 2-$CF_3$-4-F-benzamide *N*-Boc replacement. Because substitution at the $R^3$ position was known to affect $hNa_v1.7$ block, we ran a

**Table 5.** Optimization of the benzazepinone $R^3$ substituent



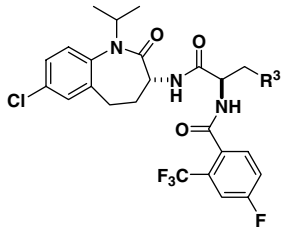
| Compound | $R^3$ | $hNa_v1.7$ ($IC_{50}$, nM) | MK-0499 (% inh at 10 μM) |
|---|---|---|---|
| **31** | 2-$OCF_3$-Ph | 169 | 7 |
| **32** | 2-F-Ph | 30 | 32 |
| **33** | 2,3-Di-F-Ph | 146 | 3 |
| **34** | 2,4-Di-F-Ph | >1000 | |
| **35** | 2,5-Di-F-Ph | 232 | 10 |
| **36** | 2,6-Di-F-Ph | 290 | 7 |
| **37** | 2,6-Di-Cl-Ph | 107 | 20 |

focused re-optimization of that domain (Table 5). Prior work had shown that a lipophilic aromatic group, preferably one bearing a 2-substituent, was required at $R^3$ for optimal potency and PK. Accordingly, the 2-$OCF_3$-Ph and 2-F-Ph derivatives **31** and **32** were synthesized and found to be potent $hNa_v1.7$ blockers. A series of di-F-Ph and di-Cl-Ph analogs were also prepared. Though the 2,4-di-F-Ph derivative **34** proved weak, the others all displayed comparably potent $hNa_v1.7$ block.

Compounds from this optimized series displayed improved rat PK profiles relative to benchmark **1**. The 2-$OCF_3$-Ph and 2-F-Ph derivatives **31** and **32**, for instance, both exhibited increased oral bioavailability and exposure (Table 6). While the 2,5-di-F-Ph compound **35** suffered a bit in comparison, the analogous 2,6-di-F-Ph and 2,6-di-Cl-Ph derivatives **36** and **37** provided additional gains in oral exposure and clearance.

Compounds **32** and **35** proved to be highly efficacious in a rat spinal nerve ligation model of neuropathic pain.[22] In this assay, rats undergo surgical ligation and transec-

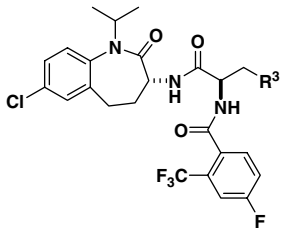
**Table 6.** Rat pharmacokinetic data for selected compounds



| Compound | $R^3$ | *F* (%) | $AUC_N$[a] | $C_{max}$[b] | $Cl_p$[c] | $t_{1/2}$[d] |
|---|---|---|---|---|---|---|
| **31** | 2-$OCF_3$-Ph | 65 | 0.57 | 0.52 | 29 | 2.6 |
| **32** | 2-F-Ph | 90 | 0.58 | 0.33 | 44 | 2.8 |
| **35** | 2,5-Di-F-Ph | 42 | 0.35 | 0.26 | 32 | 2.2 |
| **36** | 2,6-Di-F-Ph | 68 | 0.97 | 0.74 | 19 | 2.0 |
| **37** | 2,6-Di-Cl-Ph | 70 | 1.51 | 1.47 | 12 | 2.9 |

[a] (po, μM h/mpk).
[b] (μM).
[c] (mL/min/kg).
[d] (h).

**Table 7.** Pharmacodynamic efficacy data for selected compounds




| Compound | $R^3$ | Dose (po, mg/kg) | % Reversal of allodynia | |
|---|---|---|---|---|
| | | | 2 h | 4 h |
| **37** | 2,6-Di-Cl-Ph | 10.0 | 14 ± 4% | 9 ± 2% |
| **36** | 2,6-Di-F-Ph | 10.0 | 18 ± 5% | 35 ± 13% |
| **32** | 2-F-Ph | 10.0 | 56 ± 7% | 28 ± 3% |
| **35** | 2,5-Di-F-Ph | 10.0 | 55 ± 21% | 61 ± 16% |
| | Mexiletine | 30.0 | 40 ± 5% | 24 ± 3% |

tion of the L5 spinal nerve as a means of initiating neuropathy. Tactile allodynia is assessed both before and one week after surgery using calibrated Von Frey filaments. In rats that present significant allodynia, test compound is administered orally, and reversal of allodynia is then determined. When dosed orally at 10 mg/kg, compound **32** elicited significant reversal of allodynia at 2 h post dose (Table 7; % reversal @ 2 h, 4 h = 56%, 28%, $n = 8$). Compound **35** was equally efficacious at 2 h, and even more active at 4 hours post dose (% reversal @ 2 h, 4 h = 55%, 61%, $n = 8$). These results compare favorably with those we obtained using a 30 mg/kg dose of the clinical standard mexiletine (mexiletine: % reversal @ 2 h, 4 h = 40%, 24%).

Interestingly, several closely related analogs (**36** and **37**) that exhibited comparable in vitro potencies and oral exposures proved to be less efficacious in this model. The various factors underlying this discrepancy are not clear at this time.

In summary, we have reported studies aimed at improving pharmacokinetics in a previously disclosed series of benzazepinone $hNa_v1.7$ blockers. These efforts have culminated in the discovery of compounds that display significantly improved oral bioavailability and exposure when compared with a prior benchmark. Two compounds from this series also exhibit good efficacy in a rat model of neuropathic pain. Additional in vitro and pharmacological characterization of these compounds has been completed, and will be disclosed in due course.

## Acknowledgment

We thank Ramona Gray and Joe Leone for their outstanding technical contributions to this work.

## References and notes

1. Chen, H.; LaMer, T. J.; Rho, R. H.; Marshall, K. A.; Sitzman, B. T.; Ghazi, S. M.; Brewer, R. P. *Mayo Clin. Proc.* **2004**, *79*, 1533.
2. Ashcroft, F. M. *Ion Channels and Disease*; Academic Press: San Diego, 2000, pp 67–96.
3. Anger, T.; Madge, D. J.; Mulla, M.; Riddall, D. *J. Med. Chem.* **2001**, *44*, 115.
4. Amir, R.; Argoff, C. E.; Bennett, G. J.; Cummins, T. R.; Durieux, M. E.; Gerner, P.; Gold, M. S.; Porreca, F.; Strichartz, G. R. *J. Pain* **2006**, *7*(Suppl. 3), S1.
5. Harke, H.; Gretenkort, P.; Ladleif, H. U.; Rahman, S.; Harke, O. *Anesth. Analg.* **2001**, *92*, 488.
6. Kalso, E.; Tramer, M. R.; McQuay, H. J.; Moore, R. A. *Eur. J. Pain* **1998**, *2*, 3.
7. Mao, J.; Chen, L. L. *Pain* **2000**, *87*, 7.
8. Veneroni, O.; Maj, R.; Calabresi, M.; Favarelli, R. G.; Salvati, P. *Pain* **2003**, *102*, 17.
9. Dib-Hajj, S. D.; Rush, A. M.; Cummins, T. R.; Hisama, F. M.; Novella, S.; Tyrrell, L.; Marshall, L.; Waxman, S. G. *Brain* **2005**, *128*, 1847.
10. Fertleman, C. R.; Baker, M. D.; Parker, K. A.; Moffatt, S.; Elmslie, F. V.; Abrahamsen, B.; Ostman, J.; Klugbauer, N.; Wood, J. N.; Gardiner, R. M.; Rees, M. *Neuron* **2006**, *52*, 767.
11. Cox, J. J.; Reimann, F.; Nicholas, A. K.; Thornton, G.; Roberts, E.; Springell, K.; Karbani, G.; Jafri, H.; Mannan, J.; Raashid, Y.; Al-Gazali, L.; Hamamy, H.; Valente, E. M.; Gorman, S.; Williams, R.; McHale, D. P.; Wood, J. N.; Gribble, F. M.; Woods, C. G. *Nature* **2006**, *444*, 894.
12. Goldberg, Y. P.; MacFarlane, J.; MacDonald, M. L.; Thompson, J.; Dube, M.-P.; Mattice, M.; Fraser, R.; Young, C.; Hossain, S.; Pape, T.; Payne, B.; Radomski, C.; Donaldson, G.; Ives, E.; Cox, J.; Younghusband, H. B.; Green, R.; Duff, A.; Boltshauser, E.; Grinspan, G. A.; Dimon, J. H.; Sibley, B. G.; Andria, G.; Toscano, E.; Kerdraon, J.; Bowsher, D.; Pimstone, S. N.; Samuels, M. E.; Sherrington, R.; Hayden, M. R. *Clin. Genet.* **2007**, *71*, 311.
13. Hoyt, S. B.; London, C.; Gorin, D.; Wyvratt, M. J.; Fisher, M. H.; Abbadie, C.; Felix, J. P.; Garcia, M. L.; Li, X.; Lyons, K. A.; McGowan, E.; MacIntyre, D. E.; Martin, W. J.; Priest, B. T.; Ritter, A.; Smith, M. M.; Warren, V. A.; Williams, B. S.; Kaczorowski, G. J.; Parsons, W. H. *Bioorg. Med. Chem. Lett.* **2007**, 4630.
14. Armstrong, J. D.; Eng, K. K.; Keller, J. L.; Purick, R. M.; Hartner, F. W.; Choi, W.-B.; Askin, D.; Volante, R. P. *Tetrahedron Lett.* **1994**, *35*, 3239.
15. *N*-Boc-D-2-$OCF_3$-Phe was prepared using the method of Schollkopf: Schollkopf, U. *Tetrahedron* **1983**, *39*, 2085.
16. Felix, J. P.; Williams, B. S.; Priest, B. T.; Brochu, R. M.; Dick, I. E.; Warren, V. A.; Yan, L.; Slaughter, R. S.; Kaczorowski, G. J.; Smith, M. M.; Garcia, M. L. *Assay Drug Dev. Tech.* **2004**, *2*, 260.
17. Wang, J.; Della Penna, K.; Wang, H.; Karczewski, J.; Connolly, T. M.; Koblan, K. S.; Bennett, P. B.; Salata, J. J. *Am. J. Physiol. Heart Circ. Physiol.* **2002**, *284*, H256.
18. Rat PK experiments were conducted as follows: test compounds were typically formulated as 1.5 mg/mL solutions in mixtures of PEG300/water or DMSO/PEG300/water. Fasted male Sprague–Dawley rats were given either a 1.0 mg/kg iv dose of test compound solution via a cannula implanted in the femoral vein ($n = 3$) or a 3.0 mg/kg po dose by gavage ($n = 3$). Serial blood samples were collected at 5 (iv only), 15, and 30 min, and at 1, 2, 4, 6, and 8 h post dose. Plasma was collected by centrifugation, and plasma concentrations of test compound were determined by LC–MS/MS following protein precipitation with acetonitrile.

19. Jossang-Yanagida, A.; Gansser, C. *J. Heterocycl. Chem.* **1978**, *15*, 249.
20. DeVita, R. J.; Schoen, W. R.; Doldouras, G. A.; Fisher, M. H.; Wyvratt, M. J.; Cheng, K.; Chan, W. W.-S.; Butler, B. S.; Smith, R. G. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1281.
21. Armstrong, J. D.; Eng, K. K.; Keller, J. L.; Purick, R. M.; Hartner, F. W.; Choi, W.-B.; Askin, D.; Volante, R. P. *Tetrahedron Lett.* **1994**, *35*, 3239.
22. Chaplan, S. R.; Bach, F. W.; Pogrel, J. W.; Chung, J. M.; Yaksh, T. L. *J. Neurosci. Methods* **1994**, *53*, 55.