Bioorganic & Medicinal Chemistry Letters 19 (2009) 5329–5333

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of a novel class of isoxazoline voltage gated sodium channel blockers

Pengcheng P. Shao [a,*], Feng Ye [a], Ann E. Weber [a], Xiaohua Li [a], Kathryn A. Lyons [a], William H. Parsons [a], Maria L. Garcia [b], Birgit T. Priest [b], McHardy M. Smith [b], John P. Felix [b], Brande S. Williams [b], Gregory J. Kaczorowski [b], Erin McGowan [c], Catherine Abbadie [c], William J. Martin [c], Daniel R. McMasters [d], Ying-Duo Gao [d]

[a] Department of Medicinal Chemistry, Merck Research Laboratories, Rahway, NJ 07065, USA
[b] Department of Ion Channels, Merck Research Laboratories, Rahway, NJ 07065, USA
[c] Department of Pharmacology, Merck Research Laboratories, Rahway, NJ 07065, USA
[d] Department of Molecular Modeling, Merck Research Laboratories, Rahway, NJ 07065, USA

ARTICLE INFO

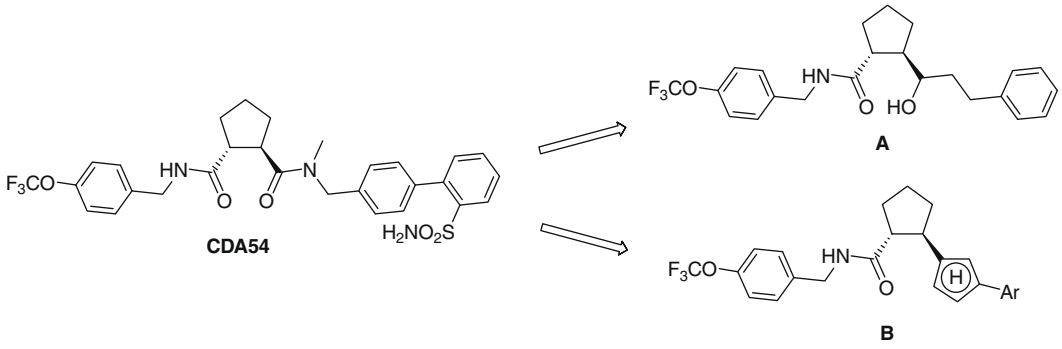
Article history:
Received 6 June 2009
Revised 27 July 2009
Accepted 28 July 2009
Available online 6 August 2009

Keywords:
Voltage gated sodium channel blocker
Neuropathic pain
Inflammatory pain
Amide replacement
Heterocycles
Isoxazoline

ABSTRACT

Analogs of the previously reported voltage gated sodium channel blocker **CDA54** were prepared in which one of the amide functions was replaced with aromatic and non-aromatic heterocycles. Replacement of the amide with an aromatic heterocycle resulted in significant loss of sodium channel blocking activity, while non-aromatic heterocycle replacements were well tolerated.

In an earlier communication, we reported our discovery of **CDA54**, a cyclopentane dicarboxamide compound, as a potent and orally bioavailable state-dependent sodium channel blocker.[1] Following this work, SAR studies with one of the amide moieties in **CDA54** replaced by alcohol (compound **A**) were reported.[2] In the present study, we wish to report our findings with heterocycle replacements of the amide group (compound **B**).



The use of heterocycles as amide surrogates is a common practice in medicinal chemistry. This substitution introduces structural rigidity, which may lead to compounds with improved selectivity, metabolic stability and enhanced pharmacokinetic properties. It is well established that the lone electron pair on the amide nitrogen can delocalize into the π^* orbital of the C=O double bond. This provides the C–N single bond with some double bond character and

* Corresponding author. Tel.: +1 732 594 2955.
E-mail address: pengcheng_shao@merck.com (P.P. Shao).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.07.125

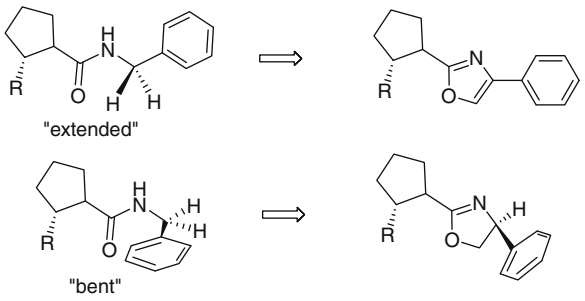


Figure 1. Mimicking amide conformation with heterocyles.

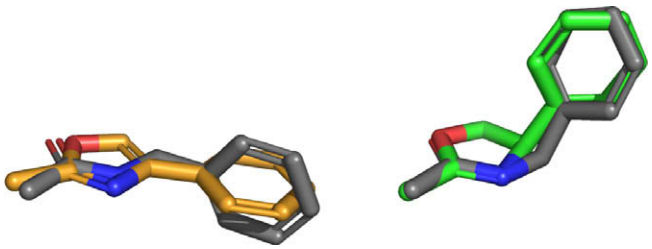
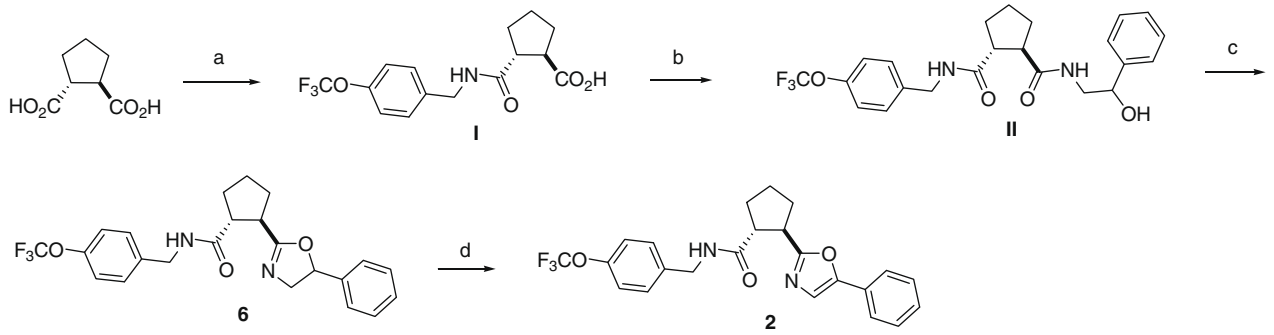


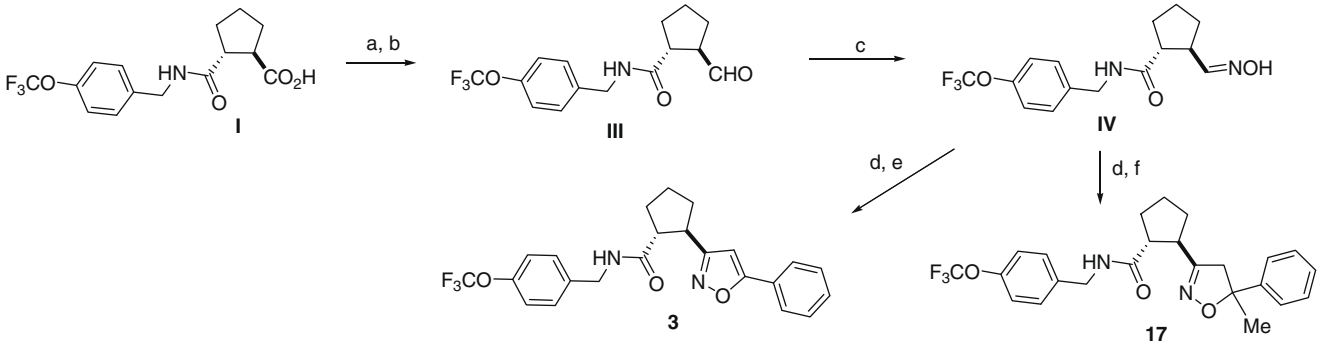
Figure 2. Overlay of *N*-benzylacetamide (grey carbon atoms) with heterocyclic analogs. Left: extended conformation overlaid with isoxazole (orange carbon atoms). Right: bent conformation overlaid with isoxazoline (green carbon atoms). The molecules were superimposed with respect to amide/heterocycle and aromatic centroid.

slows its rotation. Hence, the amide conformation (the plane defined by OCN) is relatively rigid.[3] However, the bond rotational barrier between the benzylic carbon and the amide nitrogen is relatively small. Rotation of this bond places the phenyl group at different positions relative to the plane defined by OCN in the amide. We hypothesized that the position of this phenyl ring is important as it is likely to contribute significantly to its binding to the ion channels. Among many possible conformations, two relatively low-energy conformations are illustrated in Figure 1: an 'extended' conformation with the phenyl ring centered in the plane defined by the amide, or a 'bent' conformation with the phenyl ring extending traverse to the amide plane. If the 'extended' conformation is the active conformation, replacing the amide with an aromatic heterocyclic moiety should not alter the phenyl position significantly. On the other hand, if it adopts a 'bent' conformation, typical aromatic heterocycle replacement of the amide should not be tolerated. To mimic this conformation, non-aromatic heterocyles such as oxazoline and isoxazoline may be used. This design feature becomes more apparent during molecular modeling.[4] By overlaying with respect to both the amide/heterocycle and phenyl ring, good overall superposition was observed between both pairs: extended conformation with isoxazole and bent conformation with isoxazoline (Fig. 2). Therefore, SAR studies were conducted to determine if state-dependent sodium channel blocking activity would remain after replacement of one of the amides with either type of heterocycles.

Our initial effort focused on replacing the sulfamoyl biphenylmethyl amide of **CDA54** and retaining the 4-trifluoromethoxybenzyl amide. The synthesis of 5-phenyl oxazole and oxazoline derivatives



Scheme 1. Reagents and conditions: (a) THF, CDI (1.1 equiv), 25 °C, 1 h; then 4-trifluoromethoxybenzyl amine (1.2 equiv) (67%); (b) THF, EDC (1.2 equiv), HOBt (1.2 equiv), DIEA (4 equiv), ±2-amino-1-phenyl ethanol, 25 °C, 30 min, (92%); (c) CH_2Cl_2, DAST (1.1 equiv), K_2CO_3 (1.5 equiv), −78 °C to 25 °C (52%); (d) $CHCl_3$, MnO_2 (10 equiv), 90 °C, 16 h (32%).



Scheme 2. Reagents and conditions: (a) THF, BH_3 (2.5 equiv), −78 °C, 15 min (58%); (b) CH_2Cl_2, DMSO (2 equiv), oxalyl chloride (1.5 equiv), TEA (4 equiv) −78 °C to 25 °C (89%); (c) CH_2Cl_2, hydroxylamine hydrochloride (6 equiv), Na_2CO_3 (2 M, 10 equiv), 40 °C, 16 h (95%); (d) CH_2Cl_2, NCS (1.2 equiv), 25 °C, 1 h; (e) phenylacetylene (5 equiv), TEA (2 equiv) slow addition, 25 °C, 16 h (47% -two steps); (f) 2-phenylpropene (5 equiv), TEA (2 equiv) slow addition, 25 °C, 16 h (77% -two steps).

2 and **6** is illustrated in Scheme 1. 4-phenyl oxazole and oxazoline derivative **1** and **5** were synthesized according to the same scheme except 2-amino-2-phenyl ethanol was used. Activating (±)-*trans*-cyclopentane dicarboxylic acid with one equivalent of CDI, followed by addition of excess 4-trifluoromethoxybenzylamine gave the mono acid intermediate **I**, along with a small amount of unreacted diacid and symmetrical diamide by-product. Intermediate **I** was isolated in 67% yield after acid-base work up.[5] It was then coupled with various amino alcohols by a standard EDC coupling procedure to give diamide **II**. Intermediate **II** was cyclized to form oxazoline derivative such as **6** according to a procedure developed by Wipf and co-workers.[6] Oxazole derivatives such as **2** were conveniently synthesized from oxazoline compound by treatment of activated MnO_2, although the yield for the step was rather low.

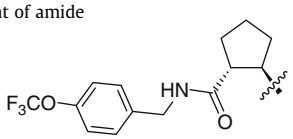
The synthesis of isoxazole and isoxazoline derivatives was demonstrated with synthesis of compound **3** and **17**, outlined in Scheme 2. A borane reduction of mono acid **I**, followed by Swern oxidation of the primary alcohol reduction product, afforded aldehyde **III**. Aldehyde **III** was treated with hydroxylamine to give oxime **IV** in excellent yield. Chlorination of the oxime with NCS gave hydroximoyl chloride. Without isolation, hydroximoyl chloride was treated with TEA to form the corresponding nitrile oxide, which underwent 1,3-dipolar addition with selected alkyne or alkene reagents in situ to give isoxazole compound (**3**) as a pair of enantiomers or isoxazoline compound (**17**) as an equal mixture of four diastereiomers.[7] Maintaining a low concentration of nitrile oxide by slow addition of base (TEA) was the key to achieving a good yield for this cycloaddition step.

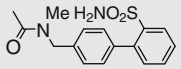
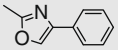
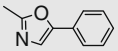
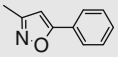
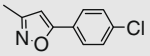
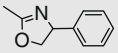
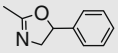
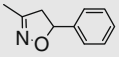
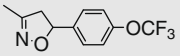
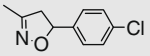
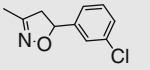
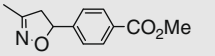
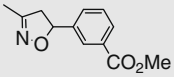
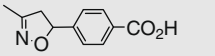
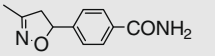
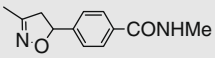
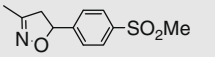
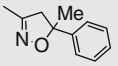
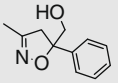
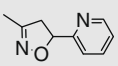
Based on our previous results, majority of compounds in cyclopentane series are state-dependent voltage gated sodium channel blockers but not subtype selective.[1] Most of compounds in this study were evaluated only for their ability to block the $hNa_v1.7$ channel.[8] It should be noted that for the initial assay screening, the oxazoles and isoxazoles such as **1** and **3** were examined as racemic mixtures. Similarly, the oxazoline and isoxazolines such as **5** and **17** were initially examined as mixtures of diastereomers. The extent of the channel block was determined in a functional, membrane potential-based assay that measures the fluorescence resonance energy transfer (FRET) between two membrane-associated dyes.[9]

Our initial results suggested that the amide may not have an "extended" conformation since replacing amide with oxazole (**1**, **2**) and isoxazole (**3**, **4**) moieties led to significant loss of potency, while oxazoline compounds (**5**, **6**) showed better $Na_v1.7$ inhibitory activity (Table 1). Since oxazoline is prone to hydrolysis in acidic media, we shifted our focus to the more stable isoxazoline series. Isoxazoline compound **7** showed potency similar to the oxazoline compounds (**5**, **6**). Lipophilic substitutions on the phenyl ring are well tolerated and improve potency slightly regardless of the position of substitution (**8**–**12**). However, polar substitutions (**13**–**16**) resulted in significant loss of Nav1.7 inhibitory activity. A methyl group at the 5-position of the isoxazoline did not affect potency (**17**). However, polar substitution (hydroxymethyl) at this position was not tolerated (**18**). Replacing the phenyl group with pyridine led to complete loss of activity (**19**).

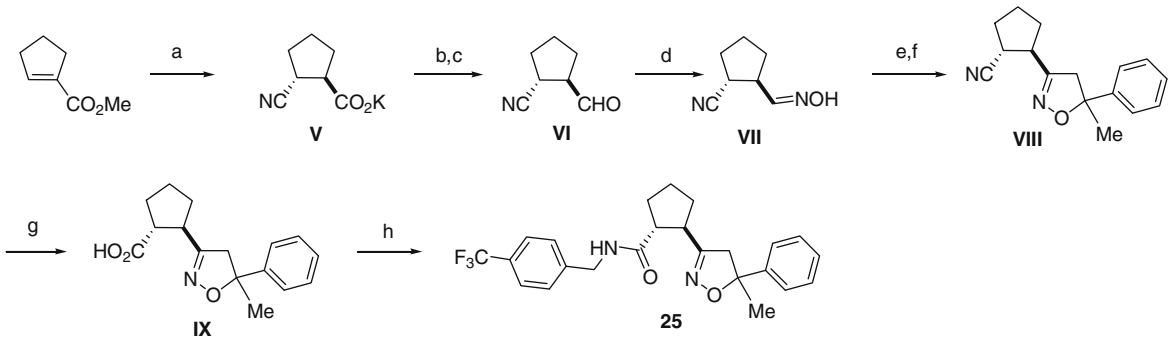
Once isoxazoline was identified as a good replacement for the amide, we set out to search for a 4-trifluoromethoxybenzamide replacements. In order to efficiently modify the amide moiety, an alternative synthetic scheme was developed. As illustrated in Scheme 3 for the synthesis of **25**, the isoxazoline was installed earlier in the synthetic sequence and the amide coupling reaction was positioned as the last step. Heating a reaction mixture of methylcyclopent-1-ene-1-carboxylate and KCN in DMF with a slight excess of water at 160 °C resulted in predominantly trans Michael addition product **V**. It is noteworthy that under these reaction

Table 1
Heterocycle replacement of amide



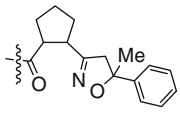
Compound		$Na_v1.7$ VIPR	
		IC_{50}* (μM)	% inh at 1 μM (%)
CDA54		0.83	
1		ND	19
2		ND	0
3		ND	38
4		ND	33
5		0.97	56
6		0.62	71
7		0.78	76
8		0.50	93
9		0.43	92
10		0.48	90
11		0.34	87
12		0.71	75
13		ND	0
14		ND	0
15		ND	14
16		ND	26
17		0.62	63
18		ND	23
19		ND	0

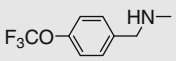
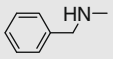
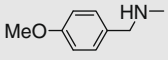
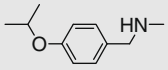
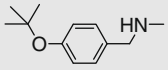
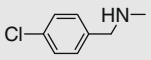
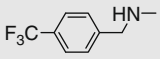
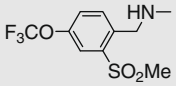
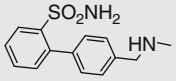
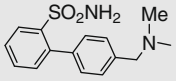
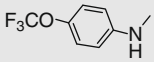
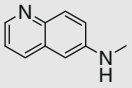
* IC_{50} and % inhibition data were average of two experiments.



Scheme 3. Reagents and conditions: (a) DMF, KCN (powder, 1.2 equiv), H_2O (1.5 equiv), 160 °C, 1 h; (b) THF, ethylchloro formate (1.2 equiv), 0 °C, 1 h, $NaBH_4$ (1.5 equiv), 25 °C, 30 min; (c) CH_2Cl_2, DMSO (2 equiv), oxalyl chloride (1.5 equiv), TEA (4 equiv) −78 °C to 25 °C; (d) CH_2Cl_2, hydroxylamine hydrochloride (1.5 equiv), Na_2CO_3 (2 M, 2 equiv), 50 °C, 0.5 h (55% -four steps); (e) CH_2Cl_2, NCS (1.2 equiv), 25 °C, 2 h; (f) 2-phenylpropene (5 equiv), TEA (2 equiv) slow addition, 25 °C, 16 h (82% -two steps); (g) HCl (concd), 90 °C, 2 h (95%); (h) THF, BOP (1.5 equiv), DIEA (3 equiv), 4-trifluoromethylbenzyl amine (2 equiv), 25 °C, 30 min (92%).

Table 2
Modification to 4-trifluoromethoxybenzamide



Compound		$Na_v1.7$ VIPR IC_{50} (μM) or % inh at 1 μM*
17		0.62
20		19%
21		16%
22		1.5
23		26%
24		15%
25		2.5
26		13%
27		0%
28		0%
29		30%
30		17%

* IC_{50} and % inhibition data were average of two experiments.

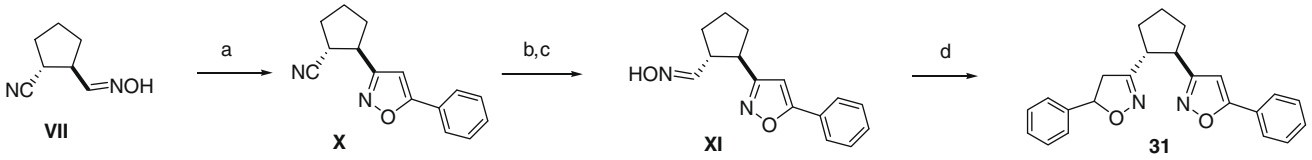
conditions, the methyl ester was hydrolyzed and **V** was isolated as the potassium salt. Potassium carboxylate **V** was elaborated to oxime **VII** under standard chemical transformation procedures in good overall yields. Chlorination of the oxime with NCS gave hydroximoyl chloride. Without isolation, the hydroximoyl chloride was treated with TEA to form the nitrile oxide, which underwent 1,3-dipolar addition with 2-phenylpropene in situ to give isoxazoline derivatives **VIII**. Nitrile **VIII** was hydrolyzed under strong acidic conditions to give carboxylic acid **IX**. It is noteworthy that isoxazoline is stable under these harsh reaction conditions. Carboxylic acid **IX** was converted to the desired amide products **25** under a standard amide coupling procedure in excellent yields, again as mixtures of diastereomers.

As shown in Table 2, it appears that 4-trifluoromethoxy substitution is optimal for conferring Nav1.7 inhibitory activity, and that we were unable to identify a superior replacement (**20**–**30**). It is also noteworthy that biphenylsulfonamide rendered compounds completely inactive even though it brought many positive attributes (potency and selectivity) to the diamide series (**CDA54**).

A series of bisheterocyclic compounds with both amides of **CDA54** replaced with heterocycles were also investigated briefly. Synthesis of these compounds is illustrated in Scheme 4 for the synthesis of **31**. These compounds (**31**–**34**) are generally less potent than the mono amide series (Table 3).

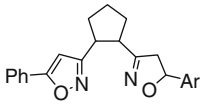
Compound **17** has good PK properties (Sprague-Dawley rat, $t_{1/2}$: 2.9 h; Clp: 17.8 mL/min/kg; normalized AUC: 0.82 μM h kg/mg and F%: 16%). It was further evaluated in the CFA rat pain model (intradermal injection of complete Freund's adjuvant).[10] It showed good efficacy with 20% and 48% reversal of mechanic allodynia at 2 h and 3 h, respectively, after a 3 mg/kg oral dose. As described above, compound **17** is a diastereomeric mixture of four isomers. In order to evaluate each diastereomer, they were separated by HPLC with a chiral column.[11] **17a** and **17c** showed slightly higher potency than **17b** and **17d** in the functional $hNa_v1.7$ assay. However, **17a** showed the best efficacy in CFA with 53% reversal of allodynia at 3 h post a 3 mg/kg oral dose. **17a** is more efficacious than **CDA54** in the CFA rat pain model at the same dose (53% and 31% reversal of allodynia, respectively). However, **17a** is less efficacious in the rat spinal nerve ligation (SNL) model of neuropathic pain than **CDA54**.[12] When dosed orally at10 mg/kg, **17a** and **CDA54** gave 21% and 35% reversal of allodynia, respectively, at 3 h post dose (Table 4).

In summary, heterocyclic replacement of the amide in **CDA54** was explored. Conformational analysis led us to the rational design of novel oxazoline and isoxazoline voltage gated sodium channel blockers. Further SAR studies showed that lipophilic substitutions on phenyl and isoxazoline rings were well tolerated, but not polar substitutions. Compound **17a** was evaluated in rat CFA and SNL pain model and showed good efficacy in rat CFA model.



Scheme 4. Reagents and conditions: (a) CH_2Cl_2, phenylacetylene (5 equiv), NaClO (10% solution, 2 equiv, slow addition), 25 °C, 8 h, (52%); (b) toluene, DIBAH (1 M toluene solution, 2 equiv) −78 °C to 0 °C (48%); (c) CH_2Cl_2, hydroxylamine hydrochloride (1.5 equiv), Na_2CO_3 (2 M, 2 equiv), 25 °C, 2 h (95%); (d) CH_2Cl_2, phenyl acetylene (3 equiv), NaClO (10% solution, 2 equiv, slow addition over 30 min), 25 °C, 16 h, (82%).

Table 3
Compounds with both amide replaced with hetercycles



Compound	Ar	$Na_v1.7$ VIPR % inh at 1 μM* (%)
31	Ph	13
32	4-CF_3OPh	18
33	3,5-Dichloro-Ph	35
34	4-$MeSO_2Ph$	43

* % inhibition data were average of two experiments.

Table 4
In vivo efficacy of the four isomers of compound **17**

Compound	$Na_v1.7$ VIPR		Dose (po, mg/kg)	% Reversal of allodynia 3 h after dosing	
	IC_{50}* (μM)	% inh at 1 μM* (%)		CFA (%)	SML (%)
17	0.62	63	3	48	
17a	0.96	64	3	53	
			10		21
17b	ND	37	3	26	
17c	1.2	48	3	20	
17d	ND	35	3	17	
CDA54	0.83		3	31	
			10	54	35

* IC_{50} and % inhibition data were average of two experiments.

Acknowledgments

The authors would like to thank Dr. Joseph Duffy for proof-reading the manuscript. The authors would also like to thank Dr. Richard Hargreaves and Dr. Joseph Duffy for their helpful suggestions on subtype selectivity issues. The authors were also grateful to Dr. Samer Eid and Mr. Christopher J. Daley for their contribution on $Na_v1.5$ assay.

References and notes

1. (a) Shao, P.; Ok, D.; Fisher, M. H.; Garcia, M. L.; Kaczorowski, G. J.; Li, C.; Lyons, K. A.; Martin, W. J.; Meinke, P. T.; Priest, B. T.; Smith, M. M.; Wyvratt, M. J.; Ye, F.; Parsons, W. H. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1901; (b) Brochu, R. M.; Dick, I. E.; Tarpley, J. W.; McGowan, E.; Gunner, D.; Herrington, J.; Shao, P.; Ok, D.; Li, C.; Parsons, W. H.; Stump, G. L.; Regan, C. P.; Lynch, J. J., Jr.; Lyons, K. A.; McManus, O. B.; Clark, S.; Ali, Z.; Kaczorowski, G. J.; Martin, W. J.; Priest, B. T. *Mol. Pharmacol.* **2006**, *69*, 823.
2. Ok, D.; Li, C.; Abbadie, C.; Felix, J. P.; Fisher, M. H.; Garcia, M. L.; Kaczorowski, G. J.; Lyons, K. A.; Martin, W. J.; Priest, B. T.; Smith, M. M.; Williams, B. S.; Wyvratt, M. J.; Parsons, W. H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1358.
3. Avalos, M.; Babiano, R.; Barneto, J. L.; Bravo, J. L.; Cintas, P. *J. Org. Chem.* **2001**, *66*, 7275.
4. Conformations of *N*-benzylacetamide, 2-methyl-4-phenyl-1,3-oxazole and 2-methyl-4-phenyl-4,5-dihydro-1,3-oxazole were optimized using the MMFFs force field with a distance-dependent dielectric of 4r. For 2-methyl-4-phenyl-4,5-dihydro-1,3-oxazole, two conformations were found which differed in ring pucker; the lower-energy conformation was used for the overlay shown in Figure 2. Molecules were superimposed by minimizing RMSD between the methyl carbon; the N, C, and O of the heterocycle or amide; and the phenyl ring centroid.
5. Intermediate **I** was extracted into basic aqueous layer, while diamide by-product was washed away with EtOAc. Aqueous portion was then acidified and intermediate **I** was extracted into EtOAc. Since unreacted diacid starting material has higher aqueous solubility, this workup gave essentially pure intermediate **I**.
6. Phillips, A. J.; Uto, Y.; Wipf, P.; Reno, M. J.; Williams, D. R. *Org. Lett.* **2000**, *2*, 1165.
7. Grundmann, C. *Synthesis* **1970**, *7*, 344.
8. Selection of compounds were evaluated for blocking $hNa_v1.5$ channel and no selectivity were observed between $hNa_v1.5$ and $hNa_v1.7$ channels.
9. 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. L.; Garcia, M. L. *Assay Drug Dev. Tech.* **2004**, *2*, 260.
10. Colpaert, F. C.; Meert, T.; De Witte, P. *Life Sci.* **1982**, *31*, 67.
11. Chiral OJ column, 15 × 250 mm; mobile phase: 12% IPA, 88% heptane; flow rate: 9 ml/min. Retention time for the four isomers: 17, 23, 31, 37 min.
12. Chaplan, S. R.; Bach, F. W.; Pogrel, J. W.; Chung, J. M.; Yaksh, T. L. *J. Neurosci. Methods* **1994**, *53*, 55.