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Discovery of isoxazole voltage gated sodium channel blockers for treatment of chronic pain

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ABSTRACT

A series of novel isoxazole voltage gated sodium channel blockers have been synthesized and evaluated. Substitutions on the benzylic position of benzamide were investigated to determine their effect on $Na_v1.7$ inhibitory potency. The spirocyclobutyl substitution had the most significant enhancement on $Na_v1.7$ inhibitory activity.

In the previous Letter, we reported our approach to replace one of the amide groups in **CDA54** with heterocycles. Our initial results showed that replacing the amide with aromatic heterocycles such as oxazole and isoxazole resulted in significant loss of potency, while oxazoline and isoxazoline (**1**) appear to be well tolerated.[1] However, all the compounds examined at that point did not have substitutions at the benzylic position of the remaining benzamide. Since substitutions at benzylic position are expected to change the free energy difference between 'bent' and 'extended' conformations (Fig. 1), this should have significant consequences on the population ratio between the two conformations and thus in the inhibitory potency of the compound.

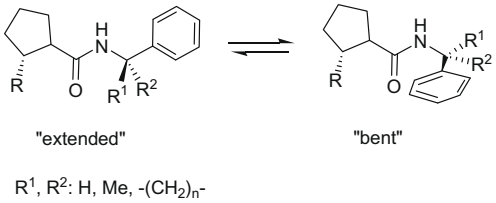
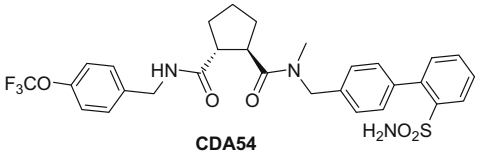
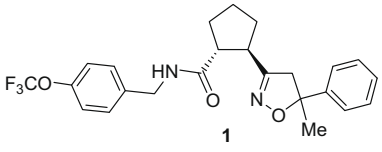


Figure 1. Equilibrium between 'extended' and 'bent' conformations.



CDA54



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The synthesis of isoxazole derivatives with substitutions on the benzylic position of benzamide is illustrated in Scheme 1. Benzyl amine derivatives such as intermediate **III** were conveniently synthesized according to the procedure developed by Ellman's group.[2]

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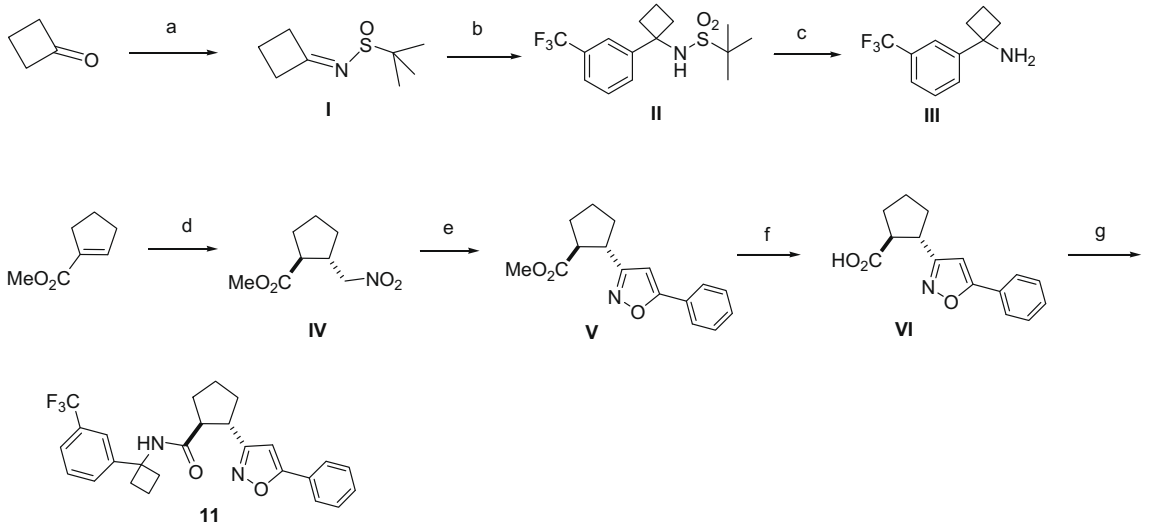
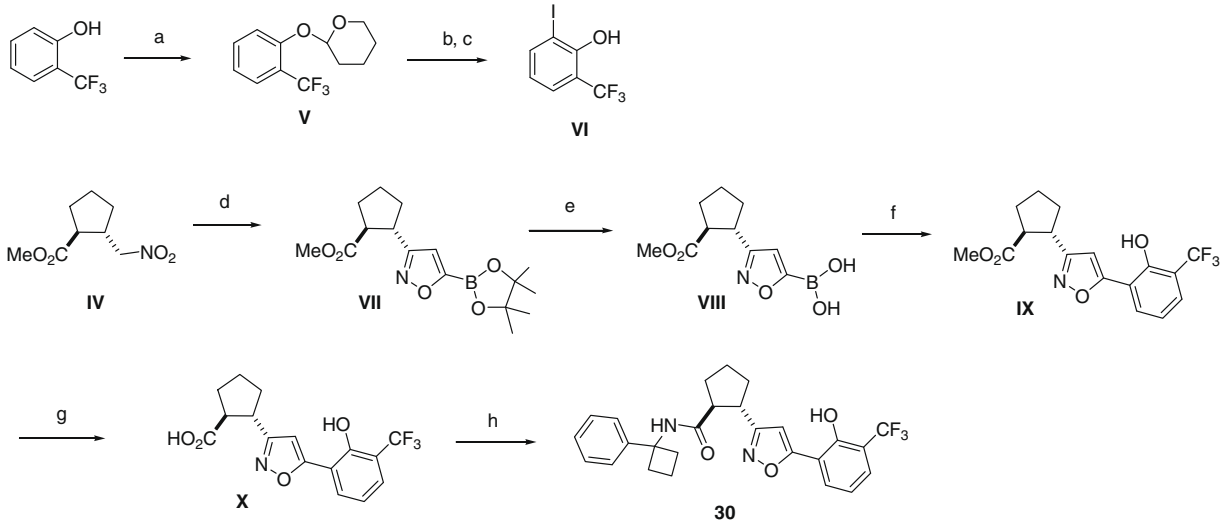
Other phenylcyclobutane amine derivatives with substitutions (F, OH, and OMe) on the cyclobutane ring were synthesized according to a previously published procedure.[3] The isoxazole intermediate such as **VI** was synthesized in a three-step sequence starting from 1-cyclopentenecarboxylic acid. TBAF-catalyzed Michael addition[4] between nitromethane and 1-cyclopentenecarboxylic acid proceeded with good selectivity (8:1) and favored the desired *trans* isomer. The minor, *cis* isomer was removed by flash chromatography. Intermediate **IV** was treated with phenylisocyanate and TEA to form the nitrile oxide, which underwent 1,3-dipolar cycloaddition with phenylacetylene in situ to form isoxazole **V**.[5] Hydrolysis of the methyl ester, followed by standard BOP-mediated amide coupling afforded the final isoxazole compound **11**.

In cases where acetylene derivatives were not readily available, Suzuki coupling reaction utilizing available halides was a convenient method to construct these compounds (Scheme 2). 1,3-Dipolar addition involving ethynyl boronic ester[6] gave the boronic acid derivative **VIII** in moderate yield after hydrolysis of the boronic ester **VII**. Suzuki coupling reaction between iodide **VI** and boronic acid **VIII** proceeded under mild conditions to provide the desired coupling product **IX** in good yield. Hydrolysis of the methyl ester, followed by standard amide coupling reaction yielded the desired isoxazole compound **30**.

Once synthesized, the compounds were evaluated for their ability to block the voltage gated sodium channels stably expressed in a HEK-293 cell line. In the present Letter, only $hNa_v1.7$ activity was evaluated. 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.[7] Compounds showing good potency in

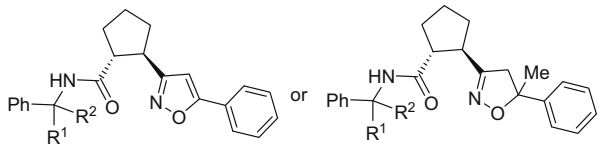


Scheme 1. Reagents and conditions: (a) THF, *t*-butylsulfinamide (1 equiv), $Ti(OEt)_4$ (2 equiv), 60 °C, 14 h (92%); (b) THF, 1-bromo-3-trifluoromethyl benzene (2 equiv), *n*BuLi (1.6 M in hexane, 2 equiv), −78 °C (46%); (c) MeOH, HCl (concd, excess), 25 °C, 1 h (95%); (d) CH_3NO_2 (3 equiv), TBAF (1 M in THF, 0.1 equiv), 70 °C, 20 h (83%); (e) toluene, phenylacetylene (5 equiv), phenylisocyanate (5 equiv), TEA (3 equiv), 120 °C, 3 h (72%); (f) MeOH, LiOH (10 equiv), 50 °C, 2 h (92%); (g) THF, intermediate **III** (1 equiv), Bop (1.5 equiv), DIEA (4 equiv), 25 °C, 1 h (35%).



Scheme 2. Reagents and conditions: (a) Toluene, THP (2 equiv), PPTS (catalytic), 60 °C, 16 h (95%); (b) THF, *n*BuLi (1.6 M in hexane, 1.05 equiv), I_2 (1.1 equiv, as THF solution), −78 °C to 0 °C; (c) acetone, 3 M HCl (excess) (72%—two steps); (d) toluene, intermediate **IV** (slow addition), 2-ethynyl-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1.2 equiv), phenyl isocyanate (2 equiv), TEA (2 equiv), 115 °C, 12 h; (e) EtOAc, 3 M HCl (excess), 15 min (48%—two steps); (f) dioxane, intermediate **VI** (1.5 equiv), $Pd(dppf)Cl_2$ (0.1 equiv), K_3PO_4 (3 equiv), 40 ?C, 2.5 h (75%); (g) $MeOH/H_2O$ (5:1), LiOH (10 equiv), 25 °C, 5 h (82%); (h) THF, 1-phenylcyclobutanamine (1.2 equiv), Bop (1.2 equiv), DIEA (5 equiv), 25 °C, 1 h (65%).

Table 1
Effect of benzylic substitutions on $Na_v1.7$ potency



Compound	R^1 R^2	Structure	$hNa_v1.7$ MK-0499 (% inh at 1 μM or IC_{50}, μM)	MK-0499 (% inh at 10 μM)
2	H, H	Isoxazole	37%	35%
3	H, CH_3	Isoxazole	1.7	47%
4	CH_3, CH_3	Isoxazole	0.43	45%
5	– CH_2CH_2–	Isoxazole	0.44	47%
6	–$(CH_2)_3$–	Isoxazole	0.12	37%
7	–$(CH_2)_4$–	Isoxazole	0.22	85%
8	–$(CH_2)_5$–	Isoxazole	0.58	ND
9	H, H	Isoxazoline	19%	ND
10	–$(CH_2)_3$–	Isoxazoline	8%	ND

Table 2
Energies (in kcal/mol) and torsion angles (in degrees) of bent and extended conformations of substituted *N*-benzylacetamides

R^1, R^2	Analog	*E* (extended)	Angle (extended)	*E* (bent)	Angle (bent)
H, H	**2**	0.07	180	0	98
H, Me	**3**	0	153	0.51	92
Me, Me	**4**	0	173	0.37	63
–$(CH_2)_2$–	**5**	3.59	180	0	87
–$(CH_2)_3$–	**6**	3.97	180	0	70
–$(CH_2)_4$–	**7**	2.93	174	0	67
–$(CH_2)_5$–	**8**	1.78	177	0	64

blocking $Na_v1.7$ sodium channel were also counter screened against several other ion channel targets, particularly hERG K^+ channel, since block of this channel has been associated with potentially lethal ventricular arrhythmias. A binding assay that measures the displacement of ^{35}S-labeled MK-0499, a known hERG K^+ channel blocker, was used as a high-throughput counter screen assay for hERG K^+ activity.[8]

As shown in Table 1, substitution on the benzylic position of the amide had a dramatic effect on $Na_v1.7$ activity. The addition of one methyl group (**3**) resulted in a small increase in potency. The *gem*-dimethyl and cyclopropyl analogs (**4, 5**) showed significant increase in potency. Maximal potency was reached with the cyclobutane compound (**6**) with an IC_{50} of 120 nM. When ring size was increased further, the potency started to decrease (**7, 8**). Interestingly, benzylic substitution did not afford potency enhancement in the isoxazoline (**9**, **10**) series. It is also noteworthy that compounds **4**–**6** were free of MK-0499 binding activity (IC_{50} >10 μM).

This dramatic potency enhancement was rather surprising, particularly with cyclobutane (**6**) and cyclopentane (**7**) analogs. We suspect that the potency of the cycloalkyl analogs is due in part to a preference of these analogs for the bioactive 'bent' conformation. Molecular modeling provided strong support for this hypothesis (Table 2). In the unsubstituted case (**2**), molecular mechanics calculations[9] show a nearly equal energy for the extended and bent conformations. Substituting with either one methyl (**3**) or *gem*-dimethyl groups (**4**) causes a small preference for the extended conformation (Fig. 2), while substituting with a spirocycloalkyl group (**5**–**8**) causes a preference for the bent conformation (Figs. 3 and 4). The preference for the 'bent' conformation reaches a maximum with the spirocyclobutane compound (**6**), in coincidence with its $Na_v1.7$ blocking activity.

The torsional energy plots are shown in Figures 2–4.

Since spirocyclobutane substitution was optimal for enhancing $Na_v1.7$ potency, this structural feature was maintained in further SAR studies. Although compound **6** is highly potent and selective against the hERG channel, it has poor pharmacokinetic properties with high clearance and low oral bioavailability (Table 5). In attempts to address this issue, substitutions on the aromatic and cyclobutane ring were explored. As shown in Table 3, a CF_3 group at the *meta* position resulted in decreased $Na_v1.7$ potency and increased MK-0499 binding potency (**11**). The effect of fluorine substitution varies depending on the position of the substitution (**12**–**14**). The *para* substitution enhanced $Na_v1.7$ potency, while *ortho* substitution caused a decrease in potency. Unfortunately, the *para*-fluoro substitution also led to enhanced MK-0499 binding activity (**14**). Next, we turned our attention to substitutions on the cyclobutane ring. Introducing a polar hydroxyl group resulted in complete loss of $Na_v1.7$ activity (**15**). The methoxy group showed a smaller effect with a twofold reduction of potency (**16**). Monofluoro substitution had little effect on $Na_v1.7$ potency (**17**, **18**). However, *gem*-difluoro substitution resulted in significant loss of $Na_v1.7$ activity (**19**). Compound **18** was evaluated for pharmacokinetic properties (Table 5). Unfortunately, its clearance rate was still very high.

Next we turned our focus to modification of the aromatic ring attached to the isoxazole ring. As shown in Table 4, the effect of lipophilic, electron-withdrawing substitutions on $Na_v1.7$ potency varies (**20**–**22**). However, these substituents generally cause strong MK-0499 binding activity. The effect of a methoxy group is highly dependent on its position (**23**–**25**). With *ortho*-methoxy substitu-

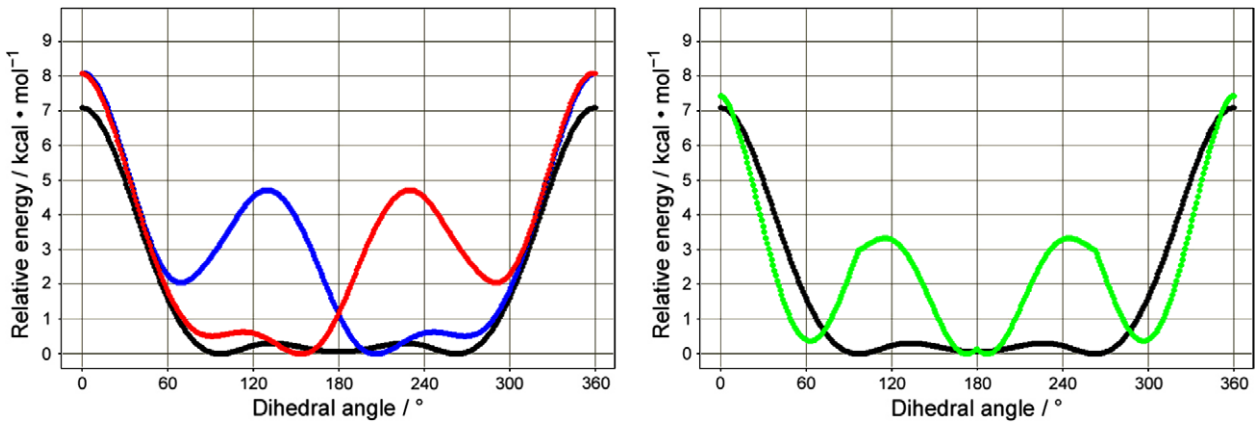


Figure 2. Left: unsubstituted (black) versus mono-Me-substituted (enantiomer 1, red; enantiomer 2, blue). Right: unsubstituted (black) versus *gem*-dimethyl (green).

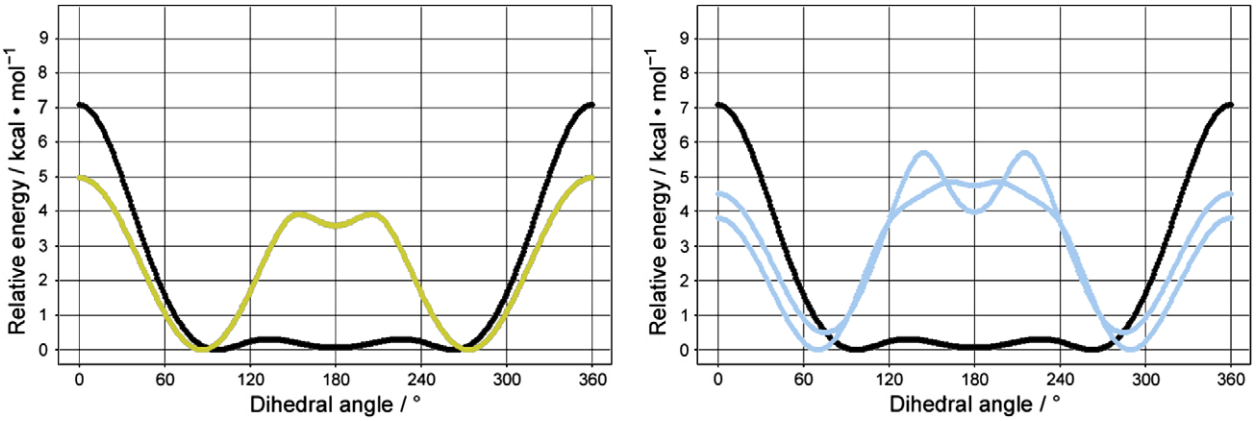


Figure 3. Left: unsubstituted (black) versus spirocyclopropyl (olive); Right: unsubstituted (black) versus spirocyclobutyl (light blue, two lines are from two cyclobutane ring puckers).

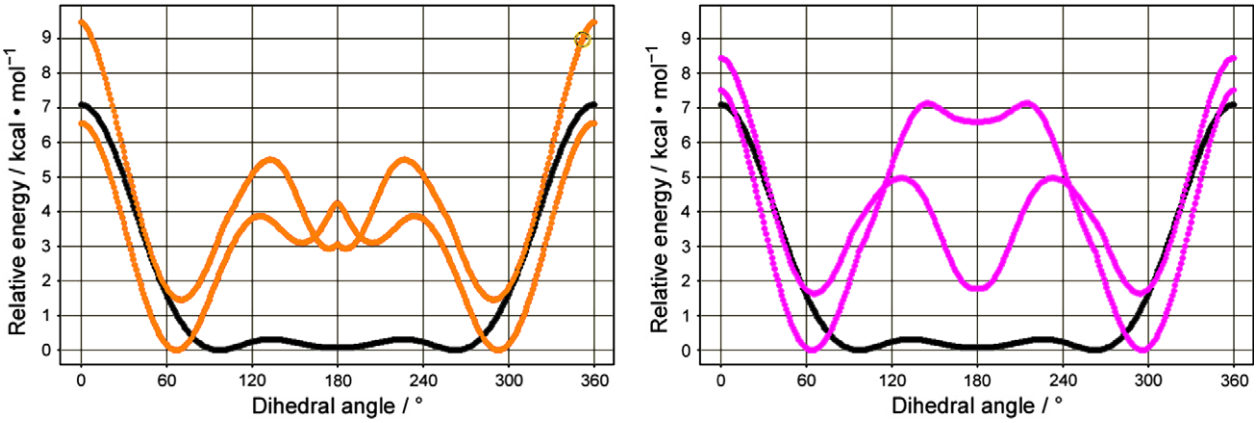
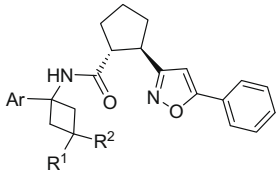


Figure 4. Left: unsubstituted (black) versus spirocyclopentyl (orange, two lines are from two cyclopentane puckers). Right: unsubstituted (black) versus cyclohexyl (magenta, two lines are from two cyclohexane puckers).

tion, the compound (**23**) was threefold less potent. *para* Substitution (**25**) had little effect on $Na_v1.7$ potency. *meta* Substitution (**24**) enhanced potency significantly. Compound **24** was one of the most potent sodium channel blockers in the series with an IC_{50} of 20 nM. The effect of a hydroxyl group also depends on substitution position (**26**–**28**). *meta* and *para* Substitution had little effect on potency. An *ortho*-hydroxyl group caused significant loss of potency. However, the potency loss caused by *ortho*-hydroxyl substitution can be compensated by introducing a lipophilic group such as OCF_3 (**29**) or CF_3 (**30**). It is interesting that the effect of the CF_3 group was highly dependent on its position with respect to the hydroxyl group (**30**–**34**). When a racemic mixture of compound **30** was separated on chiral HPLC to give a pair of enantiomers, the fast eluting isomer (**30a**) showed good $Na_v1.7$ potency and good selectivity against the hERG channel, and the slow eluting isomer (**30b**) was completely inactive on the $Na_v1.7$ channel. Pyridine analogs showed good potency regardless of having electron donating (**35**) or withdrawing (**36**) substitutions. However, the more polar pyridone analog (**37**) was completely inactive. Compound **36** was highly selective against the hERG channel.

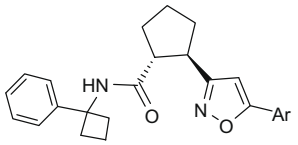
A selected group of compounds were further evaluated for pharmacokinetic properties.[10] As shown in Table 5, poor pharmacokinetic profile was a persistent issue with this series. One exception was compound **30**. It had low clearance and good oral bioavailability. The active enantiomer **30a** was evaluated in the rat spinal nerve ligation (SNL) model of neuropathic pain.[11] It showed comparable efficacy to **CDA54** with 30% and 28% reversal of mechanical allodynia at 2 and 4 h post a 10 mg/kg oral dose, respectively, while **CDA54** showed 35% reversal at 3 h after the same oral dose.[12]

Table 3
Effect of benzylamide substitutions on $Na_v1.7$ potency and MK-0499 binding activity



Compound	R^1 R^2	Ar	$hNa_v1.7$ (% inh at 1 μM or IC_{50}, μM)	MK-0499 (% inh at 10 μM)
6	H, H	Ph	0.12	37%
11	H, H	3-CF_3-Ph	0.59	89%
12	H, H	2-F-Ph	0.45	40%
13	H, H	3-F-Ph	0.11	64%
14	H, H	4-F-Ph	0.084	81%
15	H, OH	Ph	>5	
16	H, OMe	Ph	0.38	51%
17	H, F	Ph	0.18	46%
18	F, H	Ph	0.16	59%
19	F, F	Ph	41%	27%

Table 4
Effect of aromatic ring substitutions on $Na_v1.7$ potency and MK-0499 binding activity



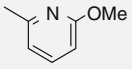
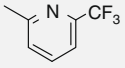
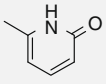
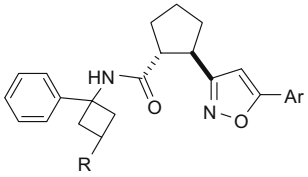
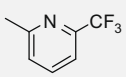
Compound	Ar	$hNa_v1.7$ (% inh at 1 μM or IC_{50}, μM)	MK-0499 (% inh at 10 μM)
6	Ph	0.12	37%
20	2-Cl-Ph	0.18	76%
21	2-CF_3-Ph	0.74	91%
22	3-OCF_3-Ph	0.37	55%
23	2-OMe-Ph	0.39	54%
24	3-OMe-Ph	0.020	62%
25	4-OMe-Ph	0.18	44%
26	2-OH-Ph	44%	54%
27	3-OH-Ph	0.21	64%
28	4-OH-Ph	0.13	15%
29	2-OH-3-OCF_3-Ph	0.26	47%
30	2-OH-3-CF_3-Ph	0.34	45%
30a	Enantiomer A	0.43	ND
30b	Enantiomer B	>10	ND
31	2-OH-4-CF_3-Ph	0.30	72%
32	2-OH-5-CF_3-Ph	1%	ND
33	2-OH-6-CF_3-Ph	1%	ND
34	4-OH-3-CF_3-Ph	0%	ND
35		0.19	54%
36		0.22	22%
37		0%	ND

Table 5
Rat pharmacokinetic data for selected compounds



Compound	R	Ar	*F*%[a]	AUC*N*[b]	Clp[c]	$t_{1/2}$[d]
6	H	Ph	3	0.024	58	1.3
18	F	Ph	12	0.056	86	1.7
22	H	3-OCF_3-Ph	46	0.20	89	2.6
24	H	3-OMe-Ph	2	0.011	79	1.6
27	H	3-OH-Ph	0	0	293	0.16
29	H	2-OH-3-OCF_3-Ph	6	0.06	35	0.88
30	H	2-OH-3-CF_3-Ph	49	1.9	9.5	0.92
36	H		36	0.1	135	1.51

[a] Bioavailability.
[b] Normalized AUC (po, μM h/mpk).
[c] Clearance (ml/min/kg).
[d] Half life (h).

In summary, a series of isoxazole voltage gated sodium channel blockers have been identified. Substitutions at the benzylic position of the benzamide showed significant effects on $Na_v1.7$ potency with the most dramatic effect deriving from the spirocyclobutyl substitution. Further SAR studies led to compound **30,** which displays improved pharmacokinetic properties. One of the enantiomers, **30a**, showed comparable in vivo efficacy in the rat SNL neuropathic pain model as the previously reported voltage gated sodium channel inhibitor **CDA54**.

Acknowledgments

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9. Torsional energy profiles of the substituted *N*-benzylacetamide model systems in which the cyclopentane–phenylisoxazole was truncated to a methyl group were calculated using MacroModel (Schrödinger, Portland, OR). The MMFFs force field was employed with a distance-dependent dielectric of 4r. The torsions were driven both forward and backward, and the lowest energy at each dihedral angle was taken. For the spirocyclopentyl and spirocyclohexyl analogs, two puckers of the carbocycle were used.
10. *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.
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