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Discovery of a novel class of biphenyl pyrazole sodium channel blockers for treatment of neuropathic pain

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ABSTRACT

A series of novel biphenyl pyrazole dicarboxamides were identified as potential sodium channel blockers for treatment of neuropathic pain. Compound **20** had outstanding efficacy in the Chung rat spinal nerve ligation (SNL) model of neuropathic pain.

In the pathogenesis of neuropathic pain, voltage gated sodium channel blockers have been shown to play a key role. Indeed, human specific genetic studies have shown that a loss of function mutation in $Na_v1.7$ is associated with congenital insensitivity to painful stimuli.[1] Neuropathic pain is caused by injury to the peripheral or central nervous system, and is often treated using weak sodium channel antagonists.[2] Certain subtypes of sodium channels ($Na_v1.7$ and $Na_v1.8$) are over expressed at the site of injuries.[3,4] Sodium channel blockers currently used in the clinic provide only partial relief and have adverse effects that limit their use. We reasoned that development of more potent state-dependent $Na_v1.7$ blockers may afford minimal CNS and cardiac side effects and improved efficacy for treating neuropathic pain.[5]

We have previously disclosed a novel class of biphenyl thiazole carboxamides, represented by **1** (Fig. 1), as state dependent sodium channel blockers.[6] These compounds displayed good $hNa_v1.7$ functional block ($Na_v1.7$ EP K_i = 0.015 μM) and good in vivo efficacy in various animal models of neuropathic pain. However, pharmacokinetic metabolite analysis revealed that this class of compounds underwent extensive hydrolysis of the amide to the acid in dog hepatocytes. The amide to acid hydrolysis was reduced by the introduction of the pyrazole ring as shown in **2**.[7] The pyrazole

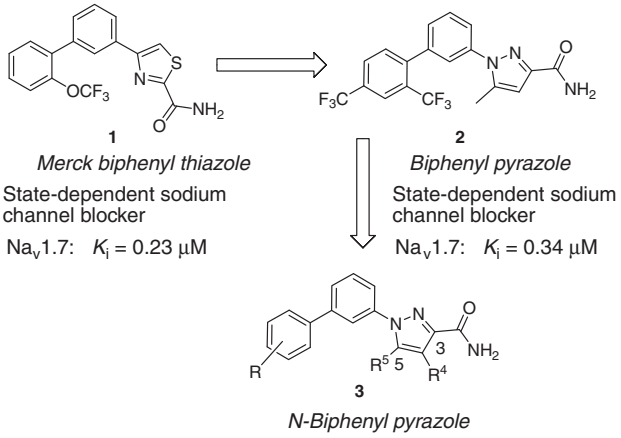

Figure 1. Design concept of *N*-biphenyl pyrazole $Na_v1.7$ blockers.

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analog **2** displayed good in vitro $Na_v1.7$ block and excellent in vivo efficacy but exhibited poor solubility. The compound was very stable during incubation with human, dog, and monkey liver microsomes, but **2** was extensively metabolized in rat liver microsomes. We set out to modify pyrazole **3** while retaining $Na_v1.7$ potency and in vivo efficacy with the hypothesis that introduction of polarity in the molecule could address some of these issues.

Our prior exploration in the area of biphenyl thiazolidine-2,4-diones, thiazoles, and pyrazoles as $Na_v1.7$ blockers established that meta arrangement of the biphenyl (1,3) is more potent than the para arrangement (1,4). We observed that lipophilic substituents like the trifluoromethoxy or trifluoromethyl group at C-2 position of the biphenyl were the preferred substituents.

The synthesized compounds were evaluated for their ability to block voltage gated $hNa_v1.7$ using a voltage-fluorescence (VIPR) assay.[8] Selected compounds were screened for activity against ancillary targets including IKr (MK-0499 binding assay), CYP inhibition, and $Ca_v1.2$ (diltiazem (DLZ) binding).[9] Compounds selective in these ancillary assays were examined in the rat spinal nerve ligation (SNL, Chung) model of neuropathic pain and their pharmacokinetic profiles were evaluated.[10]

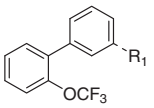
Keeping the core design of the pyrazole **3** intact, we introduced polar groups at the various positions of the pyrazole. Table 1 shows the various polar changes which were incorporated in the pyrazole and the associated activity. Introduction of a second carboxamide at the C-4 position of the pyrazole afforded compound **5**, which gave excellent $Na_v1.7$ functional block, but was weakly active in the in vivo SNL assay. Introduction of a methyl substituent at C-5 afforded **6**, which did not yield significant improvement over pyrazole **4**. The tricarboxamide pyrazole **7** had a profile similar to pyrazole **4** but with increased affinity toward MK-0499 binding. The pyrazole **8** afforded similar potency to **6** and **7**, but had an improved in vivo efficacy in the Chung model of neuropathic pain and reduced affinity toward Ikr. Pyrazoles **10** and **11** illustrate the importance of a primary carboxamide at the C-3 position of the pyrazole in this design for $Na_v1.7$ potency. Based on the above data, we decided to further examine a series of dicarboxamide pyrazole similar in design to pyrazole **8**.

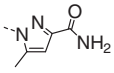
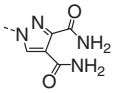
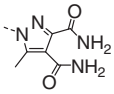
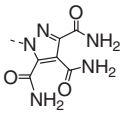
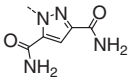
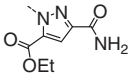
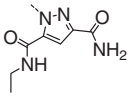
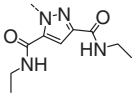
The biphenyl-3,5-carboxamide-1*H*-pyrazoles were synthesized by the route outlined in Scheme 1. Copper TMEDA complex mediated coupling of the commercially available 3,5-pyrazole carboxylic acid ethyl ester **12** with the 3-bromophenyl boronic acid, **13** afforded the biphenyl acetophenone **14**.[11] Aminolysis of the diester afforded the dicarboxamide pyrazole **15**. A palladium catalyzed cross coupling of the pyrazole **15** with aryl boronic acids afforded the desired biphenyl pyrazole dicarboxamide **16**.

Table 2 shows a series of novel *N*-aryl pyrazole-3,5-dicarboxamide sodium channel blockers that were prepared from this diamide configuration. Consistent with our prior work, we found that lipophilic substituents like the trifluoromethyl or trifluoromethoxy were preferred at the R^2 position. Most of these pyrazoles displayed good $hNa_v1.7$ block. As a measure of the subtype selectivity of these compounds, several were also tested for block of the related analgesia target $hNa_v1.8$. As illustrated in Table 2, the compounds exhibited only modest selectivity (2×–8×) in favor of potency for block of $Na_v1.7$ over $Na_v1.8$.

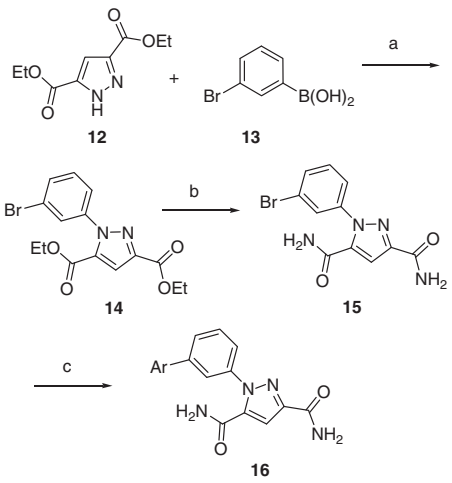
Pyrazole **8**, with a trifluoromethoxy group at the R^2 position of the biphenyl**,** had a very good sodium channel block but moderate efficacy in the in vivo Chung model. Pyrazole **17**, a dichloro analog, and pyrazole **18**, with a trifluoromethyl substituent, had similar $Na_v1.7$ functional block but poor in vivo efficacy compared to pyrazole **8**. Incorporation of a second trifluoromethyl group brought interesting results. A 2,4-CF_3 analog (**19**) had poor $Na_v1.7$ block, but the adjacent 2,5-CF_3 pyrazole **20** exhibited improved functional block of $Na_v1.7$ and modest block of $Na_v1.8$, and interestingly provided robust efficacy in vivo efficacy in the Chung model. A trifluoromethoxy substituted pyrazole incorporating a second substituent either a fluoro group at R^6 position (**23**) or a trifluoromethyl group at R^5 position (**24**) led to good improved

Table 1
The effect of substitution on the pyrazole ring of *N*-pyrazole biphenyl $Na_v1.7$ blockers



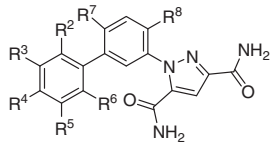
Entry	R^1	$Na_v1.7$ VIPR (IC_{50}, μM or % inh @ 10 μM)	MK-0499 (% inh at 10 μM)	Rat SNL % reversal of allodynia[a] (2 h)	Rat SNL % reversal of allodynia[a] (4 h)
4		0.224	69%	33%	11%
5		0.049	1%	18%	8%
6		0.185	15%	16%,	8%
7		0.115	49%	ND	ND
8		0.132	21%	35%,	19%
9		0.010	72%	ND	ND
10		0.029	20%	ND	ND
11		1%	27%	ND	ND

[a] Reversal of rat spinal nerve ligation induced allodynia (Chung model—Von Frey) vs pre-surgery sensitivity.



Scheme 1. Reagents and conditions: (a) $[Cu(OH)TMEDA]_2Cl_2$, DCM, 3 Å mol. sieves; (b) 7 N Ammonia, MeOH; (c) $ArB(OH)_2$, $PdCl_2(dppf)$, TEA, EtOH.

Table 2
The effect of SAR around the biphenyl region



Entry	R^2	R^3	R^4	R^5	R^6	R^7	R^8	$Na_v1.7$ VIPR (IC_{50}, μM)	$Na_v1.8$ VIPR (IC_{50}, μM)	SNL % reversal[a] (t = 2 h)	SNL % reversal[a] (t = 4 h)
8	OCF_3	H	H	H	H	H	H	0.132	0.554	35	19
17	Cl	Cl	H	H	H	H	H	0.195	ND	15	7
18	CF_3	H	H	H	H	H	H	0.155	0.915	9	2
19	CF_3	H	CF_3	H	H	H	H	9%	ND	ND	ND
20	CF_3	H	H	CF_3	H	H	H	0.810	2000	44	34
21	CF_3	H	H	H	CF_3	H	H	0.715	ND	ND	ND
22	F	OCF_3	H	H	H	H	H	0.282	1513	28	8
23	OCF_3	H	H	H	F	H	H	0.106	0.802	15	9
24	OCF_3	H	H	CF3	H	H	H	0.325	1181	29	6
25	OCF_3	H	H	H	H	F	H	0.477	ND	ND	ND
26	$OCH_2CF_2CF_3$	H	F	H	H	H	H	0.106	ND	28	8
27	$OCH_2CF_2CF_3$	H	H	F	H	H	H	0.046	0.149	25	9
28	$OCH_2CF_2CF_3$	OCF_3	H	H	H	H	H	0.064	0.203	6	3
29	$OCH_2CF_2CF_3$	CF_3	H	H	H	H	H	0.147	0.299	22	8

[a] Reversal of rat spinal nerve ligation induced allodynia (Chung model—Von Frey) vs pre-surgery sensitivity.

in vitro sodium channel functional block but only modest in vivo efficacy. A fluoro group at R^7 position (**25**) resulted in decreased $Na_v1.7$ functional block. We found that increasing the lipophilicity of the molecule with a pentafluoroethoxy group at the R^2 position and substituents at R^3 or R^4 or R^5 position (**26**–**29**) provided compounds with distinctly improved $Na_v1.7$ and $Na_v1.8$ functional block but only modest in vivo efficacy.

From these results, pyrazole **20** was identified for additional characterization based on the remarkable efficacy in the SNL (Chung) model. Counter screening compound **20** against a panel of receptors, enzymes, and ion channels (MDS Pharma Services) established pyrazole **20** to be remarkably selective for sodium channels. Binding at IKr, $Ca_v1.2$, and $Na_v1.5$ was measured as a general indicator of ion channel activities.[12] As illustrated in Table 3, pyrazole **20** has a favorable profile against calcium and potassium ion channel targets but little subtype selectivity for sodium channels.

Compound **20** did not inhibit any of the major CYP isoform enzymes as shown in Table 4. The pyrazole also exhibited a low potential for CYP3A4 induction as measured by an hPXR induction assay. Compound **20** was stable in liver microsome preparations from human, rat, and dog, with >99% parent remaining after 60 min incubation (10 μM substrate).

Table 3
Ion channel profile for compound **20**

Entry	MK-0499 binding[a]	hERG EP[b]	DLZ binding[a]	Nav1.5 VIPR (IC_{50} μM)
20	17%	15%	0%	1.32

[a] %Inh at 10 μM.
[b] %Inh at 30 μM.

Table 4
CYP profile for compound **20** (IC_{50}, μM)

Entry	CYP3A4	CYP2C9	CYP2D6	PXR
20	>100	20.3	>100	>25

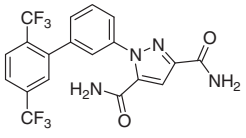
Table 5 shows the pharmacokinetic profile in rat and dog for compound **20**. In rat, the compound had low clearance (5 mL/min/kg), high oral exposure (AUC = 5 μM h), moderate plasma half life (3 h), and desirable bioavailability (60%). In dog, the PK profile followed a similar trend with low clearance, high oral exposure, and longer half life of 12 h. The oral exposure increased in a dose-dependent manner (AUC = 372 μM h) following a single 20 mpk po dose. Compound **20** afforded remarkable exposure at even higher doses (single 60 mg/kg po dose).

This pyrazole dicarboxamide was efficacious in various preclinical pain models. In the SNL model of neuropathic pain, **20** reversed mechanical allodynia at 2 and 4 h in a dose-dependent manner (Table 6) achieving maximally 59% reversal of allodynia following a 30 mg/kg dose. In the rat CFA (complete Freund's adjuvant-induced inflammatory pain) model, when dosed orally at 30 mg/kg, compound **20** produced 46% and 53% reversal of mechanical hyperalgesia at 2 h and 4 h, respectively.

Table 5
Pharmacokinetic profile of pyrazole **20**

	Dose (iv/po) (mg/kg)	$t_{1/2}$ (h)	C_{max} (μM)	Clp (mL/mg/kg)	PO AUC (μM h)	F (%)
Rat	1/3	3.1	2.64	5	5.6	60
Dog	0.5/2	12	2.92	0.5	50	33
Dog	20 po		20.33		372	
Dog	60 po		24.44		650	

Table 6
In vivo activity and exposure of compound **20**



Entry	% Reversal of allodynia (2 h)	% Reversal of allodynia (4 h)
SNL (3 mpk)	18%	30%
SNL (10 mpk)	44%	34%
SNL (30 mpk)	56%	59%
CFA (30 mpk)	46%	53%

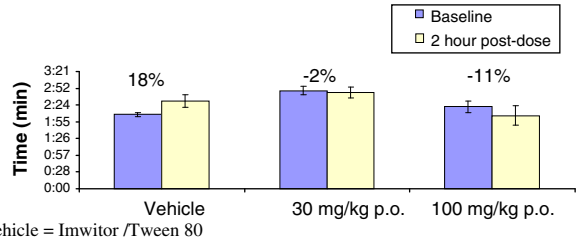


Figure 2. Effect of compound **20** on motor coordination in the rat rota-rod model.

In the rat rotarod model of motor coordination, no significant effect was observed at both 30 mg/kg and 100 mg/kg doses for pyrazole **20** which resulted in 3.5 h plasma exposure of 31.6 μM (Fig. 2). In contrast, marketed drugs mexiletine and Gabapentin at subefficacious oral dose of 100 mg/kg significantly reduced latency in rats on the rotarod model.[13,14]

The cardiovascular effects of pyrazole **20** were examined in barbiturate-anesthetized and ventilated dogs at rising doses of 1, 3, and 10 mg/kg IV. Compound **20** had no treatment-related effect on mean arterial pressure, heart rate, or QRS and QTc intervals over this dose range. A small but significant increase was observed in the PR interval, reaching maximally 6% at 10 mg/kg. Average plasma concentrations measured after infusion of 1, 3, and 10 mg/kg doses were 7.3 ± 3.6, 16.2 ± 8.0, and 78.2 ± 33.6 μM (mean ± SD), respectively.

Pyrazole **20** was also given to conscious mice to determine the potential effects on central nervous system (CNS) function, behavior, motor activity, and body temperature. No treatment-related changes were observed at 2, 5, and 24 h after a 100 mg/kg oral dose.

While compound **20** was chosen for further characterization based on the high level of pharmacological efficacy in the SNL assay, we note that efficacy in this series was not accurately predicted by potency in the $Na_v1.7$ or $Na_v1.8$ VIPR assay. Indeed, several structurally related compounds in this series afforded superior potency in these assays but exhibited diminished efficacy in vivo. There is a possibility that the sodium channel VIPR assays do not fully reproduce the physiologic state of the ion channel in vivo. Additionally, we cannot rule out the possibility that **20** could be active on additional targets for analgesia.

In summary, pyrazole **20** exhibited greater in vitro metabolic stability than pyrazole carboxamide **2**, in that hydrolysis to the corresponding acid was reduced in rat liver preparations. A new class of sodium channel blockers based on a biphenyl pyrazole core with a primary carboxamide at C-3 and C-5 position has been identified and optimized. Compound **20** has excellent efficacy in the Chung model of neuropathic pain and outstanding exposure in the rat and dog pharmacokinetic profile. The compound exhibited no CNS ancillary pharmacology and limited preclinical CV effects at exposures well above those required for preclinical efficacy.

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