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Substituted biaryl pyrazoles as sodium channel blockers

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ABSTRACT

Voltage-gated sodium channels have been shown to play a critical role in neuropathic pain. A series of low molecular weight biaryl substituted pyrazole carboxamides were identified with good in-vitro potency and in-vivo efficacy. Compound **26**, a $Na_v1.7$ blocker has excellent efficacy in the Chung model of neuropathic pain.

Voltage-gated sodium channel blockers have been shown to play a key role in the pathogenesis of neuropathic pain. Neuropathic pain is a complex state that is caused by injury to the peripheral or central nervous system.[1] Certain subtypes of sodium channels ($Na_v1.7$ and $Na_v1.8$) are over expressed at the site of injuries.[2] Neuropathic pain is often treated using existing drugs that are weak sodium channel antagonists. They provide only partial relief and have adverse effects that limit their use. Our goal is to develop state dependent $Na_v1.7$ blockers, which may afford minimal CNS and cardiac side effects and improved efficacy for treating neuropathic pain.[3] Recent support for this goal has come from human specific genetic evidence that has shown a loss of function mutation in $Na_v1.7$ has been associated with congenital insensitivity to painful stimuli.[4]

Previously, we disclosed a novel class of biphenyl thiazole carboxamides, represented by **1** (Fig. 1) as state dependent sodium channel blockers.[5] 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. Recently, scientists at Cocensys/Purdue Pharma reported the discovery of aryloxy phenyl pyrazole carboxamide **2** as sodium channel blockers with good $hNa_v1.7$ functional block ($Na_v1.7$ EP K_i = 0.013 μM) in electrophysiology.[6] By combining the two designs, we report a new class of pyrazoles where aryloxyphenyl group is replaced by a biphenyl group, yielding pyrazole carboxamide **3**, a novel class of sodium channel blockers.

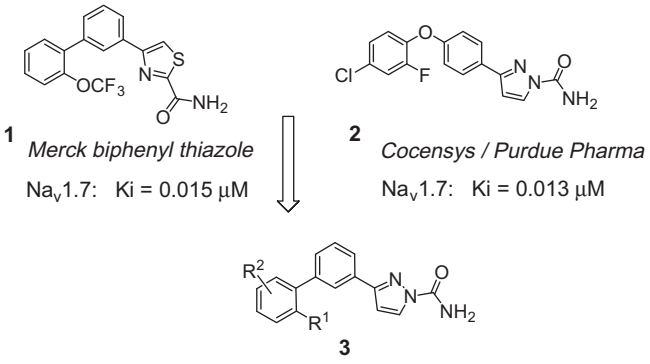

Figure 1. Design concept.

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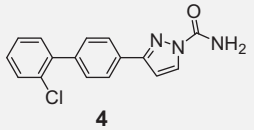
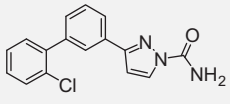
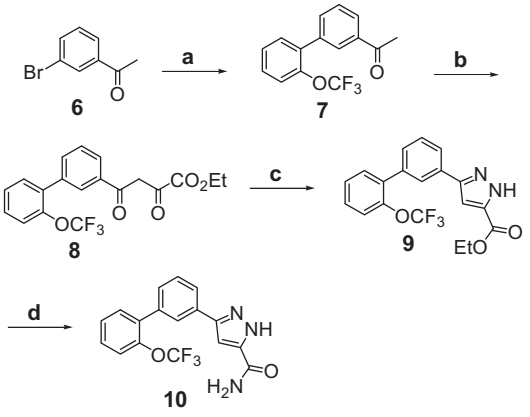
Previous work in the area of biphenyl thiazolidine-2,4-diones and thiazoles as $Na_v1.7$ blockers has shown that *meta* arrangement of the biphenyl (1,3) is more potent than the *para* biphenyl (1,4).[5,7] In case of the biphenyl pyrazoles, the same relationship was maintained (Table 1). Exploration of the biphenyl ring system consistently showed that the 2-substituted biphenyl to be the preferred position. *Meta* (1,3) biphenyl pyrazole carboxamide **5** exhibited a significantly more potent $hNa_v1.7$ block compared to *para* (1,4) biphenyl pyrazole carboxamide **4**. However, both of these *N*-pyrazole carboxamides displayed high clearance in rat pharmacokinetic studies and were rapidly metabolized during incubation in rat or human liver microsomes. An analysis of the metabolite products indicated the formation of carboxylic acid due to hydrolysis of the amide. In an attempt to solve the issue of labile amide, we synthesized a series of various pyrazole carboxamide arrangements.

Biphenyl-5-carboxamide-1H-pyrazole **10** was prepared as outlined in Scheme 1. Palladium catalyzed Suzuki cross-coupling of the commercially available 3-bromoacetophenone **6** with 2-trifluoromethoxyphenyl boronic acid afforded the biphenyl acetophenone **7**.[8] The biphenyl diketoester **8** was constructed from alkylation of the acetophenone **7** with diethyl oxalate. Condensation with hydrazine hydrate afforded the pyrazole ester **9**. The carboxamide **10** was prepared by aminolysis of the ester **9**.

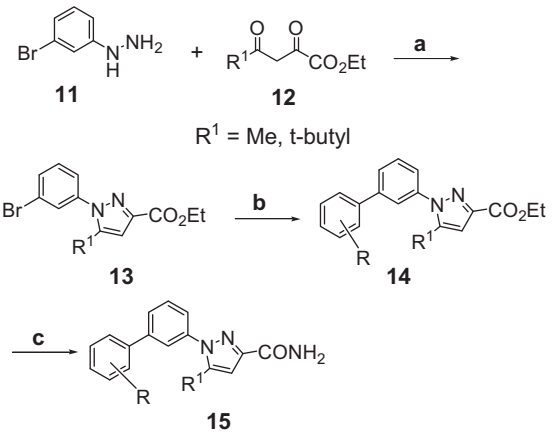
The syntheses of *N*-aryl pyrazole carboxamides is outlined in Scheme 2. These analogs were constructed from the 3-bromo pyrazole ester **13**, a common key intermediate. Condensation of the commercially available 3-bromophenyl hydrazine **11** and the diketoester **12** afforded a regioisomeric mixture of pyrazole esters. The desired regioisomer **13**, the major product, was isolated after chromatographic separation. With the pyrazole ester derivative **13** in hand, the biphenyl pyrazole esters **14** were synthesized via palladium catalyzed Suzuki cross-coupling with aryl boronic acids. Aminolysis of the ester **14** afforded the biphenyl pyrazole carboxamides **15**.

Table 1
para (1,4) versus *meta* (1,3) arrangement in pyrazoles

	Na_v 1.7 EP (K_i nM)	Rat Clp (mL/min/kg) (1 mpk, *iv*)
 **4**	0.111	125
 **5**	0.018	123



Scheme 1. Reagents and conditions: (a) $ArB(OH)_2$, Ph_3P, $Pd(OAc)_2$, *n*-propanol, H_2O, 2 M Na_2CO_3, reflux; (b) NaH, diethyl oxalate, THF; (c) hydrazine hydrate, EtOH, reflux; (d) Ammonia, MeOH.

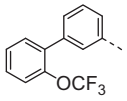


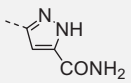
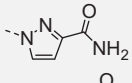
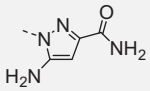
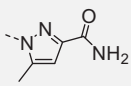
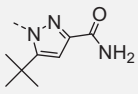
Scheme 2. Reagents and conditions: (a) AcOH, reflux; (b) $ArB(OH)_2$, Ph_3P, $Pd(OAc)_2$, *n*-propanol, H_2O, 2 M Na_2CO_3, reflux; (c) ammonia, MeOH.

The synthesized compounds were evaluated for their ability to block voltage gated $hNa_v1.7$ using an in-vitro functional membrane potential FRET based assay.[9] Selected compounds were screened for activity against ancillary targets including hERG (MK-0499 binding assay measures the displacement of ^{35}S-labeled MK-0499, a known hERG blocker), CYP inhibition and Cav 1.2 (diltiazem (DLZ)) binding.[10] Compounds selective in these ancillary assays were examined in the rat spinal nerve ligation (Chung) model of neuropathic pain and their pharmacokinetic profiles were evaluated.[11]

As shown in Table 2, the carboxamide regioisomer **10** maintained potent $hNa_v1.7$ block, but also exhibited a strong affinity towards MK-0499 binding. A series of *N*-aryl-3-carboxamides were also investigated. Compound **16** had a very good $hNa_v1.7$ block

Table 2
SAR around the pyrazole ring



Entry	R1	$Na_v1.7$ VIPR (IC_{50}, μM)	MK-0499 (% inh @ 10 μM)
10		0.508	94%
16		0.065	51%
17		0.111	44%
18		0.224	6%
19		1.368	ND

and reduced affinity towards MK-0499. Introduction of amino group at C-5 position of the pyrazole, **17**, resulted in loss of $Na_v1.7$ activity.

Replacing the amino group of **17** with a methyl group at C-5 afforded pyrazole **18**, which was a potent $hNa_v1.7$ blocker with dramatically reduced affinity for MK-0499. Introduction of a bulky *t*-butyl group at C-5 of the pyrazole, **18**, led to **19** with a dramatic loss in $hNa_v1.7$ potency. Based on these results, a series of *N*-aryl-3-methyl-5-carboxamides pyrazoles were selected for further investigation.

Table 3 shows a series of novel N-aryl pyrazole carboxamide sodium channel blockers. Consistent with our prior work, we found lipophilic substituents were preferred at the R^2 position.[5,7] Liphophilic substituents such as the trifluoromethyl or the trifluoromethoxy were found to be optimal at the 2-position of the biphenyl group. Most of these pyrazoles displayed good $hNa_v1.7$ block. Compounds with similar $hNa_v1.7$ potency were distinguished on basis of MK-0499 binding data and/or SNL (Chung) model in-vivo efficacy data (at 10 mg/kg).[11] Compounds with significant reversal of allodynia at 2 and 4 h in the SNL model were further examined for their pharmacokinetic profile.

Pyrazole **21** with a trifluoromethyl substituent is a more potent $Na_v1.7$ blocker than the 2-chloro pyrazole analog **20**. However, **21** displayed poor efficacy in the in-vivo Chung model. With a trifluoromethyl group installed at the R^2 position of the biphenyl pyrazole, a series of di-substituted analogs, **22**–**28** were explored. The inclusion of a second substituent (a fluoro or trifluoromethyl group) at R^6, pyrazole **25** and **28** resulted in excellent $hNa_v1.7$ block but little or no effect in the in-vivo Chung pain model. Comparatively, a second trifluoromethyl group installed at R^4 or R^5 position of the distal phenyl ring, compounds **26** and **27,** imparted modest $hNa_v1.7$ block but improved efficacy significantly at 2 and 4 h in the Chung model.

Compound **18**, possessing a trifluoromethoxy group at the R^2 position, afforded moderate $hNa_v1.7$ block and reduced activity in MK-0499 binding. The pyrazole **18** exhibited moderate in-vivo efficacy in the Chung model. R^2 substitution with a more liphophilic trifluoroethoxy (**29**) or pentafluroethoxy (**30**) group maintained the potency of hNa_v 1.7 block but these compounds were even less efficacious in the Chung model. Substitution of the middle ring with a fluoro or trifluoromethoxy group at R^6 or R^7 position of the biphenyl, as in compounds **33** and **34,** increased the affinity for MK-0499 binding while maintaining the same level of $hNa_v1.7$ block. Lack of a R^2 substituent, **34** or introduction of a bulky group at R^2, **35**, resulted in dramatic loss of $hNa_v1.7$ potency.

From these results, **26** and **27** were identified for additional characterization based on the efficacy in the SNL (Chung) model and favorable ion channel profile. As illustrated in Table 4, pyrazole **26** had a lower affinity for cytochrome P_{450} enzymes and a low potential for induction as measured by an hPXR induction assay, which indicated that **26** may have lower liability for drug–drug interactions.

Compound **26** was evaluated for pharmacokinetic properties in rat and dog (Table 5). In rat, it showed moderate clearance (11 mL/min/kg), good oral AUC (1.21 μM h kg/mg), good plasma half-life (2 h) and desirable bioavailability (55%). However, in dog, the PK profile showed slightly higher clearance (16 mL/min/kg) and lower plasma half-life (1.4 h).

This potent Na_v channel blocker was efficacious in various preclinical pain models. In the rat spinal nerve ligation model (SNL) of neuropathic pain, **26** attenuates mechanical allodynia to the same

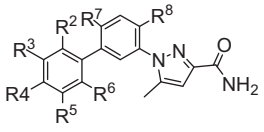
Table 4
P_{450} inhibition and hPXR induction data for compound **26** and **27**

Entry	CYP3A4 IC_{50} (μM)	CYP2C9 IC_{50} (μM)	CYP2D6 IC_{50} (μM)	PXR IC_{50} (μM)
26	>100	9	>100	>25
27	51	2.6	33	ND

Table 5
Pharmacokinetic profile of **26**

	$t_{1/2}$ (h)	Vd (L/kg)	Clp (mL/mg/kg)	nAUC (po) (μM h kg/mg)	F (%)
Rat	2.0	3.10	11	1.2	55
Dog	1.4	1.43	16	1.2	50

Table 3
Biphenyl-3-carboxamide-5-methyl pyrazoles



Entry	R^2	R^3	R^4	R^5	R^6	R^7	R^8	$Na_v1.7$ VIPR (IC_{50}, μM or % inh @ 10 μM)	MK-0499 (% inh @ 10 μM)	SNL % Reversal[a] (t = 2 h)	SNL % Reversal[a] (t = 4 h)
20	Cl	H	H	H	H	H	H	0.738	8%	ND	ND
21	CF_3	H	H	H	H	H	H	0.163	68%	14	19
22	CF_3	F	H	H	H	H	H	0.781	73%	ND	ND
23	CF_3	H	Cl	H	H	H	H	0.243	45%	8	0
24	CF_3	H	H	F	H	H	H	0.412	41%	ND	ND
25	CF_3	H	H	H	F	H	H	0.071	74%	12	2
26	CF_3	H	CF_3	H	H	H	H	0.426	53%	35	32
27	CF_3	H	H	CF_3	H	H	H	0.272	51%	39	35
28	CF_3	H	H	H	CF_3	H	H	0.041	67%	16	6
18	OCF_3	H	H	H	H	H	H	0.225	6%	33	11
29	OCH_2CF_3	H	H	H	H	H	H	0.187	43%	18	4
30	$OCH_2CF_2CF_3$	H	H	H	H	H	H	0.128	49%	15	9
31	OCF_3	H	H	H	H	F	H	0.162	66%	ND	ND
32	OCF_3	H	H	H	H	H	F	0.222	62%	17	8
33	OCF_3	H	H	H	H	OCF_3	H	0.373	77%	ND	ND
34	H	CF_3	H	CF_3	H	H	H	17%	ND	ND	ND
35	PhO	H	H	H	H	H	H	0.875	13%	ND	ND

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

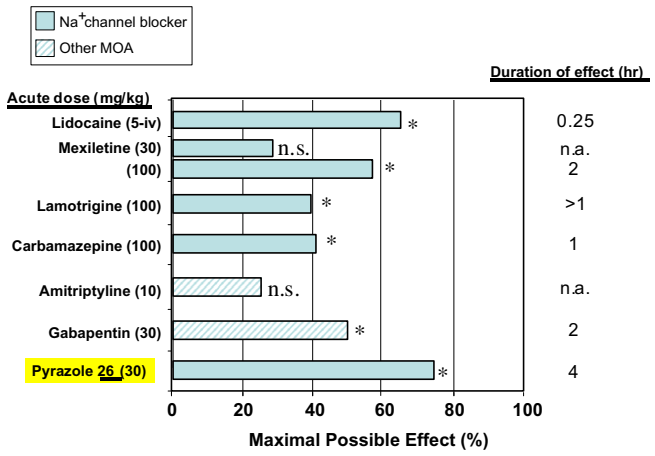


Figure 2. Comparison of pyrazole **26** with standards: SNL model of neuropathic pain.

extent as and for a longer duration (4 h) than several drugs used for treatment of neuropathic pain (Fig. 2). As can be seen from Table 6, the compound reverses mechanical allodynia at 2 and 4 h in a dose dependent manner. When dosed orally at 3 mg/kg bid for 2 days, **26** was very efficacious producing 90% (p <0.01) and 55% (p <0.001) reversal of mechanical allodynia at 2 and 4 h, respectively. Compound **26** produced 90% protection from tonic seizures at 10 mg/kg po in the mouse MES (maximal electroshock induced seizures to measure anticonvulsant activity) model (4 h post-dose).[12]

In the rat rotarod model of motor coordination, no significant change was observed at 30 mg/kg for **26**.[13] In contrast, mexiletine and Gabapentin at subefficacious dose of 100 mg/kg, po significantly reduced latency on the rotarod (Fig. 3).

In incubation of **26** with liver microsomes, significant amount of a carboxylic acid metabolite was detected which was formed via hydrolysis of the carboxamide. As illustrated in Figure 4, this conversion was modest after 60 min in microsomal preparations from

Table 6
In-vivo SNL (Chung) activity of compound **26** (po) [a]

Dose	% Reversal of allodynia (2 h)	% Reversal of allodynia (4 h)
10 mpk	35%	32%
30 mpk	69%	55%
3 mg/kg bid	80%	87%

[a] Treatment with compound 21 days after surgery.

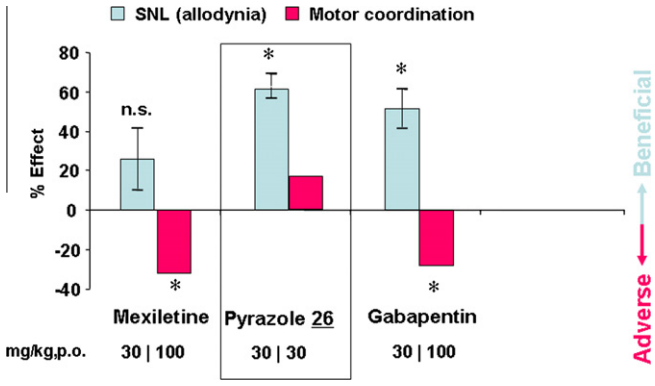


Figure 3. Efficacy of compound **26** in SNL model versus motor coordination.

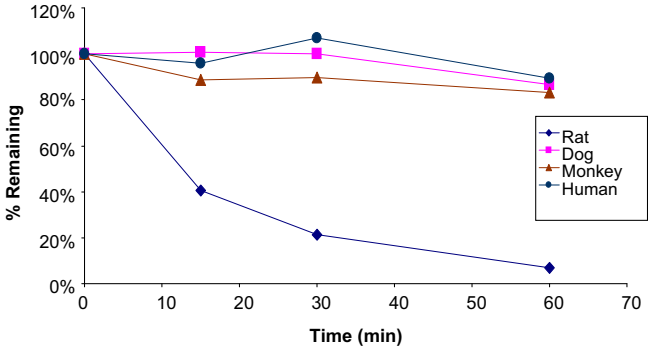


Figure 4. Metabolic stability of compound **26** in liver microsomes.

dog, human, and monkey but significantly more pronounced with rat liver microsomes.

In summary, a new class of sodium channel blockers based on a biphenyl pyrazole core with a primary carboxamide at C-3 position has been identified. Many of the analogs in this series exhibit good in-vitro potency and in-vivo efficacy. Compound **26** has excellent efficacy in the Chung model of neuropathic pain. It has a clean profile in human P_{450} inhibition and PXR induction assays, thus reducing the potential for drug–drug interactions. One of the concerns in this monoamide pyrazole carboxamide design is the hydrolysis of the amide to the acid. This issue is being addressed and is the subject of a forthcoming publication.

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