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Imidazopyridines: A novel class of hNa$_v$1.7 channel blockers

Clare London,[a,*] Scott B. Hoyt,[a] William H. Parsons,[a] Brande S. Williams,[c] Vivien A. Warren,[c] Richard Tschirret-Guth,[d] McHardy M. Smith,[c] Birgit T. Priest,[c] Erin McGowan,[b] William J. Martin,[b] Kathryn A. Lyons,[a] Xiaohua Li,[a] Bindhu V. Karanam,[d] Nina Jochnowitz,[b] Maria L. Garcia,[c] John P. Felix,[c] Brian Dean,[d] Catherine Abbadie,[b] Gregory J. Kaczorowski[c] and Joseph L. Duffy[a]

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

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Abstract—A series of imidazopyridines were evaluated as potential sodium channel blockers for the treatment of neuropathic pain. Several members were identified with good hNa$_v$1.7 potency and excellent rat pharmacokinetic profiles. Compound **4** had good efficacy (52% and 41% reversal of allodynia at 2 and 4 h post-dose, respectively) in the Chung rat spinal nerve ligation (SNL) model of neuropathic pain when dosed orally at 10 mg/kg.
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Neuropathic pain is a chronic and debilitating disease caused by injury or pathological changes to the peripheral or central nervous systems. These changes result in the abnormal processing of external stimuli (e.g., touch, temperature) by the affected pain sensing neurons (nociceptors).[1,2] Voltage-gated sodium channels (Na$_v$1·x) are strongly expressed in nociceptors and are known to be important in nociception.[3] Clinically, it has been shown that voltage-gated sodium channel blockers such as mexiletine (**1**) and carbamazepine (**2**) (Fig. 1) are efficacious in the treatment of neuropathic pain. These therapies, however, are restricted by their dose limiting CNS side effects.[4]

Data from recent human genetic studies strongly support the role of sodium channels in pain signaling. Individuals with a loss of function mutation in the gene that encodes hNa$_v$1.7 have a complete inability to sense pain. Conversely, gain of function mutations in Na$_v$1.7 lead to hyperexcitability in pain signaling neurons.[5,6] In addition to Na$_v$1.7, the Na$_v$1.8 isoform has also been identified as a possible target for neuropathic pain.[7,8]

Our research has focused on the development of hNa$_v$1.7 blockers for the treatment of neuropathic pain. Mexiletine and carbamazepine are only weak blockers of hNa$_v$1.7 (VIPR IC_{50} = 11 and 22 μM, respectively).[9] We reasoned that the identification of more potent

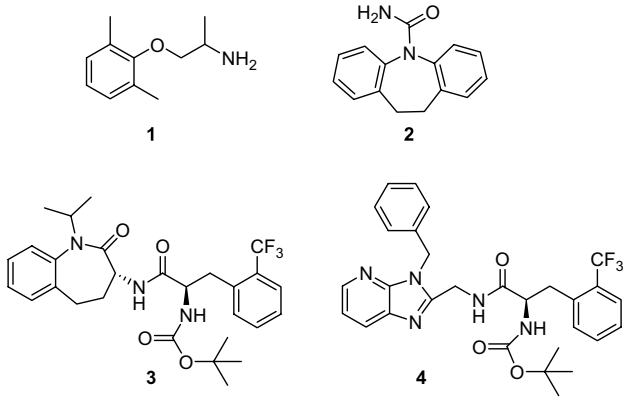

Figure 1. Sodium channel blockers.

Keywords: Sodium channel blockers; hNa$_v$1.7; Neuropathic pain; Voltage-gated sodium channels.
* Corresponding author. E-mail: clare_london@merck.com

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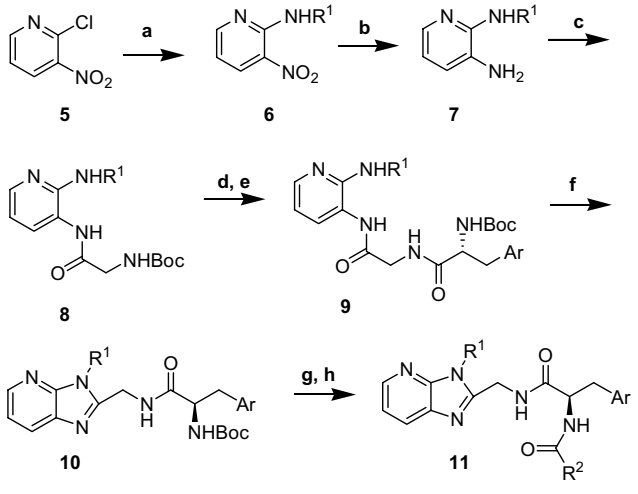
$hNa_v1.7$ blockers could afford new therapies that show significant analgesic effects as well as improved therapeutic indices over existing treatments.

We have recently reported the discovery of benzazepinone $hNa_v1.7$ blockers (e.g., **3**).[10,11] Compounds in this class suffered from modest oral exposures and high clearance rates (**3**: Clp = 50 ml/min/kg, AUC_N (po) = 0.22 μM h kg/mg). Incubation of these compounds with rat liver microsomes indicated that the major sites of oxidative metabolism lay in the benzolactam core and the *N*-Boc group. In an effort to reduce the clearance rates of these compounds, we investigated alternatives to the benzazepinone moiety. These studies resulted in the discovery of a series of imidazopyridines (e.g., **4**) that show improved pharmacokinetic (PK) profiles as well as excellent efficacy in the Chung rat spinal nerve ligation (SNL) model of neuropathic pain.

The synthesis of these compounds, outlined in Scheme 1, commenced from commercially available 2-chloro-3-nitropyridine **5**. Treatment of a DMF solution of **5** with an alkylamine in the presence of sodium carbonate afforded the 2-aminopyridine product **6**. The nitro group of **6** was reduced with Raney nickel to afford diaminopyridine **7**; subsequent amide coupling with *N*-Boc glycine yielded compound **8**. Deprotection of **8** with TFA and coupling to the appropriate amino acid afforded the acyclic precursor **9**, which was thermally cyclized in the presence of acetic acid to the *aza*-benzimidazole **10**. Amide derivatives **11** were readily prepared by removal of the Boc group with TFA, followed by coupling with a carboxylic acid. Compounds were synthesized from commercially available starting materials except in cases where the phenylalanine derivatives were unavailable. These amino acids were synthesized using the Schollkopf method.[12]



Scheme 1. Reagents and conditions: (a) R^1NH_2, Na_2CO_3, DMF, 90 °C (10–85%); (b) Raney-Nickel, H_2 (1 atm), MeOH, rt (50–76%); (c) EDC, HOBt, *N*-Boc glycine, THF, rt (60–74%); (d) 30% TFA/DCM, rt; (e) EDC, HOBt, *i*-Pr_2NEt, amino acid, THF, rt; (f) AcOH, 100 °C; (g) 30% TFA/DCM, rt; (h) EDC, HOBt, *i*-Pr_2NEt, R^2CO_2H, THF, rt.

Once synthesized, compounds were tested for their ability to block the $hNa_v1.7$ channel. This was done using $hNa_v1.7$ channels that were stably expressed in a HEK-293 cell line. The degree of channel block was measured using a functional, membrane potential-based assay, the protocols for which have been previously described.[9] Using the same assay protocol, $hNa_v1.8$ channel block was also measured for selected compounds. In addition to this primary $hNa_v1.7$ functional assay, compounds were also screened in an IK_r binding assay (MK499) and a cytochrome 3A4 inhibition assay. Since block of hERG K^+ channels can result in fatal ventricular arrythmias and inhibition of CYP 3A4 can lead to drug–drug interactions, compounds that did not have these activities would be more promising clinical candidates.[13]

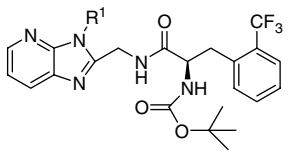
The effect of the R^1 substituent on potency and PK was investigated. As shown in Table 1, to maintain $hNa_v1.7$ potency the imidazole R^1 substituent must be no smaller than a *tert*-butyl group (**17**: $hNa_v1.7$ IC_{50} = 185 nM). Compounds bearing larger alkyl groups such as **18**–**21** showed good $hNa_v1.7$ potency, but were compromised by their poor PK profiles. These compounds such as **18** suffered from high clearance and low oral exposure.[14] In an effort to reduce clearance by blocking potential N-dealkylation, branched alkyl analogs **15** and **16** were synthesized. These analogs, however, showed reduced $hNa_v1.7$ potency.

Incorporation of a benzyl substituent at R^1 led to significant improvements in rat pharmacokinetics. Compound **4** showed a much improved rat PK profile, displaying both modest clearance and higher exposure (Clp = 15 ml/min/kg, F = 41%, AUC_N (po) = 0.86 μM h kg/mg). Incubation of **4** with rat liver microsomes revealed that the primary site of oxidative metabolism was the benzyl substituent. Assuming that oxidation of the benzylic methylene was the metabolic liability, we synthesized α-methyl analogs **22** and **23**. Unfortunately, these compounds afforded no improvement in PK. The cyclopropyl analog **24**, however, did show a reduced clearance rate (3 ml/min/kg) and fivefold higher oral exposure (AUC_N (po) = 4.63 μM h kg/mg).

As shown in Table 1, this series of compounds are potent inhibitors of cytochrome 3A4 (e.g., **4**: IC_{50} = 980 nM). Block of hERG does not appear to be an issue, as compounds in this series typically displayed <50% inhibition of MK499 at 10 μM.

With the imidazole substituent optimized for pharmacokinetics, we focused our efforts on modifying the *N*-Boc group. Previous work in the related benzazepinone series (**3**) had shown that a secondary amide or carbamate was required at this position to maintain potency. As shown in Table 2, *tert*-butyl (**25**) and neopentyl amides (**26**) were acceptable surrogates for the Boc group and resulted in analogs with similar $hNa_v1.7$ potency. While the simple phenyl amide **27** afforded a 7-fold loss in potency, incorporation of a 2-substituent increased potency (**28** and **29**). The 2-trifluoromethoxy analog, **28**, had comparable potency to the parent (**4**). Changing

Table 1. The effect of imidazole substituent on $hNa_v1.7$ binding, off-target activities, PK and in vivo efficacy



Compound	R^1	$hNa_v1.7$ IC_{50} (nM)	MK-499 (% inhibition at the rate of 10 μM)	CYP 3A4 IC_{50} (μM)	Cl_p (mL/min/kg)	AUC (norm. po, μM h kg/mg)	F (%)
12	H	>10,000	13				
13	Me	>10,000	0				
14	^{i}Pr	>10,000	0				
15		1000	54	0.86			
16		>1000	19				
17	*t*-Bu	185	32		36	0.28	31
18	CF_3CH_2	81	0		113	0.02	7
19	$CF_3CF_2CH_2$	123	19		87	0.07	10
20	*c*-$PrCH_2$	243	20	2.43	114	0.07	25
21	*t*-$BuCH_2$	83	19		64	0.08	17
4	$PhCH_2$	80	43	0.98	15	0.86	41
22		255	1		21	0.50	36
23		191	65		22	0.82	57
24		186	50	0.82	3	4.63	44

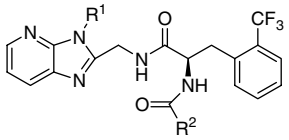
the imidazole substituent from a benzyl to a cyclopropylbenzyl consistently resulted in a decrease in $hNa_v1.7$ potency (e.g., cf. **28** with **33**). Trifluoromethylcyclopropyl and 2-trifluoromethoxyphenyl amides **32** and **33** had comparable potency to the parent (**24**), and had no MK-499 liability. These amides, which were potentially more stable to oxidative metabolism, were incorporated into subsequent compounds. However, CYP 3A4 inhibition in this series of imidazopyridines remained an issue (e.g., compounds **32** and **33**). As shown in Table 2 these compounds are more potent blockers of $hNa_v1.7$ than hNa_v 1.8.

With optimized R^1 and R^2 substituents in hand, we explored SAR at the R^3 position. Earlier work in the related benzazepinone series (**3**) had shown that a lipophilic aromatic group was necessary at R^3 to maintain $hNa_v1.7$ potency. The effect of substitution at R^3 was investigated and it was discovered that CYP 3A4 inhibition could be attenuated with the correct substitution pattern (e.g., **36** and **40**). As shown in Table 3, analogs with a 2-CF_3 substituent are all potent inhibitors of CYP3A4. Adding a substituent at the 3-position also increases CYP3A4 potency (cf. **35** with **41** or **34** with **38**). This SAR study afforded compounds with clean off-target profiles, for example, **35**, **36**, and **40** (<50% at the rate of 10 μM MK-499 binding and >10 μM inhibitor of CYP 3A4). Substitution at the *para* position affords a large decrease in potency (cf. compound **24** with **47**). Otherwise, substitution on the phenyl substituent R^3 is widely tolerated. Several analogs emerged with greater potency than the parent (**34**) with the 2,3,6-trifluoro analog affording a 3-fold increase (**43**) ($hNa_v1.7$ IC_{50} = 63 nM). Analogs such as 2-tolyl or 2-methoxyphenyl were not synthesized due to their potential PK liabilities. Compounds in Table 3 continue to greater potency for $hNa_v1.7$ over $hNa_v1.8$, with selectivity ranging from 2- to 7-fold.

With these systematic SAR studies complete, compounds were synthesized that combined all of the optimized substituents. As shown in Table 4, several analogs with $hNa_v1.7$ potency, clean off-target profiles and good rat PK were identified (e.g., **49** and **50**).

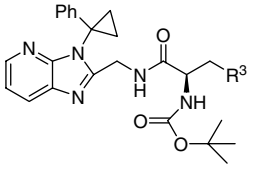
These optimized compounds were tested in the Chung rat spinal nerve ligation model of neuropathic pain.[15]

Table 2. Effect of the amide substituent on potency and off-target activities



Compound	R^1	R^2	$hNa_v1.7$ IC_{50} (nM)	$hNa_v1.8$ IC_{50} (nM)	MK-499 % inhibition at the rate of 10 μM	CYP 3A4 IC_{50} (μM)
25	Bn	^{t}Bu	261	1458	58	
26	Bn	CH_2^tBu	86	361	63	
27	Bn	Ph	552		19	
28	Bn	2-OCF_3Ph	129	230	0	
29	Bn	2-CF_3Ph	232	366	0	
30	Bn		476		45	
31			1195	2975	6	
32			261	1782	27	1.9
33		2-OCF_3Ph	181	825	36	1.8

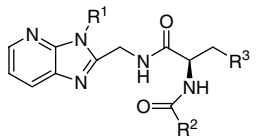
Table 3. Effect of the phenylalanine substitution on $hNa_v1.7$ and off-target activities



Compound	R^3	$hNa_v1.7$ IC_{50} (nM)	$hNa_v1.8$ IC_{50} (nM)	MK-499 % inhibition at the rate of 10 μM	CYP 3A4 IC_{50} (μM)
34	Ph	394		19	6
35	2-F-Ph	238	880	40	>10
36	2-Cl-Ph	132	500	12	>10
24	2-CF_3-Ph	186	793	50	0.82
37	2-OCF_3Ph	148	985	35	1.3
38	3-Cl-Ph	221		33	2.4
39	4-Cl-Ph	1053		48	
40	2,5-diF-Ph	148		5	>20
41	2,3-diF-Ph	275		16	4.6
42	2,6-diF-Ph	171	685	30	6.1
43	2,3,6-triF-Ph	63	446	5	3.9
44	2-Cl-6-F-Ph	147		4	5.3
45	2-Cl-5-F-Ph	102	295	13	2.0
46	2-CF_3-3-F-Ph	158	806	41	0.81
47	2-CF_3-4-F-Ph	744	1242	54	
48	2-CF_3-6-F-Ph	153		18	3.4

In this model, neuropathic pain is induced in rats by surgically ligating and transecting the rat's L5 spinal nerve. Sensitivity to mechanical stimulation is measured before and seven days after surgery using calibrated Von Frey filaments. Rats that exhibit significant allodynia are dosed orally with test compound, and the reversal of allodynia is measured. Despite good oral exposure and $hNa_v1.7$ potency, compounds **35** and **49**–**51** lacked efficacy in this model. Oral dosing (10 mg/kg) of compound **24** afforded efficacy at 2 h post-dose similar to that of mexilitene but no efficacy at the 4-h time point (% reversal at 2 and 4 h = 45% and 12%, respectively). Com-

Table 4. The effect of imidazole substituent on $hNa_v1.7$ binding, off-target activities, PK and in vivo efficacy



Compound	R^1	R^2	R^3	$hNa_v1.7$ IC_{50} (nM)	hNa_v 1.8 IC_{50} (nM)	MK-499 % inhibition at the rate of 10 μM	CYP 3A4 IC_{50} (μM)	Cl_p (mL/min/kg)	AUC norm., po (μM h kg/mg)	F (%)	% Reversal of allodynia (10 mg/kg po) in the SNL model at	
											2 h	4 h
4	Bn	O^tBu	2-CF_3-Ph	80	270	43	1.0	15	0.86	41	52	41
24		O^tBu	2-CF_3-Ph	186	793	50	0.8	3.0	4.6	44	45	12
49			2-Cl-Ph	329		19	>20	9.3	2.5	78	20	13
50			2,6-di-F-Ph	540	1869	1	>20	4.2	4.0	54	15	5
51			2,3,6-tri-F	182	446	3	6.4	6.6	3.7	85	32	4
35		O^tBu	2-F-Ph	158	880	40	11	7.5	2.0	47	25	24
Mexiletine											48	23

pound **4**, however, demonstrated significant reversal of allodynia when dosed orally at 10 mg/kg (% reversal at 2 and 4 h = 52% and 41%, respectively). As shown in Table 4, this efficacy compares favorably to the efficacy we obtained with mexiletine, a compound used clinically to treat neuropathic pain.

Compound **4** was also tested in a rat model of inflammatory pain. In this model, the rat's hind paw is subjected to intradermal injection of complete Freund's adjuvant (CFA) to induce inflammation.[16] Hyperalgesia to mechanical pressure is measured using a Randal–Sellito apparatus. Compound **4** exhibited significant reversal of hyperalgesia in this model even at a low oral dose of 3 mg/kg (% reversal at 2 and 4 h = 44% and 44%, respectively). This result is similar to the efficacy we obtained with indomethacin, a compound that is used clinically to treat inflammatory pain (3 mg/kg po: % reversal at 2 and 4 h = 43% and 56%, respectively).

In summary, we have identified a novel series of imidazopyridine $hNa_v1.7$ blockers. A benchmark compound from this class displayed robust rat pharmacokinetics, and was orally efficacious in rat models of neuropathic and inflammatory pain.

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