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A potent and selective indole N-type calcium channel ($Ca_v2.2$) blocker for the treatment of pain

Sriram Tyagarajan [a,*], Prasun K. Chakravarty [a], Min Park [a], Bishan Zhou [a], James B. Herrington [b], Kevin Ratliff [b], Randall M. Bugianesi [b], Brande Williams [b], Rodolfo J. Haedo [b], Andrew M. Swensen [b], Vivien A. Warren [b], McHardy Smith [b], Maria Garcia [b], Gregory J. Kaczorowski [b], Owen B. McManus [b], Kathryn A. Lyons [c], Xiaohua Li [c], Maria Madeira [c], Bindhu Karanam [c], Mitchell Green [c], Michael J. Forrest [d], Catherine Abbadie [d], Erin McGowan [d], Shruti Mistry [d], Nina Jochnowitz [d], Joseph L. Duffy [a]

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

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ABSTRACT

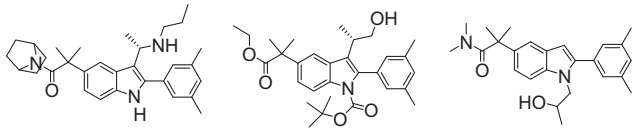
N-type calcium channels ($Ca_v2.2$) have been shown to play a critical role in pain. A series of low molecular weight 2-aryl indoles were identified as potent $Ca_v2.2$ blockers with good in vitro and in vivo potency.

Several lines of evidence point to the involvement of N-type calcium channels ($Ca_v2.2$ channels) in pain.[1] These channels are present in presynaptic terminals of primary afferent nociceptors. $Ca_v2.2$ channels are highly expressed in the dorsal horn of the spinal cord, which is the final common path for pain signal transmission. These channels are up regulated in pain conditions.[2] $Ca_v2.2$ knockout mice exhibit reduced sensitivity to pain.[3] The peptide ziconitide (Prialt®, Elan) is a potent and selective blocker of $Ca_v2.2$ channels and is a marketed drug for treating severe chronic pain.[4]

The use of ziconotide is limited by the need for intrathecal administration and the narrow therapeutic window for CNS side effects (sedation, confusion and dizziness).[5] These effects may arise from the state-independent block of $Ca_v2.2$ by ziconitide. Furthermore, systemic administration of the peptide is associated with profound hemodynamic effects.[6] It is believed that selective state-dependent block of $Ca_v2.2$ channels with small molecules will affect neuronal signaling only under conditions of hyper excitability and thus be effective in treating pain without the side effects of ziconotide.[7]

From our high throughput screening efforts (Table 1) we identified compound **1** as a $Ca_v2.2$ blocker with modest potency in the in vitro $Ca_v2.2$ fluorescent calcium-influx assay (FLIPR), and found it to be selective over cardiac ion channels with weak block of

Table 1
Screening hit to lead



1 Screening Hit | 2 | 3 Lead

Ion channel targets	1	2	3
$Ca_v2.2$ (IC_{50}, μM)	0.88	0.6	0.87
$Ca_v1.2$ (inh @ 10 μM)	62%	40%	52%
$Na_v1.5$ (inh @ 10 μM)	44%	25%	ND

* Corresponding author.
E-mail address: sriram_tyagarajan@merck.com (S. Tyagarajan).

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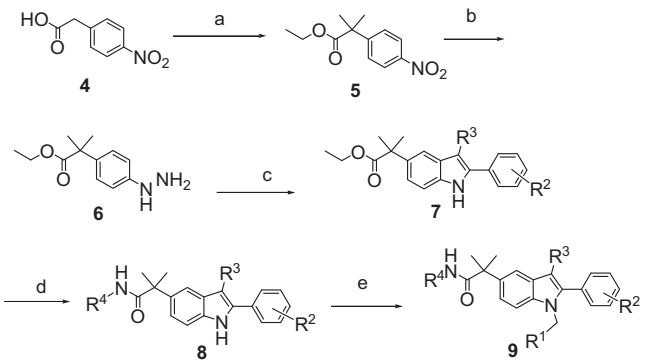
$Ca_v1.2$ and $Na_v1.5$. Utilizing this indole scaffold, modifications of substituents led to compound **2**, a somewhat simplified indole analog. Building upon the hypothesis that a C-3 substituent may not be necessary, a more simplified indole analog, compound **3**, was identified as the lead for this series. Using this simplified indole scaffold, we set out to explore a series of novel C-2 substituted indoles as small molecule $Ca_v2.2$ blockers.

The synthesis of these indoles is outlined in Scheme 1. Alkylation of the commercially available 4-nitrophenyl acetic acid **4** afforded the dimethyl ester **5**. Reduction of the nitro group and subsequent diazotization generated the hydrazine **6**. Condensation of the hydrazine **6** with aryl acetophenones afforded the 2-aryl indoles **7**. Hydrolysis of the ester to the acid and subsequent amidation yielded the corresponding amides **8**. Finally N-1 substituted analogs were generated via treatment with the corresponding alkyl halides and sodium hydride.

The synthesized compounds were evaluated for their ability to block voltage gated $Ca_v2.2$ using an in vitro fluorescent calcium-influx FLIPR assay.[8] 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 $Ca_v1.2$ (diltiazem, DLZ) binding.[9] Compounds selective in these ancillary assays were examined in various rat in vivo pain models and their pharmacokinetic profiles were evaluated.

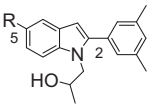
We first explored the C-5 position of the indoles as shown in Table 2. The dimethyl moiety is critical for $Ca_v2.2$ in vitro potency. Several des-methyl analogs or truncated analogs such as compound **10** were inactive in the in vitro $Ca_v2.2$ FLIPR assay. The dimethyl ester **11** and the alcohol derivative **12** had significantly reduced potency in the $Ca_v2.2$ FLIPR assay. Amides were well tolerated at C-5 position of the indole. The cyclopropyl amide **13** and the trifluromethyl amide **14** had weaker block than the dimethyl amide **3**. The more hindered lipophilic t-butyl analog **15** had improved $Ca_v2.2$ block in the FLIPR assay. These compounds were also tested for the block of L-type $Ca_v1.2$. As illustrated in Table 2, the compounds exhibited a range of activity against $Ca_v1.2$.

With an optimized *t*-butyl analog installed at C-5 position of the indole, we turned our attention to the N-1 position of the indole (Table 3). We focused our efforts on finding a suitable metabolically stable substituent at this position. Although the Boc substituent in **2** was active, both the Boc derivative **16** and the free indole **17** were less potent. Substituting this position with a liphophilic *t*-butyl ester, analog **18** improved the $Ca_v2.2$ potency. Introduction of a *t*-butyl acetamide, **19** afforded significantly improved $Ca_v2.2$ potency. Replacing the amide with an ether, analog **20** or the amide isostere dimethyl isoxazole group, analog **21** reduced



Scheme 1. Reagents and conditions: (a) (i) EtOH, H_2SO_4; (ii) NaH, DMF, EtI; (b) 10% Pd/C, EtOH; (ii) $NaNO_2$, HCl, $SnCl_2{\cdot}2H_2O$; (c) ArCOMe, EtOH, $ZnCl_2$, HOAc; (d) (i) KOH, MeOH; (ii) 1-chloro-*N*,*N*-trimethylpropenylamine, R^4NH_2, TEA, DCM; (e) NaH, R^1CH_2Br, DMF.

Table 2
SAR at C-5 position of the indole



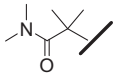
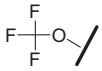
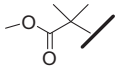
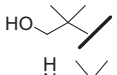
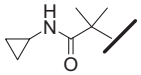
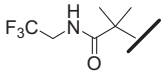
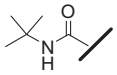
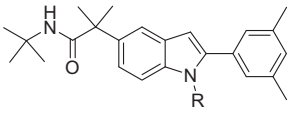
Entry	R	$Ca_v2.2$ FLIPR (% inh @ 10 μM) (IC_{50}, μM)	$Ca_v1.2$ FLIPR (% inh @ 10 μM)
3		89% (0.87)	52%
10		36%	43%
11		70%	64%
12		44%	32%
13		89% (1.15)	ND
14		91% (1.17)	ND
15		97% (0.68)	90%

Table 3
SAR at N-1 position of the indole



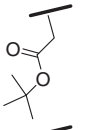
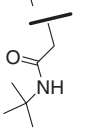
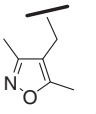
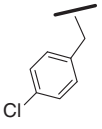
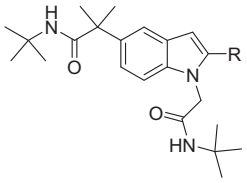
Entry	R	$Ca_v2.2$ FLIPR (% inh @ 10 μM) (IC_{50}, μM)	$Ca_v1.2$ FLIPR (% inh@ 10 μM)	MK-0499 (% inh@ 10 μM)
15		97% (0.68)	90%	64%
16		59%	ND	ND
17	H	78%	ND	ND
18		91% (0.66)	48%	60%
19		98% (0.19)	78%	33%
20		99% (0.52)	70%	81%
21		99% (0.54)	ND	65%
22		36%	ND	ND

Table 4
SAR at C-2 position of the indole



Entry	R	$Ca_v2.2$ FLIPR (IC_{50}, μM)	$Ca_v1.2$ FLIPR (% inh@ 10 μM)	PXR EC_{50} (μM)
19	3,5-MePh	0.18	79%	>30
23	Ph	0.47	79%	1.81
24	4-FPh	0.50	82%	>30
25	3,5-CF_3Ph	0.76	61%	10.76
26	3-CF3,-5-FPh	0.40	88%	> 30
27	2,4,-FPh	0.27	93%	0.94
28	2-OCF_3Ph	0.07	79%	> 30
29	3-OCF_3Ph	0.06	93%	> 30
30	4-OCF_3Ph	0.15	85%[a]	> 30
31	2-OCH_3Ph	0.28	60%	0.74
32	*t*-butyl	0.14	47%	0.57
33	CONHt-Bu	0.24	24%	6.64
34	Thiazole	0.24	40%	2.46
35	2-Pyridyl	0.57	64%	0.60

[a] IC_{50} = 0.91 μM.

$Ca_v2.2$ potency, while a benzyl analog **22** was not potent. These analogs exhibited modest selectivity in favor of $Ca_v2.2$ over $Ca_v1.2$. Although **19** was potent and selective for block of $Ca_v2.2$ channels, the compound exhibited poor in vitro metabolic stability in incubations with rat and human liver microsomes (<5% parent remaining after 1 h).

We hypothesized that the 3,5-dimethyl phenyl substituent was a metabolic liability, and we set out to find a suitable replacement for this substituent (Table 4). The resulting compounds were distinguished on basis of $Ca_v2.2$ FLIPR potency. Having optimized the N-1 amide substituent, MK-0499 activity was minimal in all subsequent compounds. The compounds were counter screened for $Ca_v1.2$ activity and for PXR activation as a measure of the potential for CYP induction.[10] The unsubstituted phenyl analog **23** at C-2 position afforded good potency but also had moderate activity in the PXR induction assay. Introduction of a fluoro group, analog **24**, maintains the $Ca_v2.2$ potency and decreased the PXR potency. Replacing the methyl groups with a more liphophilic trifluoromethyl groups, analog **25**, afforded modest in vitro block on $Ca_v2.2$, but greater potency as a PXR activator. Replacing the triflurometh groups in **25** with fluoro substituents, analog **27** improved $Ca_v2.2$ block but the compound remained a PXR activator. Introduction of a trifluoromethoxy aryl group at the C-2 position improves the in vitro $Ca_v2.2$ potency and selectivity against PXR. The 2-trifluormethoxy aryl, 3-trifluomethoxy and the 4-trifluoromethoxy aryl groups were all well tolerated at this position. Replacement with a methoxyphenyl substituent maintains the in vitro block but the compound was selective towards the PXR receptor.

We observed that alkyl groups at C-2, as in **32**, tend to be PXR activators while aryl groups at the same position can be more selective against PXR. Conversely, aryl groups at C-2 (**23**–**31**) generally exhibited more pronounced $Ca_v1.2$ block in comparison to alkyl groups at C-2 position.

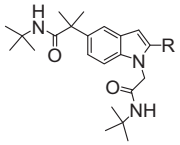
Table 5 shows the pharmacokinetic profile of selected compounds that exhibited excellent $Ca_v2.2$ block and good selectivity against PXR). Compounds **28** and **29** maintained a good pharmacokinetic profile but very poor stability in human liver microsomal incubation. The 4-trifluoromethoxy phenyl analog **30** has modest pharmacokinetic profile in rat but a better metabolic profile than indoles **28** or **29**. Compound **30** exhibited similar metabolic stability in liver preparations from human and rat, as well as dog and monkey.

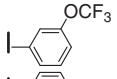
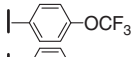
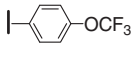
Compound **30** was further evaluated for pharmacokinetic properties in rat and dog. The compound afforded a moderate oral exposure following a 3 mpk dose in rats (C_{max} = 0.29 μM, AUC_N = 0.34 μM h kg/mg) and a high clearance and distribution (CL = 64 mL/min/kg, Vd_{ss} = 5.1 L/kg) for an overall oral bioavailabil-

Table 6
In vivo activity of compound **30**

Entry	% Reversal (1 h)	% Reversal (3 h)	% Reversal (8 h)
CFA (3 mpk, po)	36	37	17
CFA (10 mpk, po)	57	52	22
CFA (30 mpk, po)	66	68	32
SNL (10 mpk, po)	45	47	14
SNL (30 mpk, po)	68	50	27

Table 5
Pharmacokinetic data for selected compounds



Entry	R	PK species	HLM[a] (%)	RLM[b] (%)	Dose(iv/po) (mg/Kg)	AUC_N[c]	Clp[d]	$T_{1/2}$ (h)	*F* (%)
28		Rat	0.04	2.5	1.0/3.0	0.57	21	2.68	38
29		Rat	0.82	22	1.0/3.0	0.83	11	2.27	26
30		Rat	20	20	1.0/3.0	0.34	64	1.4	68
30		Dog			1.0/3.0	1.02	5.6	4.6	18%

[a] Percent of parent compound remaining in human liver microsomes following incubation after 60 min.
[b] Percent of parent compound remaining in rat liver microsomes following incubation after 60 min.
[c] μM h kg/mg.
[d] mL/min/kg.

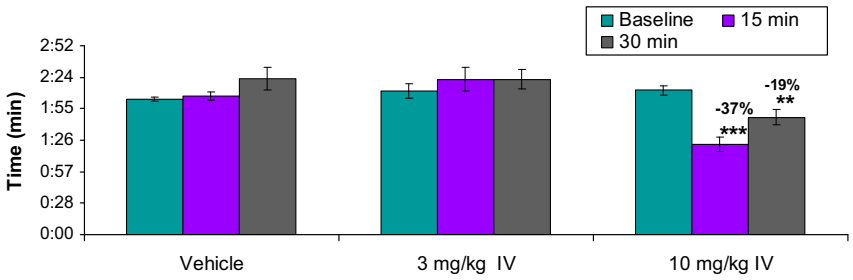


Figure 1. Effect of compound **30** at 3 and 10 mg/kg IV on motor coordination.

ity of 68% based on plasma analysis. However, in dog, the PK profile showed low clearance (4.6 mL/min/kg), good plasma half life (4.6 h) and modest oral exposure moderate oral exposure (AUC_N = 1.0 μM h kg/mg) for an overall modest bioavailability of 18%.

Compound **30** was a weak inhibitor of common isoforms of cytochrome P450 (IC_{50} > 10 μM vs CYP3A4, CYP2D6 and CYP2C9). From these collective results, compound **30** was identified for additional characterization in the in vivo rat pain efficacy models. This potent Ca_v 2.2 channel blocker was efficacious in various preclinical pain models (Table 6). In the rat complete Freund's adjuvant (CFA)-induced inflammatory pain model, compound **30** reverses mechanical allodynia in a dose dependant manner.[11] When dosed at 30 mg/kg, compound **30** afforded robust maximal 68% reversal of paw pressure analgesia (C_{plasma} = 0.75 μM, C_{brain} = 0.17 μM, t = 3 h). In the rat spinal nerve ligation (SNL) model of neuropathic pain, the compound afforded robust maximal 68% reversal of paw pressure analgesia(C_{plasma} = 0.75 μM, C_{brain} = 0.17 μM, t = 3 h). In the rat spinal nerve ligation (SNL) model of neuropathic pain, the compound afforded robust maximal 68% reversal of allodynia at 30 mpk PO (C_{plasma} = 0.41 μM, C_{brain} = 0.26 μM, t = 1 h).[12,13] Compound **30** affords excellent in vivo efficacy in all the rodent pain models relatively at low plasma and brain concentration.

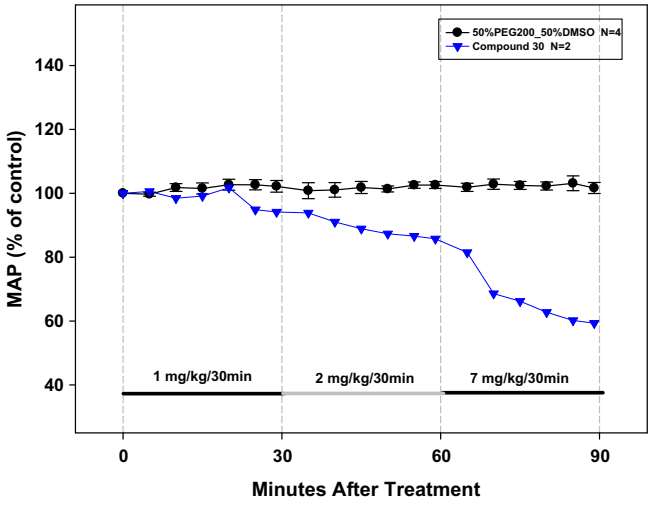


Figure 2. Effect of compound **30** on MAP in anesthetized dogs.

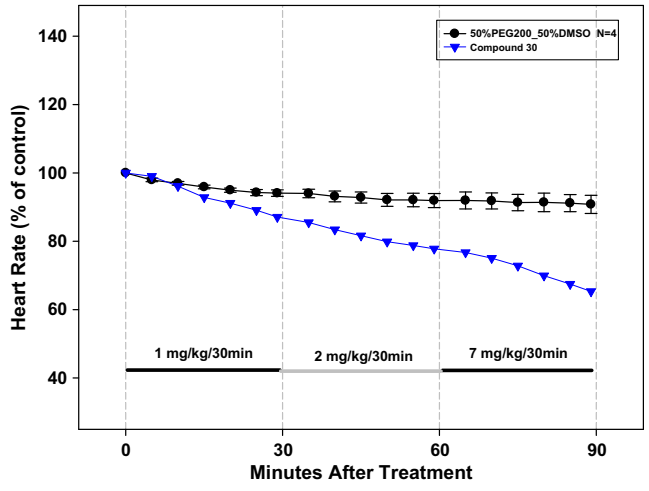


Figure 3. Effect of compound **30** on HR in anesthetized dogs.

The ancillary pharmacology of **30** was also evaluated. In the rat Rotarod model of motor coordination,[14] compound **30** was studied at doses of 3 and 10 mg/kg (IV) (Fig 1). Due to poor solubility, compound **30** could not be evaluated at higher doses. Significant impairment of motor coordination caused by compound **30** was observed at higher plasma (8.0 μM) and brain level (6.0 μM). However, no impairment of motor coordination was observed at 3 mg/kg IV (plasma and brain exposure 1.9 and 1.4 μM, respectively). Significant efficacy (EC_{50}) in the rat SNL model was obtained at a plasma exposure of 0.44 μM, which leads to a four fold window for efficacy over the no effect level for ancillary CNS effects.

In order to study the cardiovascular properties of indole **30**, the compound was administered at rising doses of 1, 3, and 10 mg/kg IV in barbiturate anesthetized and ventilated dogs (Fig 2 and Fig 3). The compound afforded an exposure-dependent decrease in mean arterial pressure (MAP) above 2 μM plasma concentration, reaching maximal −40% reduction at maximal exposure (32 μM). A concomitant decrease in HR was also observed, to a maximal −32% reduction at maximal exposure. These effects may be related to L-type Cav1.2 calcium channel activity (IC_{50} = 908 nM).

In summary, a novel aryl indole **30**, was discovered as a potent, selective and state-dependent blocker of Ca_v2.2 channels has been identified from a screening lead. Compound **30** displays excellent in vitro block in rat Ca_v2.2 FLIPR assay and has robust in vivo efficacy in the rat pain models. It has good pharmacokinetic profile in rat and dogs. Compound **30** is clean in CYP3A4 & PXR. In cardiovascular dog studies, compound **30** caused significant dose dependent reduction in blood pressure and heart rate starting at a relatively modest plasma concentration (2–3 μM). The observed CV effects are attributed to blocking of Ca_v1.2channels (L-type). Lack of selectivity between Ca_v2.2 and Ca_v1.2 block is an issue which is prevalent in this indole series and structural modifications that improve calcium channel subtype selectivity will be reported in due course.

Acknowledgments

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References and notes

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