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Discovery and SAR of novel tetrahydropyrrolo[3,4-*c*]pyrazoles as inhibitors of the N-type calcium channel

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ABSTRACT

A novel series of substituted tetrahydropyrrolo[3,4-*c*]pyrazoles were investigated as blockers of the N-type calcium channel ($Ca_v2.2$ channels), a chronic pain target.

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Voltage-dependent Ca^{2+} channels play important roles in many critical biological processes.[1] One particular type, the N-type calcium channel ($Ca_v2.2$ channel), is located mainly at pre-synaptic nerve terminals and has been implicated in the neurotransmission of pain.[2] N-type knockout mice show moderated response to pain sensation, and show reduced neuropathic pain symptoms compared to normal mice.[3] The cone snail toxin ziconotide (Prialt®) is a potent blocker of N-type calcium channels and is used for severe chronic pain. It must be administered intrathecally, and has a side-effect profile that gives it a very narrow therapeutic window.[4] Due to these features, we feel that N-type calcium channel is an attractive target for an oral therapeutic for the treatment of pain, in particular neuropathic pain. Other groups have also been pursuing this target with varying degrees of success, with one compound, CNV-2197944 (structure not disclosed), currently in clinical development for the treatment of chronic pain (Fig. 1).[5]

One hit from a screen of our compound collection was pyrazole **1**, a 100 nM inhibitor of N-type calcium channel (Fig. 2). The sidechain of **1** was rigidified by forming a fused cyclopentane ring on the pyrazole scaffold **2** that improved potency by lessening the rotational degrees of freedom of the sidechain.[6] It was hypothesized that modifying **2** by inserting a nitrogen into the cyclopentyl ring giving tetrahydropyrrolo[3,4-*c*]pyrazoles **3** should enhance the lipophilicity of the compounds and may impact other properties such as rat liver microsome (RLM) stability. These compounds were designed using a 3+2 dipolar cycloaddition reaction of a nitrilimine and an alkyne tethered together by an alkylamino chain.

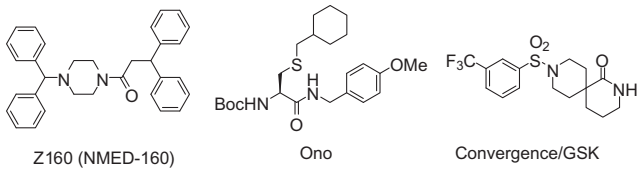

Figure 1. Some Representative N-Type Calcium Channel Blockers.

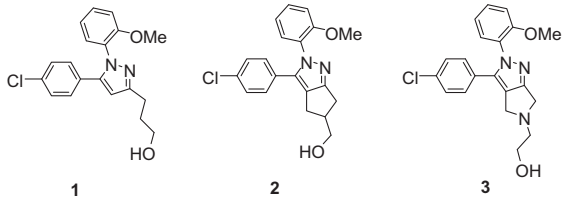

Figure 2. Initial pyrazole hit and proposed bicyclic pyrazoles.

The general synthetic schemes for the synthesis of 2,3,5-tetrahydropyrrolo[3,4-*c*]pyrazoles are described below (Scheme 1).[7] Treatment of alkyne **4** with bromine and KOH gives alkynyl bromide **5**.[8] The hydrazide, generated by displacement of the mixed anhydride with an arylhydrazine, is treated with polystyrene-

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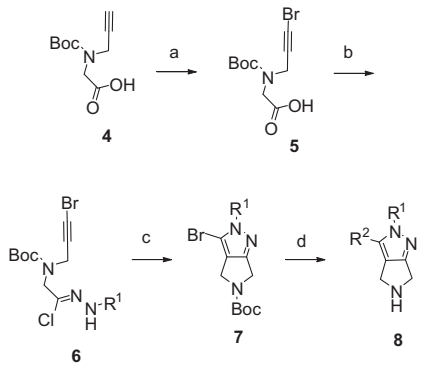
supported Ph_3P and CCl_4 to give hydrazonyl chloride **6**.[7] Upon treatment with TEA in toluene at 100 °C, **6** undergoes an intramolecular 3+2 cycloaddition of the nitrilimine on the alkyne to give the desired 3-bromotetrahydropyrrolo[3,4-*c*]pyrazole **7**.[9] The 3-aryl/heteroaryl substituent is installed by Suzuki coupling of the 3-bromopyrazole with aryl or heteroaryl boronic acids, and the Boc is removed by treatment with TFA to give key intermediate **8**.[10]

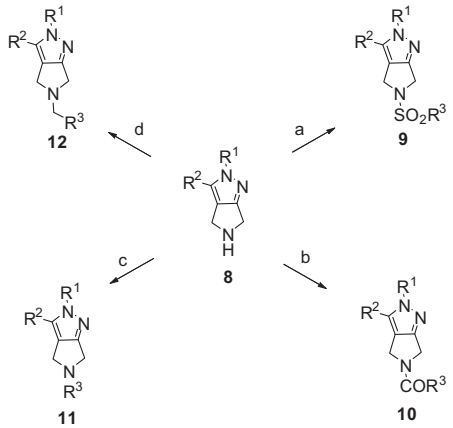
The next scheme shows the functionalization of the tetrahydropyrrole nitrogen (Scheme 2). Treatment with sulfonyl chlorides or acid chlorides in the presence of base gives the sulfonamides **9** or amides **10**, respectively. Derivatives with aryls or heteroaryls directly attached to the nitrogen were made by Buchwald/Hartwig couplings **11**. Reductive amination with aldehydes gives the aliphatic and benzylic derivatives **12**.

Our initial efforts focused on optimizing the groups at 2-, 3-, and 5-positions of the bicyclic heterocycle. Solubility was low for our initial compounds, so basic groups, such as pyridyl, were installed in an attempt to increase solubility while maintaining potency in the Functional Drug Screening System (FDSS) assay.[11] The initial hits also suffered from poor RLM stability. RLM stability was important because stable compounds were necessary to show activity in the initial in vivo model, the rat Complete Freund's Adjuvant (CFA) radiant heat model.

Substitution on the nitrogen at the 5-position of the pyrrolopyrazole gave a range of potencies and was the most tolerant of diverse substitution (Table 1). Simple alkyl groups, such as ethyl

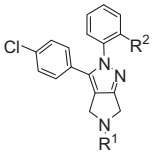


Scheme 1. Reagents and conditions: (a) Br_2, KOH; (b) (i) *i*-BuOCOCl, NMM, R^1NHNH_2, DCM; (ii) PS-Ph_3P, CCl_4, ACN, 50 °C; (c) TEA, toluene, 100 °C; (d) (i) $R^2B(OH)_2$, $Pd(OAc)_2$, (*o*-tol)$_3P$, Na_2CO_3; (ii) TFA.



Scheme 2. Reagents and conditions: (a) R^3SO_2Cl, TEA; (b) R^3COCl, TEA or R^3COOH, EDC; (c) R^3Br or R^3I, Pd_2dba_3, BINAP, Cs_2CO_3, toluene, 100 °C (d) R^3CHO, $NaBH(OAc)_3$.

Table 1
SAR at the 5-position of the pyrrolopyrazole



Compd no.	R^1	R^2	FDSS IC_{50} (nm)	RLM[b] (%)
13	Et	OMe	61% [a]	1
14	4-Cl-Ph	Et	34% [a]	NT
15	pyrid-2-yl	Et	36% [a]	18
16	CH_2-4-Cl-Ph	Et	300	18
17	CH_2-pyrid-4-yl	Et	280	10
18	$CONMe_2$	OMe	170	10
19	Boc	OMe	73	NT
20	$COCF_3$	OCF_3	180	100
21	SO_2Me	OMe	21	3
22	SO_2iPr	OMe	10	2
23	SO_2-cyclohexyl	OMe	16	12
24	SO_2NMe_2	OMe	9.0	13
25	SO_2-morpholine	OMe	1.8	5
26	SO_2-piperidine	OMe	4.0	14
27	$SO_2CH_2CH_2NHMs$	OMe	9.0	13
28	SO_2Bn	OMe	27	1

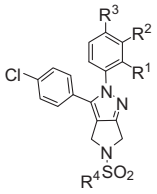
[a] % Inhibition at 1 μM.
[b] % Remaining after 10 min incubation with rat liver microsomes.

13, were not favored. Direct aryl and heteroaryl substitution as well as benzyl substitution were also not tolerated, presumably due to the basicity of the nitrogen in the ring. Amides, ureas and carbamates are somewhat more potent, as evidenced by **18**, **19** and **20**. The most fruitful substitutions in this series were the 5-position sulfonamides and sulfamides. Methanesulfonamide **21** exhibited relatively high potency, and that of isopropyl sulfonamide **22** was 2-fold higher. However, cyclic sulfamides, such as **25** and **26** were the most potent compounds made in this sub-series. Longer chain sulfonamides and sulfamides exhibited reduced potency. RLM stability remained poor for all of the potent compounds in this series; and only the weakly active trifluoroacetyl (**20**), wherein R^2 is OCF_3, had good RLM stability.

Next, a number of substituents on the aromatic ring at the 2-position of the pyrazole were explored (Table 2). Metabolite identification studies on **22** showed that loss of the methyl from the *o*-methoxy was the major metabolite, so a number of substituents on that ring were explored in an attempt to mitigate this issue. Methoxy **22** and ethoxy **29** were both quite potent; however, the more electron-withdrawing trifluoromethoxy substituent **30**, lost some potency. Contrary to our hypothesis, the ethyl substituent **31** did not afford greater RLM stability and exhibited diminished potency as well. Similarly, the dimethylamino substituent **32** also did not improve the RLM stability, although potency was restored. Substitution with methyl and fluoro at the 4-position reduced potency somewhat; however, reversing the methyl and methoxy **35** led to a much more dramatic loss of potency. Fused rings **36** and **37** did not enhance the potency. Attempts to adjust the poor RLM stability by making numerous changes to this ring did not result in any improvement except for **34**, the least potent compound in this series. Collectively, these data support the hypothesis that larger *ortho* substituents, such as methoxy, are necessary for inhibitory activity, possibly due to the angle of the aromatic ring to the pyrazole. An electron-rich ring also seems important for N-type inhibitory activity; however, this SAR runs counter to RLM stability.

Substitution on the 3-aryl ring did not improve potency over the 4-chlorophenyl starting point **22** (Table 3), and removing the aryl completely **38** or substituting bromine **39** resulted in a

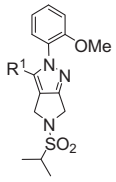
Table 2
SAR of 2-aryl analogs



Compd no.	R^1	R^2	R^3	R^4	FDSS IC_{50} (nm)	RLM[a] (%)
22	OMe	H	H	*i*-Pr	10	2
29	OEt	H	H	*i*-Pr	3.0	NT
30	OCF_3	H	H	*i*-Pr	27	NT
31	Et	H	H	Me	85	13
32	NMe_2	H	H	*i*-Pr	5.1	4
33	OMe	H	Me	*i*-Pr	32	13
34	Me	H	OMe	*i*-Pr	190	76
35	OMe	H	F	*i*-Pr	21	23
36	$-OCH_2CH_2-$		H	*i*-Pr	10	1
37	$-OCH_2O-$		H	*i*-Pr	20	7

[a] % Compound remaining after 10 min incubation with RLM.

Table 3
SAR at the 3-position of the pyrrolopyrazole



Compd no.	R^1	FDSS IC_{50} (nm)	RLM[b] (%)
22	4-Cl-Ph	10	2
38	H	33% [a]	8
39	Br	30% [a]	1
40	4-Cl-pyrid-3-yl	16	1
41	4-MeO-pyrid-3-yl	43	1
42	4-*i*-PrO-pyrid-3-yl	130	2
43	4-Me- pyrid-3-yl	46% [a]	NT
44	Quinolin-3-yl	100	1
45	Indol-5-yl	34% [a]	21
46	3-Cl-pyrid-4-yl	45% [a]	4

[a] % Inhibition at 0.33 μM.
[b] % Remaining after 10 min incubation with rat liver microsomes.

dramatic loss in potency. Installing a 3-pyridyl **40** in an attempt to increase solubility resulted in a slight loss in potency, but changing the 4-substituent to oxygen or methyl on the 3-pyridyl ring also was not favored. Fused heterocycles, such as 3-quinoline **44** and 5-indole **45** also did not exhibit improved potency nor did 3-position substitution, such as **46**. RLM stability was not affected by the changes made at the 3-position and remained poor for all compounds.

A number of the more potent compounds were selected for follow-up by screening in patch clamp assays (Table 4).[12] In general, the more potent compounds in the FDSS assay were more potent in the patch clamp assay with a few exceptions. Compound **22**, moderately potent in the FDSS assay, had greater than expected potency in the patch clamp assay; **35**, **27** and **40**, with similar potency to **22**, had very little potency in the QPatch assay. It has been postulated that relatively greater inhibition at high vs. low frequency (i.e., use- or state-dependent inhibition) might result in an improved therapeutic index.[13] Compounds such as **22**, **25**, **26** and **32** all showed some degree of state-dependent inhibition leading to an increased interest in this series.

Table 4
Patch clamp data for selected compounds

Compd no.	FDSS IC_{50} (nm)	QPatch Lo Freq[a]	QPatch Hi Freq[a]	QPatch ratio Lo/Hi (%)
22	10	11	47	23
25	1.8	28	51	55
26	4	20	45	44
27	9	0	0	0
29	3	42	50	84
32	5.1	26	47	55
35	21	0	0	0
36	10	0	7	0
40	16	14	10	140

[a] % Inhibition at 0.1 μM.

To assess selectivity, compound **31** was examined in an L-type Calcium Channel assay and was found to have only 12% inhibition of L-type at 1 μM and 48% inhibition at 5 μM concentration of compound. No other compounds from this series were assayed versus L-type, but this single data point does suggest that the series may be selective for N-type over L-type.[14]

In conclusion, the potency of a straight chain pyrazole hit for N-type calcium channel was improved by modifying the scaffold to a more rigid pyrrolo[3,4-*c*]pyrazole. A number of compounds showed state-dependent inhibition in patch clamp assays, but stability in rat liver microsomes remains an issue. Some of the learnings from this attempt to make oral N-type calcium channel inhibitors have been incorporated into efforts on other series that we hope will lead to improved therapeutics in the future.

Acknowledgments

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10. The same compounds can be accessed using an arylalkyne in place of the bromoalkyne if desired. See Ref. 7.
11. In the FDSS assay, functional activity of test compounds are determined by their inhibitory effects on Ca^{2+} influx via N-type calcium channel after

depolarizing plasma membrane with 50 mM KCl. Calcium mobilization responses to KCl depolarization are evaluated by measuring the intensity of Ca^{2+} fluorescent signal in the presence of BD Calcium Assay Dye (BD Biosciences), utilizing a Functional Drug Screening System (FDSS) by Hamamatsu. The percent inhibition at a concentration of 1 μM as well as IC_{50} values will be reported. For this screen, a stable cell line (HEK parent) expressing $Ca_v2.2$ (N-type calcium channel, Genbank accession number AAO53230) subunits is used. Cav2.2 subunits were expressed in pcDNA3.1 and pBudCE4.1 vectors under selection by 400 μg/ml of G418 and 200 μg/ml of Zeocin. Cells were clonally isolated, expanded and screened by Western blot analyses, and further tested for expression of characteristic N-type calcium currents by whole-cell patch clamp. HEK293 cells stably expressing $Ca_v2.2$ subunits are routinely grown as monolayer in Dulbecco's Modification of Eagle's Medium (DMEM; low glucose Cat # 12320-032) supplemented with 10% FBS, 2 mM L-glutamine, 100 I.U./ml penicillin, 100 μg/ml streptomycin, 400 μg/ml G418, and 200 μg/ml Zeocin (Split ratio: 1:5). Cells are maintained in 5% CO_2 at 37 °C. JNJ compounds are prepared as 10 mM stock in DMSO from neat, if available. Otherwise, the 5 or 10 mM DMSO stock solutions provided in-house are used. Calcium mobilization responses to KCl depolarization are evaluated by measuring the intensity of Ca^{2+} fluorescent signal in the presence of BD Calcium Assay Dye (BD Biosciences), utilizing a Functional Drug Screening System (FDSS) by Hamamatsu. 24 h prior to assay, cells are seeded in clear-base poly-D-lysine coated 384-well plates (BD Biosciences, NJ, USA) at a density of 5000 cells per well in culture medium and grown overnight in 5% CO_2 at 37 °C. On assay day, growth media is removed and cells are loaded with BD calcium assay dye (BD Bioscience) for 35 min at 37 °C, under 5% CO_2 and then for 25 min at room temp. Utilizing the FDSS, cells are challenged with test compounds (at varying concentrations) and intracellular Ca^{2+} is measured for 5 min prior to the addition of 50 mM KCl for an additional 3 min of measurement. IC_{50} values for compounds ran in this assay are determined from six-point dose-response studies and represent the concentration of compound required to inhibit 50% of the maximal response. Maximal fluorescence intensity (FI) achieved upon addition of 50 mM KCl was exported from the FDSS software and further analyzed using GraphPad Prism 3.02 (Graph Pad Software Inc., CA, U.S.A.). Data is normalized to the maximum average counts of quadruplicate wells for each data points in the presence of 50 mM KCl and to the minimum average counts in the presence of buffer. Theoretical curves are generated using nonlinear regression curve-fitting analysis of either sigmoidal dose response or sigmoidal dose response (variable slope) and the IC_{50} values with the best-fit dose curve determined by GraphPad Prism are reported. Conotoxin was run as a positive control in the assay and typically showed IC_{50}'s of 10–20 nM.

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14. Only certain compounds were intermittently assayed for selectivity versus L-type Calcium Channel during this program.