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Synthesis and SAR of 4-aminocyclopentapyrrolidines as orally active N-type calcium channel inhibitors for inflammatory and neuropathic pain

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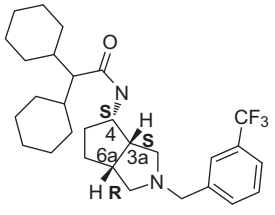
ABSTRACT

A novel series of N-type calcium channel inhibitors have been discovered. Optimization of potency and HT-ADME properties provides 4-aminocyclopentapyrrolidines with analgesic efficacy after oral dosing.

Voltage-gated calcium channels are divided broadly into high voltage-activated (HVA) and low voltage-activated (LVA) channels based on the relative voltages they require for activation. The L-($Ca_v1.1$–$Ca_v1.4$), N-($Ca_v2.2$), P/Q-($Ca_v2.1$), and R-type ($Ca_v2.3$) calcium channels require a larger depolarization for activation, whereas T-type ($Ca_v3.1$–$Ca_v3.3$) calcium channels activate in response to more moderate depolarizations.[1] Inhibition of N-type voltage gated calcium channels represents an attractive approach for the treatment of pain. Ziconotide[2] (Prialt™), a synthetic peptide version of ω-conotoxin MVIIA derived from the piscivorous marine snail (*Conus magus*), is currently used to treat intractable pain for patients for whom opiates are no longer efficacious. Ziconotide is a potent and selective blocker of neuronal N-type calcium channels; however its use is limited due to intrathecal administration. The discovery of small molecule blockers of N-type calcium channels with oral analgesic efficacy would be a valuable alternative for the treatment of pain. Recent progress in this area is very encouraging.[3–6]

We recently reported[7] a potent and selective N-type calcium channel blocker **1** (Fig. 1) with efficacy in animal pain models when delivered intraperitoneal (i.p.). Herein we describe the discovery of orally efficacious N-type calcium channel blockers using a set of in vitro high throughput-ADME (HT-ADME) data in conjunction with $Ca_v2.2$ potency. The HT-ADME data allowed for optimization of pharmacokinetic properties to provide compounds for oral dosing in efficacy studies. Select compounds were then tested in preclinical models of inflammatory, osteoarthritic, and neuropathic pain. Select compounds were also tested in the rat aorta ring relaxation assay to measure activity at L-type calcium channels which when inhibited can contribute to cardiovascular responses[8].

In an effort to improve the oral bioavailbility of this class of compounds while maintaining good potency and PK properties, we explored the SAR of benzyl, sulfonamide, and aryl substituents on the ring nitrogen, in conjunction with changes to the amide group. We also explored the other synthetically accessible



1
Cav2.2 IC_{50}= 0.46 μM
L-type IC_{50} >30μM

Figure 1. $Ca_v2.2$ inhibitor with analgesic activity when dosed i.p.

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diastereomers of the 4-aminotetrahydropyrrolidine core. Synthesis of the (3a*S*,4*R*,6a*R*)-diastereomer of 4-aminocyclopentapyrrolidine is outlined in Scheme 1. Resolved sulfinylimine **2**[7] was deprotected to give the chiral ketone **3**. The ketone was then stereoselectively reduced to the alcohol **4** using sodium borohydride at low temperature, favoring hydride delivery from the less hindered convex face of the bicycle by approximately >20:1. The alcohol **4** was converted to the amine **6** by mesylation, azide inversion, and Staudinger reduction. The amine was then acylated with bis-cyclcohexyl acetic acid to give the diastereomeric bis-cyclohexyl compound **7**.

Leucine derived amides **10–12** were then synthesized as outlined in Scheme 2. The appropriately Boc-protected leucine derivative was coupled to the amine **6** and then deprotected under acidic conditions. The primary amine of compound **10** was then sulfonylated to give compound **12**.

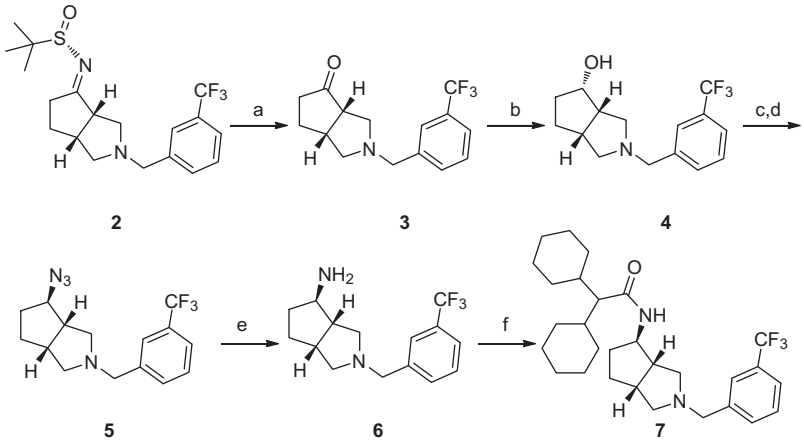
N-sulfonamide derivatives were then synthesized as outlined in Scheme 3. (3a*R*,6a*S*)-Ketone **13**[9] was converted to (3a*S*,4*R*,6a*R*)-amine **14** using the same azide inversion sequence outlined in Scheme 1. Appropriately protected leucine was then coupled to the amine to give amides **15** and **16** and the *N*-benzyl group was removed using catalytic hydrogenation with Degussa's catalyst. The resulting pyrrolidines **17** and **18** were then sulfonamidated using benzene sulfonylchlorides and then the Boc group removed under acidic conditions to give compounds **22–24**. The primary amine **22** was bis-methylated using formaldehyde in a reductive alkylation to give *N*,*N*-dimethyl derivative **25**.

N-sulfonamide derivatives with the opposite stereochemistry were then synthesized as outlined in Scheme 4. (3a*S*,6a*R*)-Ketone **26**[9] was converted to (3a*R*,4*S*,6a*S*)-amine **27** using the same azide inversion sequence outlined in Scheme 1. The common intermediate *p*-trifluoromethylbenzene sulfonamide **28** was synthesized by using the protection/deprotection steps outlined in Scheme 4. The (3a*R*,4*S*,6a*S*)-enantiomeric amine **28** was used to vary the amino acid portion of the molecule by coupling with the appropriate amino acid and then deprotection of the Boc group to give the desired sulfonamide analogs **30–36**. The amino acid could then be alkylated with acetone or ethyl iodide to give compounds **37** and **38**.

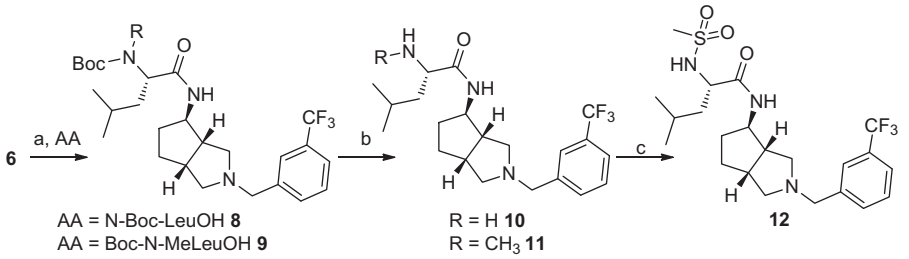
N-Aryl 4-aminocyclopentapyrrolidine analogs were synthesized as described in Scheme 5. Starting with (3a*R*,4*S*,6a*S*)-amine **27**, acylation with Boc-*N*-methyl leucine gives compound **39**. Subsequent debenzylation, palladium catalyzed N-arylation with aryl bromides and final deprotection of the Boc group provides novel analogs **40**, **41** and **42** with our most effective trifluoromethyl groups. Palladium catalyzed N-arylation with aryl bromides in the presence of a mild base such as potassium phosphate tribasic and *tert*-amyl alcohol as solvent enabled chemoselective cross coupling of a secondary amine in the presence of an unprotected amide.

In order to discover more potent N-type calcium channel blockers while maintaining drug-like properties, HT-ADME in vitro data were incorporated in the SAR evaluation of the new analogs as shown in Table 1. Starting with compound **1**, by changing the relative stereochemistry of at C3 from (*S*) to (*R*) (compound **7**), we observed decreasing dofetilide binding (K_i = 0.69 and 9.52 μM, respectively). In order to impart more favorable physicochemical properties on our 4-aminocyclopentylpyrrolidine analogs, we changed our bis-cyclohexyl amide to a less lipophilic group and added a basic nitrogen by using L-leucine.[10,11] This gave rise to compound **11** with greatly improved solubility and microsomal stability and comparable potency to compound **7**. The methanesulfonamide derivative **12** showed an improvement in solubility, but did not improve the potency or microsomal stability. However, changing the *N*-benzyl group into a benzene sulfonamide in compound **22** gave a more potent N-type calcium channel blocker with acceptable human liver microsomal stability and solubility. The *N*-Me leucine derivative **23** had improved potency and microsomal stability with a decrease in solubility. Moving the trifluoromethyl group from the *meta*-position in compound **23** to the *para*-position in compound **24** decreased both potency and solubility but not metabolic stability. The opposite(3a*R*,4*S*,6a*S*)-enantiomer **30** gave increased microsomal stability without significantly decreasing potency or solubility.

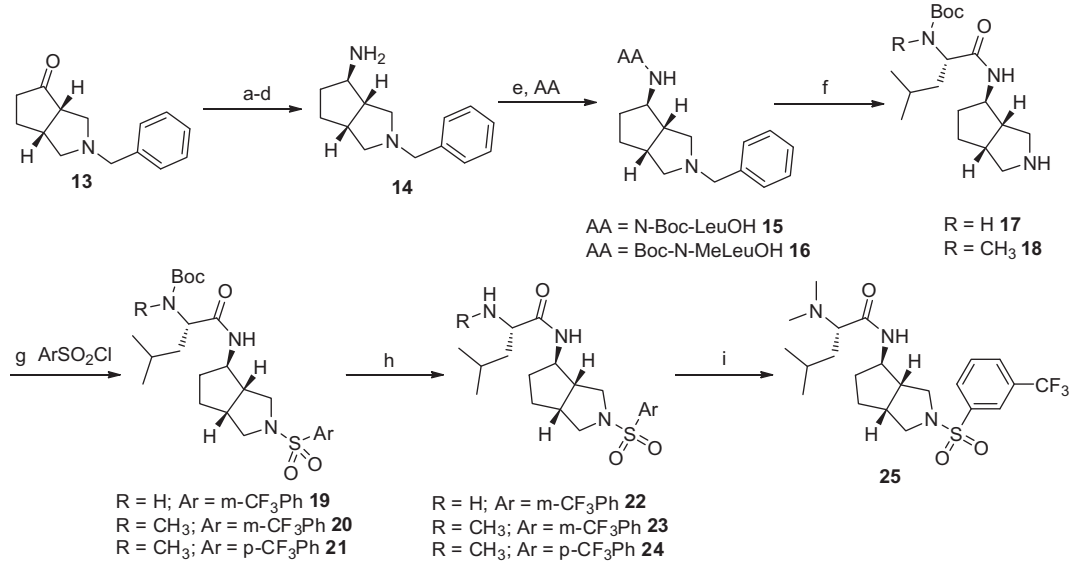
Potency gains were then made by varying the amino acid portion of the molecule on the most metabolically stable (3a*R*,4*S*,6a*S*)-enantiomer of the 4-aminocyclopentapyrrolidine core. When the amino acid was shortened by one methyl group from leucine for compound **30** to norvaline for compound **31** the solubility and microsomal stability increased while the potency decreased. When the steric bulk of the amino acid is decreased to the norleucine analog **32** the potency and microsomal stability remained the same, but the solubility decreased from 77 to 38 μM. Adding steric bulk and lipophilicity as in the neopentylglycine compound **35** had no effect on the potency and solubility but the human microsomal stability decreased. Primary amines with bulky alkyl group amino acids such as *tert*-leucine (compound **33**) had excellent solubility but weaker potency with no real decrease in microsomal stability. For the neopentylglycine analogs, the *N*-methyl analog **35** had comparable potency and solubility compared with the amino analog **34** and lower metabolic stabilities



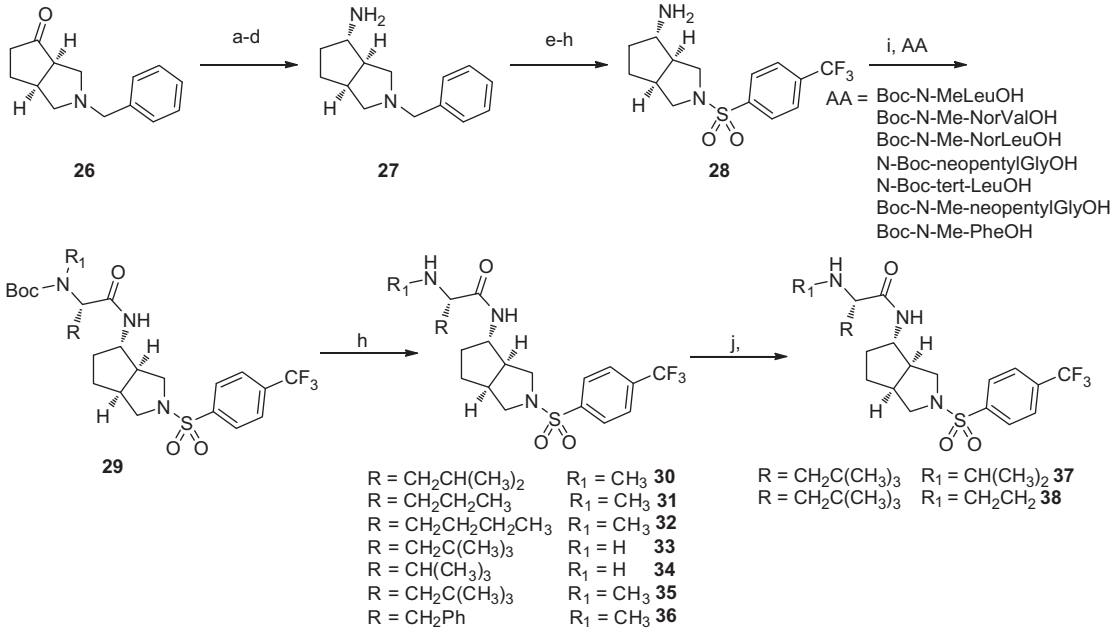
Scheme 1. Synthesis of (3a*S*,4*R*,6a*R*) chiral 4-aminocyclopentyl pyrrolidine via azide inversion. Reagents and conditions: (a) HCl, H_2O, THF, rt, 2 h; (b) $NaBH_4$, MeOH, −78 °C to rt, 16 h; (c) Et_3N, MsCl, CH_2Cl_2, 25 °C, 30 min; (d) NaN_3, DMA 90 °C 16 h; (e) PPh_3, H_2O, THF, 80 °C, 1 h; (f) EDC, HOBt, CH_2Cl_2, rt, 16 h.



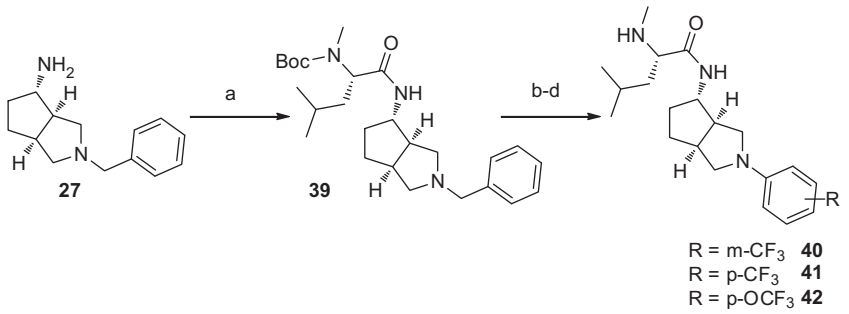
Scheme 2. Synthesis of leucine analogs. Reagents and conditions: (a) EDC, HOBt, CH_2Cl_2, rt, 16 h; (b) 4 N HCl/dioxane, rt, 1 h; (c) Et_3N, MsCl, CH_2Cl_2, 1 h.



Scheme 3. Synthesis of (3a*S*,4*R*,6a*R*)-enantiomeric *N*-sulfonamide analogs. Reagents and conditions: (a) $NaBH_4$, MeOH, −78 °C to rt, 16 h; (b) Et_3N, MsCl, CH_2Cl_2, 25 °C, 30 min; (c) NaN_3, DMA 90 °C 16 h; (d) PPh_3, H_2O, THF, 80 °C, 1 h; (e) EDC, HOBt, CH_2Cl_2, rt, 16 h; (f) 20% $Pd(OH)_2$/carbon, EtOH, 30 psi H_2, rt, 4h; (g) Et_3N, rt 3 h; (h) 4 N HCl/dioxane, rt, 1 h; (i) H_2CO, PS-$CNBH_3$, HOAc, CH_2Cl_2, rt, 16 h.



Scheme 4. Synthesis of (3a*R*,4*S*,6a*S*)-enantiomeric *p*-trifluoromethyl benzene sulfonamide analogs. Reagents and conditions: (a) $NaBH_4$, MeOH, −78 °C to rt, 16 h; (b) Et_3N, MsCl, CH_2Cl_2, 25 °C, 30 min; (c) NaN_3, DMA 90 °C 16 h; (d) PPh_3, H_2O, THF, 80 °C, 1 h; (e) Et_3N, Boc_2O, CH_2Cl_2; (f) 20% $Pd(OH)_2$/carbon, EtOH, 30 psi H_2, rt, 4 h; (g) p-CF_3–benzenesulfonyl chloride, Et_3N, rt, 3 h; (h) 4 N HCl/dioxane, rt, 1 h; (i) EDC, HOBt, CH_2Cl_2, rt, 16 h; (j) acetone, PS-$CNBH_3$, HOAc, CH_2Cl_2, rt, 16 h or EtI, Et_3N, THF, 65 °C, 3 days.



Scheme 5. Synthesis of (3a*R*,4*S*,6a*S*)-enantiomeric *N*-aryl analogs. Reagents and conditions: (a) Boc-N-MeLeuOH, EDC, HOBt, CH_2Cl_2, rt, 16 h; (b) 20% $Pd(OH)_2$/carbon, EtOH, 30 psi H_2, rt, 4 h; (c) ArBr, $Pd_2(dba)_3$, X-PHOS, K_3PO_4, *tert*-amylalcohol, 80 °C, 16 h; (d) 4 N HCl/dioxane, rt, 1 h.

Table 1
N-type calcium channel activity of 4-aminocyclopentapyrrolidine analogs

Compd	N-type FLIPR		% Rem @ 30 min		CLND
	IC_{50}[a] (μM)	pIC_{50} ± SEM[a]	RLM	HLM	Sol. (μM)
1	0.46	6.33 ± 0.06	0.1	0.5	<3
7	1.87	5.73 ± 0.08	2.6	1.2	<3
11	1.89	5.72 ± 0.04	60.7	19.4	67
12	2.84	5.55 ± 0.05	42.1	23.6	80
22	1.06	5.98 ± 0.05	10.3	45.0	44
23	0.52	6.20 ± 0.02	26.5	25.5	75
24	0.77	6.07 ± 0.05	47.3	50.5	27
25	0.45	6.34 ± 0.05	1.0	0.1	79
30	0.75	6.07 ± 0.04	69.9	54.4	77
31	0.96	6.02 ± 0.12	83.4	74.6	97
32	0.75	6.13 ± 0.08	66.4	51.1	38
33	1.51	5.82 ± 0.03	77.1	75.6	94
34	0.87	6.03 ± 0.10	79.4	41.8	68
35	0.79	6.08 ± 0.05	66.5	33.6	76
36	0.86	6.06 ± 0.03	26.2	26.8	nt
37	0.39	6.41 ± 0.13	64.3	79.6	21
38	0.49	6.24 ± 0.02	89.2	22.8	20
40	1.19	5.93 ± 0.05	81	73	56
41	1.24	5.91 ± 0.11	>85	>85	33
42	5.15	5.29 ± 0.11	66	>85	31

[a] All values are means ± SEM of at least three separate experiments.

in human liver microsomes. Alkylation of the neopentylglycine analog **34** with either acetone or ethyl iodide gave compounds **37** and **38** as shown in Scheme 4. The isopropyl amine analog **37** had similar potency to the ethyl amino analog **38** but improved stability in human liver microsomes. The phenyl alanine analog **36** had similar potency to compound **34** but poorer microsmal stability. The *N*-aryl compounds **40**–**41** were less potent in the N-type calcium channel FLIPR assay than the sulfonamide analogs (Table 1) but exhibited excellent microsomal stability and solubility.

Increases in microsomal stability were consistent with the rat PK as shown in Table 2. The *N*-methyl amino acid derivatives, represented by compound **23** were considered to have the best PK compared with the amino (compound **22**) and *N*,*N*-dimethylamino (compound **25**) analogs. Also, the increase in microsomal stability for compound **24** is accompanied by an increase in oral bioavailability. For the opposite enantiomer **30**, doubling the microsomal stability was accompanied by an increase in oral bioavailability. Compound **37** represents a compromise between solubility, potency and oral bioavailability. Rat phamacokinetics of the *N*-aryl compounds was remarkably different from the sulfonamide analogs. As shown in Table 2, compound **41** had a marked increase in half life, high volume of distribution, high clearance and high oral bioavailability. Further analysis showed a brain/plasma ratio of 16.5/1 at 30 min after an i.p. dose of 3 μmol/kg. In comparison, the brain/plasma ratio of compound **37** was 0.9/1 at 60 min after oral dosing.

Table 2
Rat PK for selected calcium channel blockers

Compd	$t_{1/2}$ (h)	V_{ss} (L/kg)	CL_p (L/h/kg)	%*F*	Oral AUC (ng h/mL)
22[a]	0.5	15.0	34.1	0	0
23[a]	2.0	9.2	3.6	10.5	405
24[b]	2.1	5.6	2.2	21.0	221
25[a]	1.4	5.2	3.4	0.8	35
30[b]	3.3	13.2	2.8	50.0	422
37[a]	2.7	7.1	2.0	46.7	240
41[b]	15.4	26.5	1.3	83.0	799

[a] 5 μmol/kg IV dose and 30 μmol/kg oral dose.
[b] 5 μmol/kg IV dose and oral dose.

Compound **37** was tested in manual electrophysiology using whole cell patch-clamp recordings of rat N-type calcium channels overexpressed in HEK293 cells (Fig. 2). Cells were held depolarized to partially inactivate the channels and allow for inhibition of multiple channel states to be detected. Under these conditions, compound **37** inhibited N-type calcium channels with an IC_{50} value of 340 nM. Under more hyperpolarized conditions, where channels are biased towards the closed-state, compound **37** was 30× less potent, consistent with state-dependent block of N-type calcium channels. Similarly, manual electrophysiology with compound **41** showed an IC_{50} value of 1.0 μM under depolarized conditions, with a shift to 4.4 μM under more hyperpolarized conditions.[12]

To determine analgesic activity of an orally bioavailable N-type calcium channel inhibitor, compound **37** was tested against preclinical pain models. Compound **37** showed efficacy in the capsaicin secondary mechanical hyperalgesia pain model[13] with an ED_{50} of 13 mg/kg. Compound **37** was also efficacious in the MIA-OA (monoiodoaceate induced osteoarthritis) pain model[7] with an ED_{50} of 8 mg/kg. When tested in the chronic constriction injury (CCI)-induced neuropathic pain model for neuropathic pain,[14]

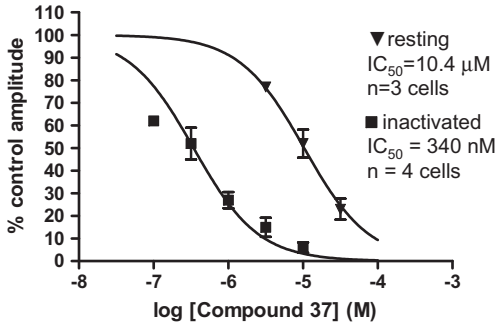


Figure 2. Inhibition of N-type calcium channel current by compound **37** assessed by manual electrophysiology.

compound **37** showed efficacy with an ED_{50} of 32 mg/kg. Compound **41** showed efficacy in the MIA-OA model with an ED_{50} of 3 mg/kg. When tested in the CCI model of neuropathic pain, compound **41** had an ED_{50} of 10 mg/kg.[12]

In cardiovascular safety studies, compound **37** showed a dose dependent decrease in mean arterial pressure in the anesthetized rat cardiovascular model[15] starting at a plasma concentration of 1.57 μg/mL. In the rat aorta ring tissue relaxation assay[7], compound **37** had an IC_{50} value of 2.6 μM. This assay is a measure of L-type calcium channel activity in native vascular tissue and the cardiovascular effects observed in rats may be due to interaction of compound **37** with L-type calcium channels.

In conclusion, we have discovered N-type calcium channel inhibitors with efficacy in inflammatory and neuropathic preclinical pain models with a therapeutic window for cardiovascular safety. This was achieved by optimizing for in vitro ADME parameters of microsomal stability and solubility along with potency at the biological target.

Disclosures

X.B., C.M.Y., D.D., S.S., T.A.V., L.M., I.M., A.M.S., C.Z.Z., P.B., J.M.W., K.C.M., M.F.J., V.E.S., M.S.R. and C.-H.L. are employees of AbbVie and may hold AbbVie stock. The research described herein is solely funded by AbbVie. AbbVie contributed to the study design, research and interpretation of data.

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