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# T-type calcium channel blockers: spiro-piperidine azetidines and azetidinones—optimization, design and synthesis

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ABSTRACT

A series of spiro-azetidines and azetidinones has been evaluated as novel blockers of the T-type calcium channel ($Ca_V3.2$) which is a new therapeutic target for the potential treatment of both inflammatory and neuropathic pain. Confirmation and optimization of the potency, selectivity and DMPK properties of leads will be described.



A family of integral membrane proteins, collectively referred to as voltage-gated calcium channels, represent major pathways for calcium influx in diverse tissues throughout the body. Based on biophysical properties, pharmacology, and molecular sequences, the family of voltage-gated calcium channels is divided into the following subtypes: L- ($Ca_V1.1$–$Ca_V1.4$), P/Q- ($Ca_V2.1$), N- ($Ca_V2.2$), R- ($Ca_V2.3$), and T-type ($Ca_V3.1$–$Ca_V3.3$).[1] Control of calcium influx across the plasma membrane is a critical component for spatial and temporal regulation of intracellular calcium levels and also for determining transmembrane potential. Cellular functions regulated by intracellular calcium include muscle contraction, neurotransmitter release, gene expression, and chemotaxis.[2,3]

T-type calcium channels serve two primary roles in excitable tissues; providing current for rhythmic pacemaking and generation of low level depolarizations from which neuronal burst firing can occur.[3] $Ca_V3.x$ channels are widely distributed in excitable tissue but in an isoform-dependent manner.[4,5]

Among the therapeutic indications proposed for T-type calcium channels are: hypertension, heart failure, insomnia, metabolic syndrome, epilepsy, and neuropathic pain.[5–7] T-type channels are present in DRG and in regions of the CNS involved in pain processing.[4,5] Effects of small molecules or intrathecal administration of antisense DNA directed against $Ca_V3.2$ support a role for T-type calcium channels in neuropathic pain models.[8] Therefore, we sought to identify selective blockers of T-type channels for the treatment of neuropathic pain.

Recently, *spiro*-piperidine azetidinones **1** have been reported as potent TRPV1 antagonists.[9] From an in-house screening effort, the *spiro*-piperidine azetidines **2–4**, were identified as blockers of $Ca_V3.2$. These hits, as mixtures of stereoisomers, exhibited good activity in IonWorks HT and manual patch clamp assays.[10] Our initial goal was to separate the mixtures and determine if the T-type calcium channel blocker activity resided solely in one of the stereoisomers, and then evaluate the DMPK profiles of these hits.

The chemistry to synthesize compounds **2–4** is shown in Scheme 1. The enolate of ester **5** was treated with the *N*-alkyl (or *N*-aryl) aldimine **6** to afford the *spiro*-piperidine azetidinone 7.[13] The azetidinone **7** was reduced to azetidine **8**. Removal of the Boc group of **7** and **8**, and then reaction with a variety of isocyanates affords the respective ureas **9** and **10**.

Accordingly, *spiro*-piperidine azetidine **2** was prepared and subsequently separated by chiral HPLC chromatography to give isomers **2a** and **2b**[14] in a 1:1 ratio. Between the two isomers, **2a** was a more potent blocker of $Ca_V3.2$ than **2b** in both IonWorks HT and manual voltage clamp assays as shown in Table 1. Compound **2a** was also shown by manual voltage clamp to discriminate between $Ca_V3.2$ and two other voltage-gated cation channels (70× selective vs $hCa_V2.1$; >80× selective vs $hNa_V1.5$).

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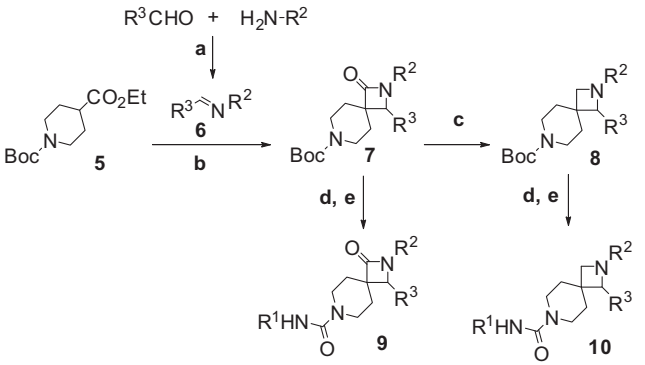
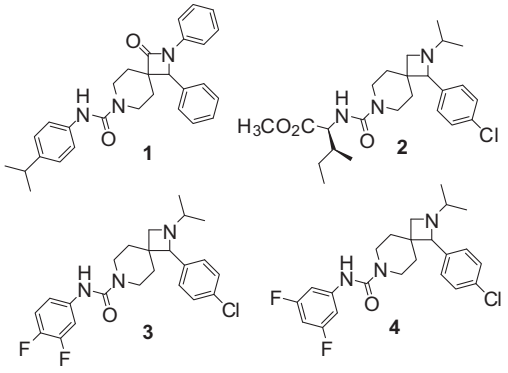



**Scheme 1.** Reagents: (a) toluene, molecular sieves; (b) LiN(*i*-Pr)$_2$, THF; (c) Rh(CO)(PPh$_3$)$_3$, Ph$_2$SiH$_2$, THF; (d) TFA, CH$_2$Cl$_2$; (e) R$^1$N=C=O, ClCH$_2$CH$_2$Cl, CH$_3$CN.

**Table 1**




| Compds | IW (VC) hCa$_V$3 2 IC$_{50}$ nM | Rapid rat po AUC nM h | TRPV1[a,11] |
|---|---|---|---|
| **2**[b,12] | 104 (123) | | 0.70 |
| **3**[12] | 174 (340) | 0 | 87.10 |
| **4**[12] | 138 (328) | 0 | 64.00 |
| **2a**[b] | 115 (124) | | 19.10 |
| **2b** | 733 (3197) | | |

[a] TRPV1 capsaicin activation, % inhibition at 3.3 μg/mL.[11]
[b] Compound **2** was separated on HPLC Chiralpak AS column to give **2a**, isomer A, faster eluting and **2b** isomer B.

After confirming that the Ca$_V$3.2 activity resides predominately in **2a** (isomer A), we developed the SAR using the modular synthetic routes shown in Scheme 1 to improve the potency and optimize the DMPK profiles for these compounds.

Using the methodology in Scheme 1, we were able to explore the SAR of the azetidinones **9** and azetidines **10**. First, we investigated azetidine series by varying the azetidine nitrogen substituent (R$^2$) from methyl, isopropyl, and isobutyl; and also the C4 substituent (R$^3$) being either 4-chlorophenyl and 4-fluorophenyl (Table 2).

When R$^3$ 4-chlorophenyl azetidine series, the R$^2$ isopropyl **2–4** is equipotent with isobutyl **15** and more potent than methyl analogs **11** and **12**. The respective R$^2$ methyl azetidinones **13** and **14** are less potent than the azetidines. The R$^3$ 4-chlorophenyl azetidines **2–4, 11, 12**, and **15** are more potent than the respective R$^2$ methyl and R$^2$ isopropyl, R$^3$ 4-fluorophenyl azetidines **16–18**.

To improve the PK profile, we explored introducing a cyclopropyl group[15] in place of isopropyl at R$^2$ (Table 2). In the azetidine series, this modification was made in both the alkyl **19** and aryl urea **20** analogs which both showed a modest improvement in the PK profile, however, a loss in Ca$_V$3.2 potency was observed. The respective aryl urea azetidinone **21** showed a significant improvement in the PK profile. However, this modification gave a significant loss in Ca$_V$3.2 potency.

To remove a potential site of metabolism and improve the PK, the aliphatic groups at R$^2$ in the azetidinones **9** azetidines **10** were replaced with an aryl group as shown in Table 2. When R$^2$ equals 3-fluorophenyl, these compounds retain activity provided that the alkyl (isoleucine methyl ester) and aralkyl ureas are used. In this series the branched chain alkyl ureas and aryl ureas are less potent. As seen before, isomer A is still more potent than isomer B. In this case, the azetidinone series **23a** and **23b** is slightly more potent than the corresponding azetidine **22a** and **22b** (Ca$_V$3.2). Compound **23a** was shown to have >92-fold selectivity for blocking hCa$_V$3.2 compared to hCa$_V$2.2 and hNa$_V$1.5. The R$^2$ 3-fluorophenyl series is slightly more potent than 2-fluorophenyl series.[16]

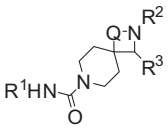
In the azetidinone series, the SAR of the isoleucine methyl ester was explored showing that replacement of the methyl ester **23a** with cyano **24a** or isopropyl ester **25a** maintained activity. Methyl amide **26a** resulted in slight loss of activity as did the use of *t*-butylserine **27a**. Replacement of isoleucine with alanine **28a** and serine **29a** resulted in a significant decrease in activity. It was interesting to see that the isoleucine could be replaced with a α-methylbenzyl amine with only a slight decrease in the ability to block the Ca$_V$3.2 channel. As shown in Table 2 and 4-fluoro-α (*R*)-methylbenzyl urea **32a** was the most potent aralkyl urea against Ca$_V$3.2. Importantly, we found that this modification provided very good TRPV1 selectivity. In Table 2, for R$^2$ aryl series, the aryl ureas are less potent. However, the R$^2$ aryl azetidinones **36a** and **36b** are more potent (Ca$_V$3.2) than the respective azetidines **35a** and **35b**.

Gratifyingly, we found that replacement of the R$^3$ aryl with a 2-pyridyl group, further improved the potency against the Ca$_V$3.2 channel (Table 2). As we had demonstrated before, for both azetidines and azetidinones, the Ca$_V$3.2 potency resides in isomer A for this series as well. As seen before, azetidinones **39a** and **39b** are more potent than azetidines **38a** and **38b**. Incorporation of the 2-pyridyl group provided a dramatic improvement in the rat PK profile with 0–6 h AUC's from 1 to 18 μM h observed after an oral dose of 10 mpk.

With this overall improvement, we then turned our attention to the in vivo evaluation of these compounds in the rat SNL model of neuropathic pain.[17] Three compounds were selected: (1) best hCa$_V$3.2 potency: **39a** (isomer A.VC hCa$_V$3.2 IC$_{50}$ 42 nM, rat PPB 99.6%); (2) best AUC: **40a** (isomer A; rat AUC (0–6 h, po) @ 10 mpk 18,055 nM h, rat PB 99.6%); and (3) good hCa$_V$3.2 and AUC: **42a** (isomer A). Compounds **39a** and **42a** were inactive in this model at 100 and 30 mpk, respectively. We speculated that the inactivity of these compounds in the SNL was either due to their poor TRPV1 selectivity or their high protein binding. There was a trend toward efficacy with compound **40a** at 2 h (50% PWT: 9.2 ± 2.6 Gm; vehicle −4.9 ± 2.1 Gm) and 3 h (50% PWT: 8.9 ± 2.8 Gm; vehicle −5.0 ± 1.7 Gm) after dosing 30 mpk but statistical significance was not achieved.

Two isomer B compounds that displayed better selectivity over TRPV1: **41b** (R$^1$ is 3-trifluoromethylphenyl) with moderate Ca$_V$3.2 activity, good AUC and *c*log *P*, and better TRPV1 selectivity; and for **44b** (R$^1$ is 3,5-difluorophenyl) with moderate Ca$_V$3.2 activity, good AUC and *c*log *P* and moderate TRPV1 activity were also tested in the SNL. These compounds were found to be inactive in the rat SNL at 30 mpk, but **44b**, a positive trend was seen at 1 h 50% PWT 7.4 ± 2.6 Gm, vehicle 3.0 ± 1.4 Gm) which suggested that the high protein binding was the problem. In support of this we found that the ability of these compounds to block the Ca$_V$3.2 channel in the voltage clamp assay was considerably right shifted by 40- to >469-fold **24**, 40, **44**, **46**, and **49** when tested in whole cell patch clamp the presence of 100% rat serum (Table 3). The example of

**Table 2**



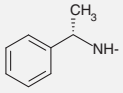
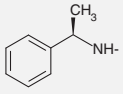
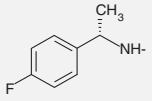
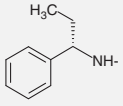
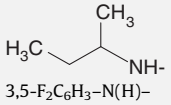

| Compds[a] | $R^1N(H)$– | $R^2$ | $R^3$ | Q | IW (VC) $hCa_V3$ 2 $IC_{50}$ nM | *clog P* | Rapid rat po AUC nM h | r (h) protein binding pct[b] | TRPV1[c] | Rat SNL, PO |
|---|---|---|---|---|---|---|---|---|---|---|
| **11** | Ile-OMe | Me | 4-Cl-$C_6H_4$– | $CH_2$ | 410 | 3.86 | | | 25.20 | |
| **12** | 3,5-$F_2C_6H_3$–N(H)– | Me | 4-Cl-$C_6H_4$– | $CH_2$ | 214 | 3.72 | 0 | | 25.00 | |
| **13** | ILe-OMe | Me | 4-Cl-$C_6H_4$– | C=O | 800 | 2.42 | | | 3.12 | |
| **14** | 3,5-$F_2C_6H_3$–N(H)– | Me | 4-Cl-$C_6H_4$– | C=O | 920 | 2.50 | | 96.60 | 20.68 | |
| **15** | 3,5-$F_2C_6H_3$–N(H)– | *i*-Bu | 4-Cl-$C_6H_4$– | $CH_2$ | 177 | 5.17 | | | 53.00 | |
| **16** | ILe-OMe | *i*-Pr | 4-F-$C_6H_4$– | $CH_2$ | 256 | 4.12 | | | 13.38 | |
| **17** | 3,5-$F_2C_6H_3$–N(H)– | *i*-Pr | 4-F-$C_6H_4$– | $CH_2$ | 172 (995) | 3.98 | 0 | | 46.49 | |
| **18** | 3,5-$F_2C_6H_3$–N(H)– | Me | 4-F-$C_6H_4$– | $CH_2$ | 410 | 3.15 | | 95.00 | 21.78 | |
| **19** | Ile-OMe | Cyclopropyl | 4-Cl-$C_6H_4$– | $CH_2$ | 325 (713) | 4.35 | 245 | | | |
| **20** | 3,5-$F_2C_6H_3$–N(H)– | Cyclopropyl | 4-Cl-$C_6H_4$– | $CH_2$ | 398 | 4.20 | 477 | 99.80 | | |
| **21** | 3,5-$F_2C_6H_3$–N(H)– | Cyclopropyl | 4-Cl-$C_6H_4$– | C=O | 2258 | 2.76 | 3070 | | 34.20 | |
| **22a** | Ile- OMe | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | $CH_2$ | 146 | 5.55 | | | 8.50 | |
| **22b** | Ile- OMe | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | $CH_2$ | 3200 | 5.55 | | | 12.47 | |
| **23a** | Ile- OMe | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | C=O | 45 (109) | 5.06 | | | 6.18 | |
| **23b** | ILe-OMe | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | C=O | 872 | 5.06 | | | 8.79 | |
| **24a** | Ile-nitrile | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | C=O | 35 (83) | 4.40 | | | | |
| **25a** | Ile-OPr-*i* | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | C=O | 45 | 5.90 | | | 18.00 | |
| **26a** | Ile-NHMe | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | C=O | 182 | 4.20 | | | 4.80 | |
| **27a** | Ser(*t*-Bu)-OMe | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | C=O | 251 | 4.61 | | | 4.40 | |
| **28a** | Ala-OMe | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | C=O | 827 | 3.84 | | | | |
| **29a** | Ser-OMe<br>AND Enantiomer | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | C=O | 3333 | 2.75 | | | 6.00 | |
| **30a** |  <br>AND Enantiomer | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | C=O | 139 | 5.29 | | | | |
| **31a** |  <br>AND Enantiomer | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | C=O | 351 | 5.29 | | | | |
| **32a** |  <br>AND Enantiomer | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | C=O | 82 (340) | 5.43 | | 99.90 | 2.40 | |
| **33a** |  | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | C=O | 224 | 5.74 | | | | |
| **34a** |  | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | C=O | 1251 | 4.50 | | | 5.20 | |
| **35a** | 3,5-$F_2C_6H_3$–N(H)– | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | $CH_2$ | 3503 | 5.63 | | | 6.22 | |
| **35b** | 3,5-$F_2C_6H_3$–N(H)– | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | $CH_2$ | 3200 | 5.63 | | | 30.08 | |
| **36a** | 3,5-$F_2C_6H_3$–N(H)– | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | C=O | 586 | 5.14 | 0 | | 19.29 | |
| **36b** | 3,5-$F_2C_6H_3$–N(H)– | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | C=O | 390 | 5.14 | 1616 | | 6.79 | |
| **37a** | 3-$CNC_6H_3$–N(H)– | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | C=O | 183 | 4.58 | 617 | | 4.79 | |
| **37b** | 3-$CNC_6H_3$–N(H)– | 3-F-$C_6H_4$– | 4-Cl-$C_6H_4$– | C=O | 1060 | 4.58 | 485.11 | | 12.69 | |
| **38a*** | 3,5-$F_2C_6H_3$–N(H)– | 3-Cl,4-F-$C_6H_3$– | 2-Pyridyl | $CH_2$ | 60 (41) | 4.19 | 4830 | 99.90 | | |
| **38b*** | 3,5-$F_2C_6H_3$–N(H)– | 3-Cl,4-F-$C_6H_3$– | 2-Pyridyl | $CH_2$ | 291 | 4.19 | | 99.9 | | |
| **39a** | 3,5-$F_2C_6H_3$–N(H)– | 3-Cl,4-F-$C_6H_3$– | 2-Pyridyl | C=O | 4 (42) | 3.64 | 9058 | 99.6 (99.90) | | 100 mpk: na[d,e] |
| **39b** | 3,5-$F_2C_6H_3$–N(H)– | 3-Cl,4-F-$C_6H_3$– | 2-Pyridyl | C=O | 176 | 3.64 | | | | |
| **40a** | 3,4-$F_2C_6H_3$–N(H)– | 3-Cl,4-F-$C_6H_3$– | 2-Pyridyl | C=O | 41 | 3.57 | 18,056 | 99.6 (99.90) | 48.00 | 10 and 50 mpk: na |
| **40b** | 3,4-$F_2C_6H_3$–N(H)– | 3-Cl,4-F-$C_6H_3$– | 2-Pyridyl | C=O | 402 | 3.57 | | | 39.00 | |
| **41a** | 3-$F_3CC_6H_3$–N(H)– | 3,4-$F_2$-$C_6H_3$– | 2-Pyridyl | C=O | 17 | 3.72 | | | | |
| **411b** | 3-$F_3CC_6H_3$–N(H)– | 3,4-$F_2$-$C_6H_3$– | 2-Pyridyl | C=O | 176 (283) | 3.72 | 3686 | 99.50 | | 30 mpk: na[f] |
| **42a** | 3,4-$F_2C_6H_3$–N(H)– | 3-Cl-$C_6H_4$– | 2-Pyridyl | C=O | 116 (118) | 3.43 | 1488 | 98.8 (99.90) | 82.68 | 30 mpk: na |
| **42b** | 3,4-$F_2C_6H_3$–N(H)– | 3-Cl-$C_6H_4$– | 2-Pyridyl | C=O | 652 | 3.43 | 6445 | | 88.96 | |
| **43a** | 3-$NCC_6H_4$–N(H)– | 3-Cl-$C_6H_4$– | 2-Pyridyl | C=O | 57 | 2.94 | 2036 | 99.2 (98.70) | 30.00 | |
| **43b** | 3-$NCC_6H_4$–N(H)– | 3-Cl-$C_6H_4$– | 2-Pyridyl | C=O | 855 | 2.94 | | 98.80 | 25.00 | 30 mpk: na |
| **44a** | 3,5-$F_2C_6H_3$–N(H)– | 3-Cl-$C_6H_4$– | 2-Pyridyl | C=O | 18 (29) | 3.50 | 1153 | 98.9 (99.70) | 38.00 | |
| **44b** | 3,5-$F_2C_6H_3$–N(H)– | 3-Cl-$C_6H_4$– | 2-Pyridyl | C=O | 200 | 3.50 | | 99.50 | 36.00 | 30 and 60 mpk: na |

**Table 2** (*continued*)

| Compds[a] | $R^1N(H)$– | $R^2$ | $R^3$ | Q | IW (VC) $hCa_V3$ 2 $IC_{50}$ nM | *c*log *P* | Rapid rat po AUC nM h | r (h) protein binding pct[b] | TRPV1[c] | Rat SNL, PO |
|---|---|---|---|---|---|---|---|---|---|---|
| **45a** *N*-oxide | 3,4-$F_2C_6H_3$–N(H)– | 3-Cl-$C_6H_4$– | 2-Pyridyl | C=O | 698 | 1.55 | | 93.40 | | |
| **45b** *N*-oxide | 3,4-$F_2C_6H_3$–N(H)– | 3-Cl-$C_6H_4$– | 2-Pyridyl | C=O | 1498 | 1.55 | | | | |
| **46a** | 3,5-$F_2C_6H_3$–N(H)– | 3-NC-$C_6H_4$– | 2-Pyridyl | C=O | 247 (478) | 2.20 | | | | |
| **46b** | 3,5-$F_2C_6H_3$–N(H)– | 3-NC-$C_6H_4$– | 2-Pyridyl | C=O | 732 | 2.20 | | | | |
| **47** | 3,5-$F_2C_6H_3$–N(H)– | 5-Cl-pyrid-3-yl | 2-Pyridyl | C=O | 519 | 2.1 | | | 6.7 | |
| **48** | 3,5-$F_2C_6H_3$–N(H)– | 5-CN-pyrid-3-yl | 2-Pyridyl | C=O | 2377 | 0.92 | | | 8.8 | |

[a] **a** is isomer A; **b** is isomer B: otherwise racemic.
[b] rPB: rat protein binding; (h): human protein binding.
[c] TRPV1 capsaicin activation, % inhibition at 3.3 μg/mL.
[d] na: not active.
[e] Plasma concn. 3.4 μM at 100 mpk at 3 h.
[f] Plasma concn. 10 mpk, 2.1 μM at 2 h.

**Table 3**

| Compds[a] | VC $hCa_V3.2$ rSerum $IC_{50}$ nM | Serum shift |
|---|---|---|
| **44a** | 3490 | 120 |
| **40a** | 17,700 | 246 |
| **24a** | 17,000 | 205 |
| **46a** | 19,300 | 40 |
| **49a** | >30,000 | >469 |

[a] **a** is isomer A; **b** is isomer B.

compound **46a** suggested that serum binding could be reduced considerably by decreasing the *c*log *P*. We turned our attention to lowering the *c*log *P* in the hope that this would lower protein binding and provide activity in the SNL. The *c*log *P* was reduced to 2.94 when $R^1$ was replaced by 3-cyanophenyl **43a** (Table 2). This modification lowered $Ca_V3.2$ potency, lowered *c*log *P*, while maintaining moderate TRPV1 selectivity, however, this compound still had high protein binding.

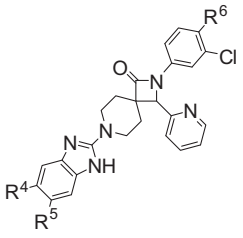
The *c*log *P* of **42a** and **42b** was further reduced to 1.55 by formation of their *N*-oxides **45a** and **45b** which resulted in a significant loss of $Ca_V3.2$ activity and was not evaluated further.

To further lower the *c*log *P* and have good TRPV1 selectivity, a series of $R^3$ 2-pyridyl, $R^2$ heterocyclic compounds were evaluated (Table 2, **47** and **48**).[18] These compounds showed very low *c*log *P* and good TRPV1 selectivity, however, their $Ca_V3.2$ activity was significantly reduced.

Finally, modification of the urea moiety by replacement with a benzimidazole group (Table 4)[19] provided compounds that were equipotent against $Ca_V3.2$. Two compounds had very good rat PK. Compound **52b** was evaluated in the SNL, but it was found to be inactive at 10 mpk which again may be a result of the very high protein binding.

By structural modifications of the azetidine and azetidinone hits, we succeeded in obtaining compounds having low nM $Ca_V3.2$ potency, good rat PK, and improved TRPV1 selectivity. We demonstrated that the isoleucine moiety in the initial screening hit could be replaced by a α-methylbenzyl group while maintaining the activity against the $Ca_V3.2$ channel. Further modification replaced the urea moiety with the benzimidazole group. Together these modifications provided compounds with significant improvements in both the activity against $Ca_V3.2$ and the oral PK in the rat. Nevertheless, none of the compounds produced statistically significant effects in the rat SNL model. We attribute the low efficacy in this series to the high protein binding and the resulting increases the in vivo exposures required to block $Ca_V3.2$. Efforts to lower the *c*log *P* and protein binding met with limited success and did not improve the SNL activity for this series.

**Table 4**




| Compds[a] | $R^4$ | $R^5$ | $R^6$ | IW (VC) $hCa_V3$ 2 $IC_{50}$ nM | *c*log *P* | Rapid rat po AUC nM h | r (h) protein binding%[b] | r trpv cap activation[c] | Rat SNL, PO |
|---|---|---|---|---|---|---|---|---|---|
| **50a** | F | $CF_3$ | H | 44 (63) | 5.1 | | | 37.9 | |
| **51a** | H | Cl | F | 78 (71) | 4.8 | 41,161 | 100 | 86.2 | |
| **51b** | F | Cl | F | 60 (84) | 5 | | 99.8 | 8.6 | |
| **52a** | F | $CF_3$ | F | 29 | 5.24 | 8014 | | 92.00 | 10 mpk: na[d,e] |
| **52b** | F | $CF_3$ | F | 31 (106) | 5.24 | | | 59.80 | 10 mpk: na[d,e] |

[a] **a** is isomer A; **b** is isomer B: otherwise racemic.
[b] rPB: rat protein binding; (h): human protein binding.
[c] TRPV1 capsaicin activation, % inhibition at 3.3 μg/mL.
[d] na: not active.
[e] Plasma concn. 1.75 μM at 2.5 h.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.06.012.

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10. (a) IonWorks Screen for hCaV3.2.; (b) Manual Whole Cell Patch Clamp experimental, see Supplementary data.
11. (a) TRPV1 assay.; (b) Mouse TRPV1: NL version experimental, see Supplementary data.
12. DMPK for compounds **2**, **3**, and **4**.
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14. For example, **2** refers to racemic mixture; **2a** refers to isomer A, faster eluting; and **2b** refers to isomer B.
15. Procedure for preparation of compounds **20** and **21**.
16. Azetidine $R^1$ is isoleucine methyl ester; $R^2$ is 2-fluorophenyl, $R^3$ is 4-chlorophenyl: IW hCav3.2 $IC_{50}$: isomer A, 262; isomer B, 3200 nM. Azetidinone $R^1$ is isoleucine methyl ester, $R^2$ is 2-fluorophenyl, $R^3$ is 4-chlorophenyl: IW hCav3.2 $IC_{50}$: isomer A, 125 nM; isomer B, 1626 nM.
17. Rat spinal nerve ligation model experimental, see Supplementary data.
18. Preparation of *tert*-butyl 2-(5-chloropyridin-3-yl)-1-oxo-3-(pyridin-2-yl)-2,7-diazaspiro[3.5]nonane-7-carboxylate, see Supplementary data.
19. Preparation of 2-(3-chloro-4-fluorophenyl)-3-(pyridin-2-yl)-2,7-diazaspiro-[3.5]nonan-1-one experimental, see Supplementary data.