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N,N-DIALKYL-DIPEPTIDYLAMINES AS NOVEL N-TYPE CALCIUM CHANNEL BLOCKERS

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Abstract: Selective N-type voltage sensitive calcium channel (VSCC) blockers have shown utility in several models of stroke and pain. We are especially interested in small molecule N-type calcium channel blockers for therapeutic use. Herein, we report a series of *N,N*-dialkyl-dipeptidylamines with potent functional activity at N-type VSCCs and in vivo efficacy. The synthesis, SAR, and pharmacological evaluation of this series are discussed.

Excessive calcium entry into depolarized neurons contributes significantly to neuronal injury. Voltage-sensitive calcium channels (VSCC) regulate intracellular calcium concentration, which affects various important neuronal functions such as cellular excitability, neurotransmitter release, hormone secretion, intracellular metabolism, neurosecretory activity, and gene expression.[1] Neuronal VSCC are classified into L, N, P, Q, R, and T subtypes. These channels differ in their protein structures, function, conductance, activation/inactivation voltage, and sensitivity to various drugs or toxins. N-type channels are associated with central and peripheral neurons, being primarily located on presynaptic nerve terminals. These channels regulate the calcium flux subserving depolarization-evoked release of transmitter from synaptic endings. They are tissue specific, mediate neuronal processes having well defined functional roles, and can be selectively blocked by high-affinity ligands.[1] It has been suggested that N-type VSCCs could be ideal targets for developing new pharmacological agents for clinical use.[2] ω-Conotoxin MVIIA,[3] a 25 amino acid-residue containing peptide found in the venom of piscivorous marine snail (*Conus magus*), is a potent and selective N-type voltage-sensitive calcium channel blocker. The synthetic version of MVIIA, SNX-111,[3] has demonstrated efficacy in animal models of traumatic brain injury, focal cerebral ischemia and pain.[2] Currently, SNX-111 is in clinical trials for the treatment of pain.

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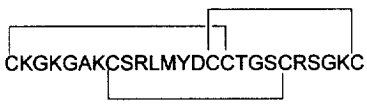
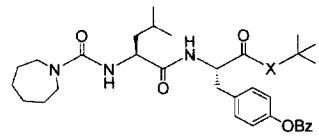


SNX-111

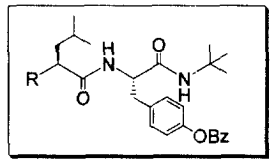


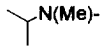
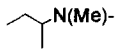
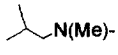
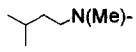
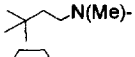
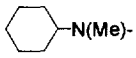
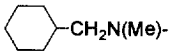
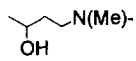
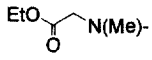
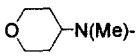
PD 151307 X = O
PD 167341 X = NH

In the process of searching for small molecule N-type calcium channel blockers, we ran a high volume screen of the Parke-Davis compound library and identified PD 151307 as a potential chemical lead. PD 151307 is a significant potent antagonist for N-type calcium channels in the IMR-32 human neuroblastoma cells (IC_{50} = 0.32 μM).[4] Unfortunately, due to its unfavorable physicochemical properties (high ClogP and poor aqueous solubility, measured aqueous solubility < 1 μg/mL), PD 151307 has limited therapeutic potential. PD 151307 is a *t*-butyl ester; our previous studies indicated that its *t*-butyl amide derivative (PD 167341, IC_{50} = 0.59 μM) was also an N-type Ca^{+2} channel blocker. Herein, we used the *t*-butyl amide analog for the SAR study in this work since an amide is more stable than an ester in physiological systems in general. While investigating the SAR of PD 151307 and PD 167341, we discovered a novel series of *N,N*-dialkyl-dipeptidyl-amines and found that they were active in vitro in the IMR-32 assay and efficacious in vivo in an audiogenic seizure mouse model using DBA/2 mice.

Replacement of the azepane-1-caboxylic amide group of PD 167341 and with a NH_2, NHMe, or NMe_2 group, resulting **1**, **2**, and **3** which showed IC_{50}'s of 2.3-3.8 μM in the IMR-32 assays. Furthermore, replacement of the NMe_2 group of compound **3** with a piperidine resulted in **4**,[5,6] which exhibited slightly enhanced potency (IC_{50} = 1.4 μM). A piperidinyl group is larger than a dimethylamino group and smaller than the azepane-1-caboxylic amide by size; the activity of **4** fell in between the dimethylamino analog (**3**) and PD 151307. The size of the above substitutions at this position is likely important for potency of N-type Ca^{+2} channels. Therefore, a detailed SAR of substitution pattern was explored in this series, and several R groups were evaluated for potency in the IMR-32 assay (hydrogen (**2**), methyl (**3**), isopropyl (**6**), 2-butyl (**7**), 1-butyl (**8**), isobutyl (**9**), 3-methylbutyl (**10**), and 3,3-dimethylbutyl (**11**)). The in vitro results demonstrated that 1-butyl (**8**, IC_{50} = 0.28 μM) and 3-methylbutyl (**10**, IC_{50} = 0.32 μM) are the optimal substitutions for high potency in the IMR-32 assay. Other substitutions such as isopropyl and 3,3-dimethylbutyl (**6** or **11**) are less preferred.

Table 1: The in vitro, in vivo, and elemental analysis of *N,N*-dialkyl-dipeptidylamines



	R	IMR-32 IC_{50} (μM)	Audiogenic seizure model % protection (Tonic phase) (N = 5 mice / dose tested)[7]
1	H_2N-	3.8	
2	MeHN-	2.3	
3	Me_2N-	2.3	
4		1.4	
5		6.4	
6		2.8	
7		1.5	
8		0.28	
9		0.38	
10		0.32	80% @ 10 mg/Kg 20% @ 3 mg/Kg
11		0.40	
12		0.20	100% @ 10 mg/Kg 20% @ 3 mg/Kg
13		0.70	20% @ 10 mg/Kg
14		2.2	40% @ 10 mg/Kg
15		4.4	
16		3.1	

An additional SAR exploration of linker chain length versus activity was conducted by comparing the piperidinyl (**4**), cyclohexylamino (**12**), and cyclohexylmethylamino (**13**) analogs (IC_{50}'s: 1.4, 0.20, and 0.68 μM, respectively). The cyclohexylamino derivative (**12**) was the most active compound with potency approximately equal to our initial high volume screening lead (PD 151307). To provide more aqueous soluble analogs, we investigated various hydrophilic functional groups (**14**, **15**). The hydroxy and ester derivatives (**14**, IC_{50} = 2.2 μM and **15**, IC_{50} = 4.4 μM) were significantly less active than the parent compound (**8**, IC_{50} = 0.28 μM). A similar phenomenon was observed in the comparison of piperidinyl and morpholinyl analogs (**4**, IC_{50} = 1.4 μM and **5**, IC_{50} = 6.4 μM) as well as in the comparison of cyclohexyl and tetrahydropyran-4-yl derivatives (**12**, IC_{50} = 0.20 μM and **16**, IC_{50} = 3.1 μM). Thus, we conclude that the potency of N-type Ca^{+2} channel blockade correlates with the lipophilic character of the antagonist.

To assess central bioavailability, several compounds of this series (**10**, **12**, **13**, and **14**) were evaluated in an audiogenic seizure mouse model and showed significant activity in preventing tonic seizures in DBA/2 mice (iv). (Table 1)[7] Compounds **10** and **12** were fairly active in vivo; they were further characterized in electrophysiological assays to determine if in vivo activity in the DBA/2 seizure model was due to Ca^{2+} channel activity or activity at other ion channels.[8] Both compounds blocked neuronal N-type Ca^{2+} channels measured electrophysiologically in superior cervical ganglion neurons with compound **10** blocking 71 ± 13.8% (N = 4) at 10 μM and compound **12** blocking 54 ± 13.9% at 10 μM. Both compounds also blocked voltage-gated sodium channels, with compound **10** blocking 53 ± 22.0% (N = 4) at 10 μM and compound **12** blocking 54 ± 9.2% at 10 μM. These results suggest these compounds block seizures by activity at voltage-gated calcium channels with a contribution from activity at voltage-gated sodium channels.

In summary, a series of *N,N*-dialkyl-dipeptidylamines based N-type calcium channel blockers has been described. These compounds demonstrate potent in vitro activity in the IMR-32 assay and in vivo efficacy in the audiogenic seizure mouse model. We found that the N-type Ca^{+2} channel activity correlates with the lipophilicity and size of the N-substitution in this series. Compounds **8**, **10**, and **12** are the most potent analogs in this report.

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References and Notes

1. (a) Bowersox, S. S.; Valentino, K. L.; Luther, R. R. *Drug News and Perspectives* 1994, *7(5)*, 261. (b) Scraibine, A. *Neuroprotection: Fundamental and Clinical Aspects*; Marcel Dekker: New York, 1997; pp 27 - 51. (c) Gilmore, J.; Dell, C.; Bowman D.; Lodge, D. *Ann. Report Med. Chem.* 1995, *30*, 51.
2. (a) Bowersox, S. S.; Singh, T.; Luther, R.R. *Brain Res.* 1997, *747*, 343. (b) Bowersox, S. S.; Gadbois, T.; Singh, T.; Pettus, M.; Wang, Y.; Luther, R. R. *J. Pharmacol. Exp. Ther.* 1996, *279*, 1243. (c) Verweij, B. H.; Muizelaar, J. P.; Vinas, F. C.; Peterson, P. L.; Xiong, Y.; Lee, C. P. *Neurological Res.*, 1997, *19*, 224.
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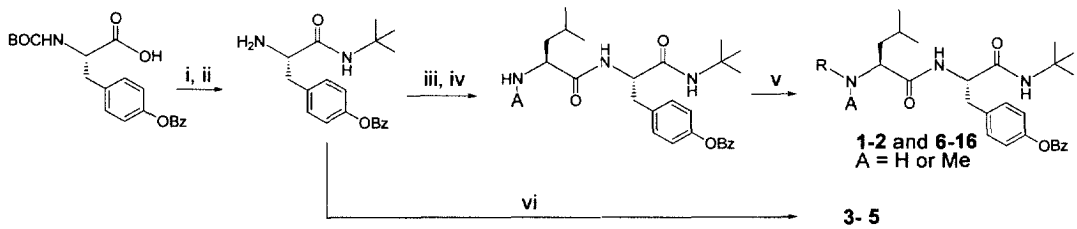
4. N-type Ca^{+2} channel blocking potencies of the compounds were determined using a fluorescence based Ca^{+2}-flux assay, using Indo-1 as indicator in IMR-32 human neuroblastoma cells. Inhibition of Ca^{+2} fluxes induced by K^{+}-evoked depolarization were measured in the presence of an L-type Ca^{+2} channel blocker (nitrendipine). PD 151307 was run in parallel as a standard in each assay.
5. The preparation of compounds in this series is outlined below:



i. HBTU (O-benzotriazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate), (iPr)$_2$NEt, DMF, *t*-butylamine; ii. TFA, CH_2Cl_2; iii. HBTU, (iPr)$_2$NEt, DMF, N^{α}-BOC-N^{α}-Me-leucine; iv. TFA, CH_2Cl_2; v. $NaBH(OAc)_3$, CH_2Cl_2, an aldehyde or ketone (yield 50-95%); vi. HBTU, (iPr)$_2$NEt, DMF, and N,N-dialkyl-leucine (*N*,*N*-dimethyl-L-leucine, (*S*)-4-Methyl-2-morpholin-4-yl-pentanoic acid, or (*S*)-4-Methyl-2-piperdinyl-pentanoic acid).

The preparation of (*S*)-4-Methyl-2-morpholin-4-yl-pentanoic acid and (*S*)-4-Methyl-2-piperdinyl-pentanoic acid were described in Kwapiszewski, W.; Bialasiewicz, W. *Acta Poliniae Pharmaceutica-Drug Res.* 1994, *51*, 227.

N,N-Dimethyl-leucine was prepared by the following method: Leucine (10.42 g, 80 mmol) was dissolved in H_2O (120 mL) and treated with formaldehyde (80 mL, 37% solution) and Raney Nickel. The reaction mixture was stirred for 21 h, acetone was added and the product was precipitated and collected (77%).

6. Compound **1 - 16** were analyzed by ^1H NMR, MS, HPLC, and elemental analysis.
   Examples of elemental analysis are:
   Compound **8** ($C_{31}H_{47}N_3O_3$) Calcd: C 73.05, H 9.29, N 8.24; Found: C 72.99, H 9.34, N 8.16.
   Compound **10** ($C_{32}H_{49}N_3O_3$) Calcd: C 73.38, H 9.43, N 8.02; Found: C 73.17, H 9.62, N 7.89.
   Compound **12** ($C_{33}H_{49}N_3O_3$) Calcd: C 73.98, H 9.22, N 7.84; Found: C 73.67, H 9.23, N 8.09.
   Compound **13** ($C_{34}H_{51}N_3O_3$) Calcd: C 74.41, H 9.18, N 7.66; Found: C 74.73, H 9.42, N 7.32.
7. Compound **10**, **12**, **13**, and **14** showed 100% protection from the Tonic phase at the dose of 30 mg/kg. The protocol for audiogenic seizure mouse studies by DBA/2 mice: (a) De Sarro, G. B.; Meldrum, B. S.; Nistico, G. *Br. J. Pharmacol.* 1988, *93*, 247. (b) N-type Ca^{+2} channel blocker, ω-conotoxin GVIA, is active in the DBA/2 model. Jackson, H. C.; Scheideler, M. A. *Phychopharmacology* 1996, *126*, 85. (c) Na^+ channel blockers are active in the DBA/2 model. Meldrum, B. S. *Neurology* 1994, *44*, S14.
8. Whole-cell voltage-clamp electrophysiology experiments evaluating drug actions on Ca^{2+} channels were done as outlined in Stoehr, S. J.; Campbell, G. W.; Rock, D. M. *Drug Develop. Res.* 1997, *41*, 85. Experiments on Na^+ channels used similar techniques, with voltage-clamp protocols and solutions modified to isolate Na^+ channel currents.