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SYNTHESIS AND BIOLOGICAL EVALUATION OF SUBSTITUTED 4-(OBz)PHENYLALANINE DERIVATIVES AS NOVEL N-TYPE CALCIUM CHANNEL BLOCKERS

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Abstract: Selective N-type Voltage Activated Calcium Channel (VACC) blockers have shown utility in several models of stroke and pain. In the process of searching for small molecules as N-type calcium channel blockers, we have identified a series of *N,N*-dialkylpeptidylamines (e.g., PD 175069) with potent functional activity at N-type VACC. Further modification of the leucine moiety of PD 175069 with a cyclized ring structure provides a series of novel molecules. Syntheses and pharmacological evaluation of the series are presented.

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] In mammalian systems, neuronal VSCCs are classified into L, N, P, Q, R, and T subtypes. These channels differ in their protein structures, biophysical properties, and pharmacological profiles. N-type channels appear to localize in central and peripheral neurons. They are found throughout the forebrain and are primarily abundant in the synaptic connection. These channels regulate the calcium flux for depolarization-evoked neurotransmitter release from synaptic endings. N-type Ca^{+2} channels are tissue specific, mediate neuronal processes having well defined functional roles, and can be selectively blocked by high-affinity ligands.[1] Selective N-type voltage sensitive calcium channel (VSCC) blockers have shown utility in several models of stroke and pain.[2] ω-Conotoxin MVIIA is a 25 amino acid- peptide found in the venom of piscivorous marine snail (*Conus Magus*). It is a potent and selective N-type voltage-sensitive calcium channel blocker (K_d ~ 10 pM).[3] The synthetic version of MVIIA, SNX-111,[3] has demonstrated efficacy in animal models of traumatic brain injury, focal cerebral ischemia and pain. For severe neuropathic pain, it is more potent than morphine and chronic administration does not elicit tolerance or lead to addiction. Currently, SNX-111 (ziconotide) is in clinical trials for the treatment of pain.

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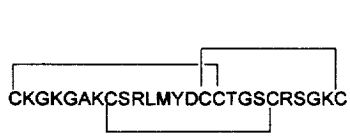
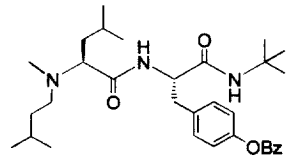
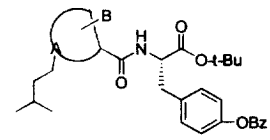


SNX-111



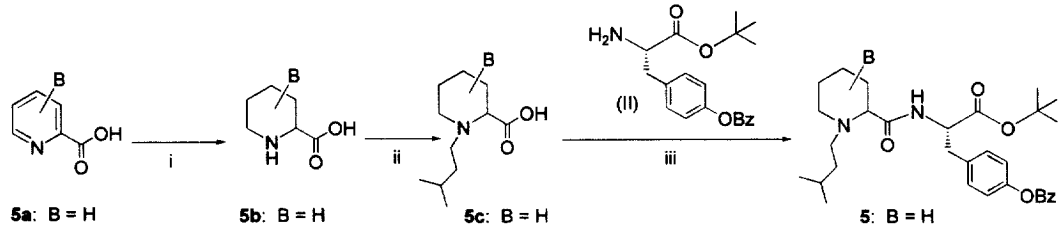
PD 175069



(I)

Due to the promising efficacy shown by N-type calcium channel blockers such as SNX-111, we were especially interested in small molecule N-type calcium channel antagonists. Previously, we reported that PD 175069[4] (a *N,N*-dialkyl-dipeptidyl-amine) was a potent antagonist for N-type Ca^{+2} channels in IMR-32 human neuroblastoma cells (IC50 = 0.32 μM)[5] and was efficacious in vivo in an audiogenic seizure model using DBA/2 mice.[4] Further exploration of this series prompted us to investigate rigid analogs of PD 175069. The design involved tying together the *sec*-butyl group of the leucine side chain with the N-methyl group to generate structures of type **I**. These structures provide a unique, conformationally constrained motif for the N-methyl-leucine fragment of the dipeptide. In this paper, we disclose several analogs with this novel template and discuss their activity for N-type Ca^{+2} channel blockade in preliminary pharmacological evaluation.

Scheme 1



5a: B = H
7a: B = 3-Me
8a: B = 6-Me
9a: B = 4-Me
11a:B = 4-i-pr

5b: B = H
7b: B = 3-Me
8b: B = 6-Me
9b: B = 4-Me
11b:B = 4-i-pr

5c: B = H
7c: B = 3-Me
8c: B = 6-Me
9c: B = 4-Me
11c:B = 4-i-pr

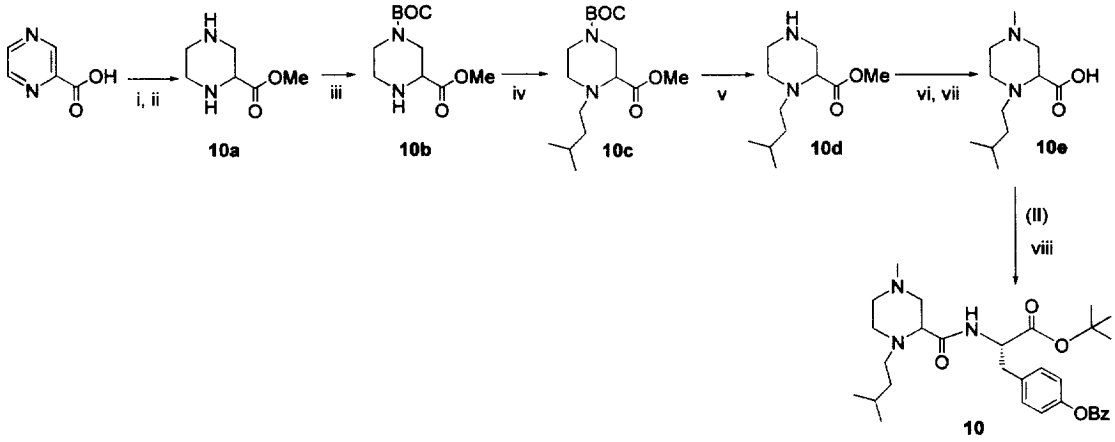
5: B = H
7: B = 3-Me
8: B = 6-Me
9: B = 4-Me
11:B = 4-i-pr

i. H_2O, NH_4OH, 20% Pd/C, H_2; ii. Isovaleraldehyde, H_2, 20% Pd/C, EtOH; iii. HBTU (O-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate), $(i\text{Pr})_2\text{NEt}$, DMF.

Compound **1–4** and **12–13** (Table 1) were prepared by the method described for the synthesis of **1**.[4] The preparation of compounds **5**, **7**, **8**, **9**, and **11** is illustrated in Scheme 1. Thus, the reduction of an appropriately substituted pyridine-2-carboxylic acid[6] in water provided the desired substituted piperidine-2-carboxylic acid derivatives (**5b**, **7b**, **8b**, **9b**, **11b**). The alkylation of the substituted piperidine-2-carboxylic acid was conducted via a reductive-amination procedure using initially isovaleraldehyde in ethanol followed by catalytic

hydrogenation with Pd/C (20%) in ethanol. Compounds **5c**, **7c**, **8c**, **9c** and **11c** were isolated by re-crystallization from acetone. Coupling of the substituted 1-(3-methyl-butyl)-piperidine-2-carboxylic acid with (*S*)-2-amino-3-(4-benzyloxy-phenyl)-N-*tert*-butyl-propionic ester (**II**) provided the final products (**5**, **7**, **8**, **9**, and **11**). The preparation of compound **6** was conducted by the alkylation of commercially available piperidine-4-carboxylic acid, followed by coupling with the aminoacid derivative **II**. The synthesis of compound **10** is outlined in Scheme 2. Intermediate **10b**[7] was alkylated at N-1 of the piperazine ring to generate **10c**. Compound **10c** was then deprotected and subsequently methylated at the N-4 position. Further hydrolysis of the methyl ester provided 1-(3-methyl-butyl)-4–methyl-2-piperazinecarboxylic acid (**10e**), which was the synthetic precursor to compound **10**.

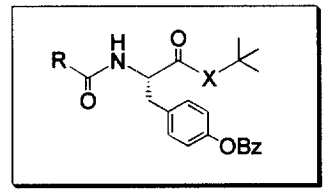
Scheme 2

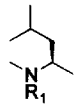
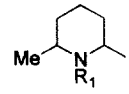
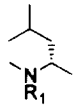
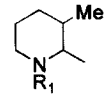
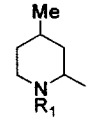
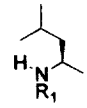
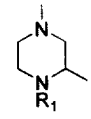
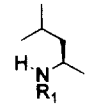
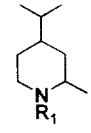
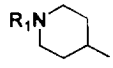


i. H_2, 20% Pd/C, NaOH, H_2O; ii. CH_2N_2, THF; iii. BOC-ON,[8] Dioxane, H_2O; iv. Isovaleraldehyde, H_2, 20% Pd/C, EtOH; v. TFA, CH_2Cl_2; vi. Formaldehyde, H_2, 20% Pd/C, MeOH; vii. Concentrated HCl, acetone, H_2O; viii. HBTU, $(i\text{Pr})_2\text{NEt}$, DMF.

PD 175059 (**1**) contained a dipeptidyl structure of Leucine-(OBz)-Tyrosine. To confirm that the leucine moiety was most advantageous for N-type Ca^{+2} channel blockade, we replaced the leucine moiety of PD 175069 with D-leucine, valine, and glycine. The in vitro results indicated that the stereochemistry of L- or D-isomers (**1** or **2**) had little effect on their IMR-32 activities, whereas the leucine derivative was more active than its valine (**3**, IC_{50} = 0.83 μM) and glycine (**4**, IC_{50} = 1.6 μM) analogs by at least two- to fivefold. Therefore, we chose compound **1** as the template and built seven conformationally semi-constrained analogs (Table 1: **5–11**) for SAR studies. Based on the SAR discussed, we retained the chirality of the (OBz)-tyrosine and used the racemic isomers of the 6-membered heterocyclic ring. The previous SAR also indicated that, the ester analog (**13**) is about equipotent as the amide analog (**12**) in PD 175069 series.

Table 1: IMR-32 activity of N-type calcium channel blockers



	R	X	IMR-32 IC_{50} (μM)		R	X	IMR-32 IC_{50} (μM)
1		NH	0.32	**7**		O	**0.32**
2		NH	0.37	**8**		O	1.1
3		NH	0.83	**9**		O	0.78
4		NH	1.6				
12		NH	0.30	**10**		O	1.0
13		O	0.40	**11**		O	0.49
5		O	1.0				
6		O	1.5				

R_1 = 3-methyl-butyl ,[9]

Initially, the two positional isomers, 2-piperidinyl (**5**) and 4-piperidinyl (**6**), were compared and were found to be approximately equipotent. Further conformational constraints were studied by the addition of a methyl group to the 6-, 3- and 4-position of the piperidine ring of **5**, which resulted in compounds **7**, **8**, and **9**. The trend of their N-type Ca^{+2} channel potencies was: **7** > **9** > **8** (Table 1: IC_{50}'s of 0.32, 0.78, 1.1 μM, respectively) with the 6-methyl-piperidinyl analog (**7**) being the most active of the three. The SAR of C-4-substitution in **5** was compared by synthesizing hydrogen (**5**), methyl (**9**), and isopropyl (**11**) analogs. Their IMR-32 results indicate that the isopropyl derivative (**11**, IC_{50} = 0.49 μM) was about twofold more active than **5** and **9**. Replacement of the carbon at C-4 of 4-methyl-piperidinyl compound (**9**) with a nitrogen provided the corresponding 4-methyl-piperazine analog (**10**), which slightly reduced IMR-32 activity. These results indicate that conformationally constrained tertiary amine functionality in the reduced heterocyclic ring and a branched alkyl group at C-4 are preferred for N-type Ca^{+2} channel activity.

In summary, a series of PD 175069 analogs incorporating novel conformationally constrained leucine surrogates has been synthesized as N-type calcium channel blockers. These compounds demonstrate potent in vitro activity in the IMR-32 assay. Compounds **7** and **11** with 2,6- and 2,4-disubstitution in the piperidine ring 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: N.Y., 1997; pp 27–51. (c) Gilmore, J.; Dell, C.; Bowman D.; Lodge, D. *Ann. Report Med. Chem.* **1995**, *30*, 51. (d) Mathur, V. S.; McGuire, D., Bowersox, S. S.; Miljanich, G. P.; Luther, R. R. *Pharm. News* **1998**, *5(5)*, 25. (e) Bowersox, S. S., Luther, R. *Toxicon* **1998**, *36(11)*, 1651.

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5. 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.[4]

6. Pyridine-2-carboxylic acid (**5**), 6-methyl-pyridine-2-carboxylic acid (**7**), and 3-methyl-pyridine-2-carboxylic acid (**8**) are commercially available. The preparation of 4-methyl-pyridine-2-carboxylic acid (**9**) and 4-isopropyl-pyridine-2-carboxylic acid (**11**) followed the procedures described in Shuman, R. T.; Ornstein, P. L., Paschal J. W.; Gesellchen, P. D. *J. Org. Chem.* **1990**, *55*, 738. and references cited therein.

7. Bigge, C. F.; Hays, S. J.; Novak, P. M.; Drummond, J. T.; Johnson, G.; Bobovski, T. P. *Tetrahedron Lett.* **1989**, *30*, 5193.

8. BOC-ON is a registered trademark of the Aldrich Chemical Company, Inc.

9. Compound **1–13** were analyzed by ^{1}H NMR, MASS, and elemental analysis. Examples of elemental analysis are:

   Compound **2** ($C_{32}H_{46}N_2O_4.H_2O.HCl$) Calcd: C 73.38, H 9.43, N 8.02; Found: C 73.22, H 9.49, N 7.90.

   Compound **7** ($C_{32}H_{46}N_2O_4.0.25H_2O.HCl$) Calcd: C 68.19, H 8.42, N 4.97; Found: C 68.00, H 8.63, N 4.91.

   Compound **10** ($C_{31}H_{45}N_3O_4$) Calcd: C 71.09, H 8.66, N 8.03; Found: C 71.25, H 8.40, N 7.72.