Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2151–2156

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

STRUCTURE-ACTIVITY RELATIONSHIP OF *N*-METHYL-*N*-ARALKYL-PEPTIDYLAMINES AS NOVEL N-TYPE CALCIUM CHANNEL BLOCKERS

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Received 1 March 1999; accepted 16 June 1999

Abstract: Selective N-type voltage sensitive calcium channel (VSCC) blockers have shown efficacy in several animal models of stroke and pain. In the process of searching for small molecule N-type calcium channel blockers, we have identified a series of *N*-methyl-*N*-aralkyl-peptidylamines with potent functional activity at N-type VSCCs. The most active compound discovered in this series is PD 173212 (**11**, IC_{50} = 36 nM in the IMR-32 assays). SAR and pharmacological evaluation of this series are described.

A number of distinct classes of voltage-sensitive calcium channels (VSCC) have been identified in neurons. Neuronal VSCC are classified into L, N, P, Q, R, and T subtypes in mammalian systems, and they are different in their protein structures, biophysical properties, and pharmacological profiles.[1] N-type channels appear to be localized in the central and peripheral nervous system and are particularly abundant in synaptic nerve terminals, suggesting a role in regulation of neurotransmitter release.[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 potent and selective N-type voltage-sensitive calcium channel blocker (K_d ~ 10 pM).[3] The synthetic version of ω-Conotoxin 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 lead to tolerance or result in addiction. An NDA for SNX-111 (ziconotide) will be filed for its pain indication in the near future.

Due to the promising efficacy shown by peptide N-type calcium channel blockers, we focused our efforts on developing small molecule N-type calcium channel blockers for therapeutic use. Previously, we reported that compound **1** was a potent antagonist (IC_{50} = 0.68 μM) to functionally block N-type calcium channels in IMR-32

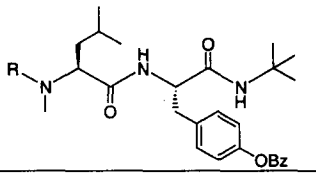
0960-894X/99/$ - see front matter
PII: S0960-894X(99)00359-5

human neuroblastoma cells[4] and to show protection in an audiogenic seizure model using DBA/2 mice.[5] Further exploration of the SAR in this series led us to discover several more potent N-type Ca^{+2} channel blockers. We took compound **1** as the prototype and replaced the cyclohexylmethyl group of **1** with aralkyl groups to generate a series of *N*-methyl-*N*-aralkyl-peptidyl derivatives. These analogs are highly active in vitro in the IMR-32 assay and moderately efficacious in vivo as anticonvulsants. In this paper, we disclose several novel structures and discuss their in vitro and in vivo activity.

The initial SAR was conducted with aralkyl analogs of **1** (Table 1). Replacement of the cyclohexylmethyl group of **1** with a benzyl group offered **2**[6,7] (IC_{50} = 0.36 μM, Table 1) which showed a 2-fold enhanced activity. This prompted us to investigate heteroaryl-containing analogs of **2** and target compounds with better aqueous solubility. Unfortunately, the 2-furanylmethyl (**3**) and 3-pyridylmethyl (**4**) analogs showed lower activity than the benzyl derivative (**2**) by 5-10 fold. Likely, compounds with heteroaryl groups are less favorable than its corresponding benzyl derivative for N-type Ca^{+2} channel blockade. A further SAR exploration of linker chain-length versus activity was conducted by comparing the methylene (**2**, IC_{50} = 0.36 μM), ethylene (**5**, IC_{50} = 1.7 μM), and propylene (**6**, IC_{50} = 1.9 μM) analogs. We found that the methylene linkage is the most preferred one; the motif of compound **2** is advantageous for the interaction between antagonist and the pharmacophore of N-type Ca^{+2} channels.

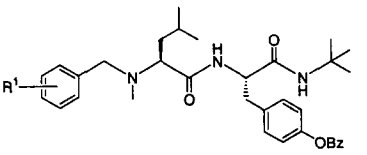
Moreover, the SAR of the substitution pattern on the N-benzyl ring of **2** was examined. We used a chlorine atom as a probe to explore the activity of the three regioisomers on the phenyl ring (**7**, **8**, **9**, Table 2). The results indicated that the 4-Cl (**9**) analog is more active than its 3-Cl (**8**) and 2-Cl (**7**) derivatives. The 4-chloro group of **9** was further substituted with various functional groups resulting in six new compounds (**10-14**). Interestingly, activities of these derivatives were correlated with the lipophilicity of the substituents, with a trend of 4-*tert*-butyl (**11**) > 4-bromo (**10**) > 4-chloro (**9**) > 4-methylamino (**12**) > 4-hydrogen (**2**) > 4-methoxy (**13**) > 4-hydroxy (**14**). The activities declined (IC_{50} = 0.036 (**11**), 0.13 (**10**), 0.23 (**9**), 0.28 (**12**), 0.36 (**2**), 0.5 (**13**), 1.0 (**14**) μM, respectively) as the lipophilicity of R_1 (π value: 1.98, 0.86, 0.71, 0.18, 0, -0.02, -0.67, respectively)[8] decreased. It appeared that *tert*-butyl and bromo were the optimal groups for substitution. It is worth mentioning that compound **11** (PD 173212, IC_{50} = 36 nM) is the most active small molecule for N-type calcium channel blockade reported so far. PD 173212 (**11**) potently blocked recombinant B-class (N-type) calcium channel currents 78 ± 7.8% at 300 nM (N = 3), with an IC_{50} of 74 nM (N = 2)[9] using whole-cell voltage-clamp techniques. PD 173212 also showed moderate efficacy in preventing tonic seizures in the

Table 1: The in vitro and in vivo results of *N*-methyl-*N*-substituted-dipeptidylamines



	R	IMR32 IC_{50} (μM)	Audiogenic seizure model % protection (IV) (Tonic phase) (N = 5 mice / dose tested)
1	cyclohexylmethyl	0.68	100% @ 30 mg/kg, 20% @ 10 mg/kg
2	benzyl	0.36	
3	2-furanylmethyl	1.6	60% @ 30 mg/kg
4	3-pyridylmethyl	3.8	
5	phenethyl	1.7	
6	phenpropyl	1.9	

Table 2: The in vitro and in vivo results of *N*-methyl-*N*-(substituted-benzyl)-dipeptidylamines



	R^1	IMR32 IC_{50} (μM)	Audiogenic seizure model % protection (IV) (Tonic phase) (N = 5 mice / dose)
7	2-Cl	0.56	
8	3-Cl	1.0	
9	4-Cl	0.23	
10	4-Br	0.13 ± 0.03 (n = 2)	
11	4-t-butyl	**0.036** ± 0.01 (n = 2)	60% @ 30 mg/kg
12	4-$N(Me)_2$	0.28	80% @ 30 mg/kg
2	4-H	0.36	
13	4-OMe	0.50	100% @ 30 mg/kg, 40% @ 10 mg/kg
14	4-OH	1.0	

audiogenic seizure model (iv).[10] Further studies suggest that PD 173212 possesses selectivity for non L-type Ca^{+2} channels versus neuronal Na^{+}, K^{+}, and L-type Ca^{+2} channels.[9] In addition, several other compounds in this series (**3**, **10**, **12**, **13**) were evaluated in an audiogenic seizure mouse model and showed activity in preventing tonic seizures in DBA/2 mice (iv). (Table 1, 2)

In summary, a series of substituted peptidyl-amine based N-type calcium channel blockers has been discovered. These compounds demonstrate potent in vitro activity in the IMR-32 assay as well as in electrophysiology, and they are efficacious in the audiogenic seizure mouse model. PD 173212 (**11**), the 4-*tert*-butylbenzyl substituted analog, is the most potent analog in this series.

Acknowledgements: The authors wish to thank the Parke-Davis Analytical Research for spectral and microanalysis data. We acknowledge Gregory Campbell for preparing the cell culture for electrophysiology.

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.

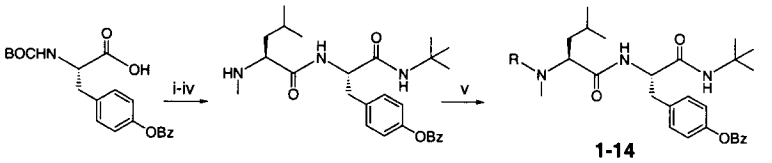
2. (a) Bowersox, S. S.; Singh, T.; Luther, R. R. *Brain Res.* **1997**, *747*, 343. (b) Bowersox, S. S.; Gadbois, T.; Singh, T.; Pettus, M.; Wang, Y. Luther, R. R. *J. Pharmacol. Exp. Ther.* **1996**, *279*, 1243. (c) Verweij, B. H.; Muizelaar, J. P.; Vinas, F. C.; Peterson, P. L.; Xiong, Y.; Lee, C. P. *Neurological Res.*, **1997**, *19*, 224.

3. (a) Miljanich, G. P.; Ramachandran J. *Ann. Rev. Pharmacol. Toxicol.* **1995**, *35*, 704. (b) Olivera, B. M.; Miljanich, G. P.; Ramachandran J.; and Adam, M. E. *Ann. Rev. Biochem.* **1994**, *63*, 823. (c) Pringle, A. K.; Benham, C. D.; Sim, L.; Kennedy, S. J.; Iannotti, F.; Sundstrom, L. E. *Stroke* **1996**, *27*, 2124.

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[5] was used as controlled reference in all these assays.

5. Hu, L.-Y.; Ryder, T. R.; Cody, W. L.; Lotarski, S. M.; Millerman, E.; Rafferty, M. F.; Rock, D. M.; Song, Y.; Stoehr, S. J.; Taylor, C. P.; Weber, M. L.; Szoke, B. G.; Vartanian, M. G. *Bioorg. Med. Chem. Lett.* **1999.**

6. The preparation of compounds in this series is outlined below:



1-14

i.HBTU (O-benzotriazol-1-yl-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate), $(iPr)_2NEt$, DMF, *t*-butylamine; ii. TFA, CH_2Cl_2; iii. HBTU, $(iPr)_2NEt$, DMF, N^{α}-BOC-N^{α}-Me-leucine; iv. TFA, CH_2Cl_2; v. CH_2Cl_2, $NaBH(OAc)_3$, an aldehyde (yield 50-95%).

7. Compounds **1-14** were analyzed by ^1H NMR, mass spectroscopy, and elemental analysis. Examples of analysis in this series: Compounds **10** ($C_{34}H_{44}N_3O_3Br_1$) Calcd: C 72.70, H 7.90, N 7.48; Found: C 72.95, H 7.93, N 7.42. Compound **11** ($C_{38}H_{53}N_3O_3$) Calcd: C 76.09, H 8.91, N 7.00; Found: C 75.98, H 9.04, N 6.88.

8. (a) *A Textbook Of Drug Design And Developmen*; Krogsgaard, P. and Bundgaard, H., Ed.; Harwood Academic Publicher, 1991, pp 80. (b) Inami, Y.; Tomita, T.; and Terada, Q. *Chem. Pharm. Bull.* **1991**, *39*, 1426.

9. The selectivity of PD 173212 for N-type Ca^{+2} channels versus other types of channels is listed below: PD 173212 blocked recombinant B-class (N-type) Ca^{+2} channels with a IC_{50} of 74 nM (N = 2), and blocked Na^+ channels by 8% at 1 μM (N = 4), and blocked K^+ channels by 10% at 1 μM (N = 4) in superior cervical ganglion neurons. PD 173212 had effects on L-type Ca^{+2} channels only at higher concentrations with an IC_{50} greater than 10 μM as measured in A-10 fetal rat thoracic aorta smooth muscle cells using fluorescent techniques similar to those used with N-type Ca^{+2} channels in IMR32 cells. In the A-10 assay the L-type calcium channel blocker nitrendipine reduced the intracellular calcium signal with an IC_{50} of 0.93 nM. Whole-cell voltage-clamp experiments evaluating drug actions on recombinant Ca^{2+} channels were done as outlined in Rock, D. M.; Horne, W. A.; Stoehr, S. J.; Hashimoto, C.; Zhou, M.; Cong. R.; Palma, A.;

Hidayetoglu, D.; and Offord, J. In: Tsien, R. W.; Clozel, J-P and Nargeot, J. (Eds) *Low-Voltage-Activated T-type Calcium Channels*, **1998**, *279-289*. Experiments on superior cervical ganglion neurons were performed as outlined in Stoehr, S. J.; Campbell, G. W.; Rock, D. M. *Drug Develop. Res.* **1997**, *41*, 85. except solutions and voltage protocols were used to isolate Na^+ and K^+ channels from acutely isolated neurons.

10. The protocol for DBA/2 mouse studies: 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.