Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2453–2458

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

STRUCTURE–ACTIVITY RELATIONSHIP AT THE PROXIMAL PHENYL GROUP IN A SERIES OF NON-PEPTIDYL N-TYPE CALCIUM CHANNEL ANTAGONISTS

Todd R. Ryder,[a,*] Lain-Yen Hu,[a] Michael F. Rafferty,[a] Susan M. Lotarski,[b] David M. Rock,[b] Sally J. Stoehr,[b] Charles P. Taylor,[b] Mark L. Weber,[b] George P. Miljanich,[c] Elizabeth Millerman,[c] and Balazs G. Szoke[c]

Departments of Chemistry[a] and Neuroscience Therapeutics[b], Parke-Davis Pharmaceutical Research, Division Of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105, U.S.A.
[c]Elan Pharmaceuticals, Inc., 3760 Haven Avenue, Menlo Park, CA 94025, U.S.A.

Received 21 June 1999; accepted 15 July 1999

Abstract: Selective N-Type Voltage Sensitive Calcium Channel (VSCC) antagonists have shown utility in several models of pain and ischemia. We report the structure–activity relationship at the proximal phenyl group in a series of non-peptidyl VSCC blockers.

Introduction

Neuronal voltage sensitive calcium channels (VSCC) regulate intracellular calcium concentration, which in turn affects important neuronal functions such as neurotransmitter release, cellular excitability, hormone secretion, metabolism, and gene expression.[1] N-type calcium channels are one of several subtypes of VSCC, and are located primarily on presynaptic nerve terminals of central and peripheral neurons. These channels are sensitive to changes in membrane potential and regulate the calcium flux involved in depolarization-evoked release of neurotransmitter. Thus, they play a key role in coupling the electrical signal of an action potential to chemical changes inside nerve cells. It is well-documented in the literature that neuronal injury is often associated with excessive influx of calcium following an ischemic or traumatic event.[1]

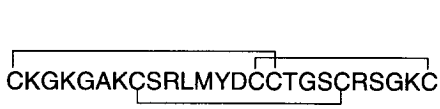
It has been suggested that selective N-type calcium channel blockers could be useful as therapeutic agents.[2] For example, ω-Conotoxin MVIIA, a 25 amino acid residue- containing peptide found in the venom of a piscivorous marine snail (*Conus Magus*), is a highly potent and selective N-type VSCC blocker.[3] The synthetic equivalent, SNX-111 (ziconotide, **1**) has demonstrated therapeutic benefit in animal models of traumatic brain injury, focal cerebral ischemia, and pain.[3] Recent progress in the area of small molecule N-type calcium channel blockers has been reviewed.[4]

As part of our efforts to develop small molecule N-type VSCC antagonists, PD 151307 (**2**) was identified as a chemical lead by high volume screening of the Parke-Davis compound library.[5] In the process of exploring the SAR of this compound, we discovered a novel series of 4-aminopiperidine derivatives, such as

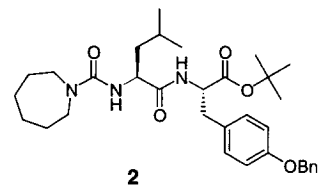
0960-894X/99/$ - see front matter
PII: S0960-894X(99)00405-9

181283 (**3**), and found that they are active in vitro in the IMR-32 assay, and efficacious in vivo in an audiogenic seizure model using DBA/2 mice.[6]

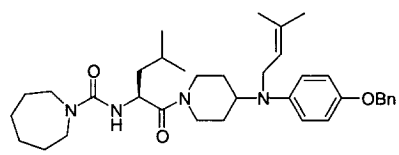
Figure 1. N-Type Calcium Channel Blockers



1



2



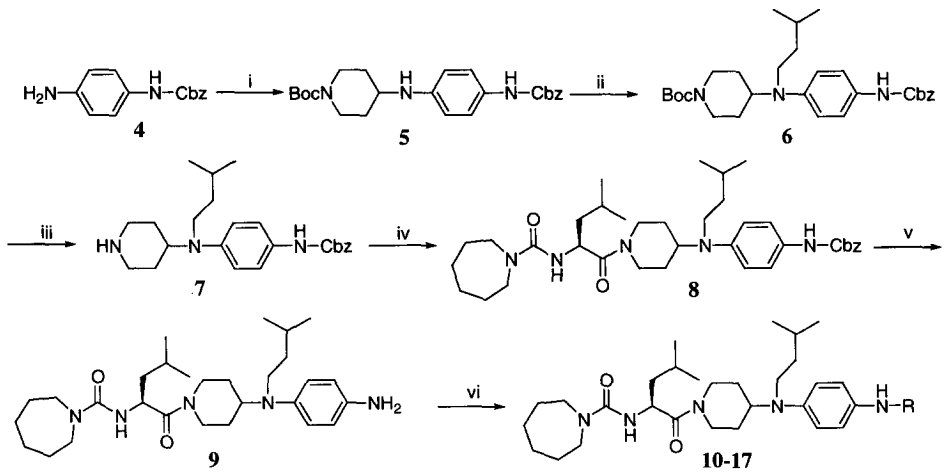
3

In this report, we present the results of an exploration of the SAR at the proximal, aniline phenyl group of PD 181283 (**3**). Our goal was to determine the effect of variations at this position on potency at N-type calcium channels, as well as to incorporate functionality which could improve the physicochemical properties or metabolic stability of compounds in this series. We disclose three novel analogs with improved in vitro activity relative to **3**.

Chemistry

Our synthetic approach is illustrated in Scheme 1. Commercially available *tert*-butyl 4-oxo-1-piperidinecarboxylate was reductively aminated with mono-protected phenylenediamine[7] **4** to give piperidine **5**. In a second reductive amination reaction, this material was alkylated on the aniline nitrogen with isovaleraldehyde to give tertiary amine **6**. This intermediate was deprotected under acidic conditions to give **7**, and then coupled with (S)-2-[(Azepane-1-carbonyl)-amino]-4-methyl-pentanoic acid[6] to give amide **8**. The Cbz protecting group was cleaved by hydrogenolysis to give aniline **9**, which was then alkylated in a third reductive amination reaction with a variety of aldehydes to yield the desired final products, **10–17**. Other final compounds were prepared by analogous methods. Thus, the phenethyl[8] (**18**), unsubstituted phenyl (**19**), and benzyloxy (**20**) analogs were each prepared using the appropriate aniline starting material in the initial reductive amination reaction. 4-bromo-2-methyl-2-butene was used as the alkylating agent in the second step of the sequence for **18** and **19**. The benzyl group in **20** was cleaved by hydrogenolysis to yield the phenol analog, **21**.[9]

Scheme 1.



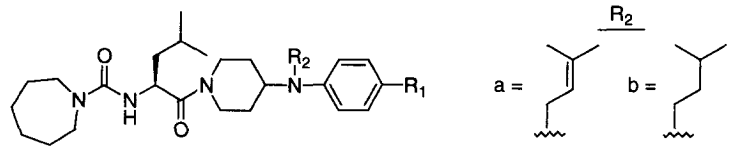
i. *tert*-Butyl 4-oxo-1-piperidinecarboxylate, $NaBH(OAc)_3$, CH_2Cl_2; ii. isovaleraldehyde, $NaBH(OAc)_3$, CH_2Cl_2; iii. 1:1 trifluoroacetic acid/CH_2Cl_2; iv. (S)-2-[(Azepane-1-carbonyl)-amino]-4-methyl-pentanoic acid, *O*-benzotriazol-1-yl- *N,N,N',N'*- tetramethyluronium hexafluorophosphate, $(iPr)_2EtN$, DMF; v. 20% Pd/C, H_2, THF/MeOH; vi. aldehyde, $NaBH(OAc)_3$, CH_2Cl_2.

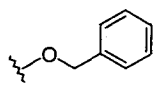
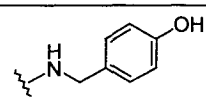
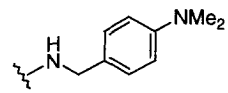
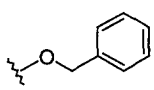
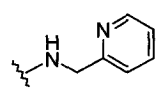
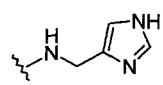
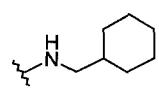
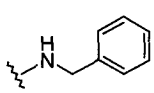
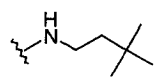
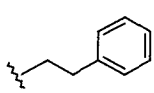
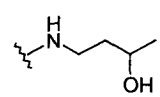
Results

The activity of these compounds at N-type VSCC was evaluated in the IMR-32 assay[10] (Table 1). The structural starting point for this SAR study was the previously reported compound **3**, which had an IC_{50} of 0.34 μM in this assay. In an attempt to reduce the molecular weight, we prepared analog **19** (IC_{50} = 1.0 μM), which lacked the 4-benzyloxy substituent. This compound was about three fold less active than the parent compound, establishing that substitution is required for optimal potency.

In other studies, we noted that saturation of the 3-methyl-but-2-enyl substituent of **3** led to a two fold improvement in potency (**20**, IC_{50} = 0.19 μM). Since this change could also potentially improve the metabolic stability of our compounds by removing a site for oxidation in vivo, we chose to use the saturated side chain to prepare two other lower molecular weight compounds which lacked the benzyl group but retained a heteroatom. Compound **21** (IC_{50} = 1.0 μM) was substituted with a phenolic hydroxy group: it was about six fold less potent than **20**. Changing the hydroxy group of **21** to an amino group in compound **9** (IC_{50} = 2.2 μM) resulted in an even steeper, twelve fold decrease in potency as compared to **20**.

Table 1. IMR-32 IC_{50} values



	R_1	R_2	IC_{50}		R_1	R_2	IC_{50}
3.		a	0.34 ± 0.06 μM (n = 4)	**11.**		b	0.69 μM
19.		a	1.0 μM	**12.**		b	0.49 μM
20.		b	0.19 μM	**13.**		b	1.1 μM
21.		b	1.1 μM	**17.**		b	120 μM
9.		b	2.2 μM	**14.**		b	0.29 μM
10.		b	0.40 μM	**15.**		b	0.18 μM
18.		a	0.67 μM	**16.**		b	1.8 μM

Next, we prepared two analogs with changes to the oxygen atom of the O-benzyl group of **20**. To assess the effect of changing the O-benzyl oxygen to a nitrogen, we compared **20** with the N-benzyl analog **10** (IC_{50} = 0.40 μM). This compound was about two fold less active than the parent. Similarly, an analog with a carbon replacement for the oxygen in the O-benzyl group (**18**, 0.67 μM) was about two fold less active than its parent (**3**, 0.34 μM).

We then explored the SAR at the distal phenyl group of N-benzyl analog **10**. Our goal was to improve the physicochemical properties of the series by incorporating more polar functionality on the ring or in place of the phenyl group itself. The 4-hydroxybenzyl (**11**, IC_{50} = 0.69 μM) and 4-dimethylaminobenzyl (**12**, IC_{50} = 0.49

μM) analogs demonstrated that incorporation of polar substituents at the *para* position of this phenyl group resulted in a 3 to 4 fold loss in potency. Replacement of the phenyl group with a 2-pyridyl group resulted in about a six fold loss in potency (**13**, IC_{50} = 1.1 μM), while the still more polar imidazolyl-methyl analog was essentially inactive (**17**, IC_{50} = 120 μM).

Finally, we prepared a few analogs with alkyl and cycloalkyl groups which could be more metabolically stable than the phenyl group. Two of these, the cyclohexylmethyl (**14**, IC_{50} = 0.29 μM) and 3,3-dimethyl-butyl (**15**, IC_{50} = 0.18 μM) compounds, were among the most potent novel analogs in this report. They were about equipotent when compared with **20**, and slightly more potent than the N-benzyl analog (**10**) or the initial lead, PD 181283 (**3**). Interestingly, incorporation of a polar 3-hydroxybutyl side chain resulted in a ten fold loss in potency (**16**, IC_{50} = 1.8 μM) as compared with analog **15,** which lacked a hydroxy group. This result was consistent with the decreases in potency observed for other polar substitutions at this position.

The most potent novel analog (**15**) was evaluated in an audiogenic seizure model using DBA/2 mice.[11] It showed significant activity in preventing tonic seizures (80% protection at 30 mg/kg) in this assay. This compound was also evaluated in electrophysiological assays.[12] It blocked neuronal N-type calcium channels measured electrophysiologically in superior cervical ganglion neurons (71 ± 11.3 % at 3 μM, n = 3), with a calculated IC_{50} = 1.4 μM. Compound **15** also blocked voltage-gated sodium channels (41% at 3 μM, n = 2). These results suggest that the compound is active at both voltage-gated calcium channels and voltage-gated sodium channels.

Conclusions

In conclusion, we report three new compounds (**14**, **15**, and **20**) that are more potent in the IMR-32 asay than the starting point for this SAR study, PD 181283 (**3**). The most potent of these analogs (**15**) was also active in an audiogenic seizure mouse model. Our work suggests that a substituent of sufficient size and lipophilicity on the proximal phenyl group is necessary for potent N-type calcium channel blockade in the IMR-32 assay. Within this broad requirement, a variety of structural changes are tolerated without a substantial loss in potency. Two of the most active compounds disclosed here (**14** and **15**), lack both the distal phenyl group and alkene functionality of our previously reported compounds, which could be sites of metabolic oxidation in vivo. The additional amine functionality in these compounds could also improve their physicochemical properties by providing another site for acid salt formation and hydrogen bond donation to solvent.

Acknowledgements: We thank Norm Colbry and Don Johnson for running the catalytic hydrogenation reactions, Thomas Malone and Arindam Chatterjee for preparation of (S)-2-[(Azepane-1-carbonyl)-amino]-4-methyl-pentanoic acid, Krista M. Siebers for preparation of analog 19, and 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*, 261. (b) Scraibine, A. In *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) Verwejj, B. H.; Muizelaar, J. P.; Winas, 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.; Adam, M. E. *Ann. Rev. Biochem.* **1994**, *63*, 823. (c) Pringle, A. K.; Benham, C. D.; Sim, L.; Kennedy, S. J.; Iannoti, F.; Sundstrom, L. E. *Stroke* **1996**, *27*, 2124.
4. Cox, B.; Denyer, J. C. *Exp. Opin. Ther. Patents* **1998**, *8*, 1237.
5. Malone, T. C.; Booth, R. J.; Campbell, G.; Cody, W. L.; Geer, J. J.; He, J.; Hu, L.-Y.; Lescosky, L. J.; Miljanich, G. P.; Nadasdi, L.; Rafferty, M. F.; Rock, D. M.; Sercel, A.; Stoehr, S. J.; Song, Y.; Szoke, B. G.; Tarczy-Hornoch, K.; Taylor, C. P.; Urge, L.; Vartanian, M. G.; Weber, M. L.; Yi, C. S. Abstracts of Papers, 217th National Meeting of the American Chemical Society, Anaheim, CA, March 21-25, 1999; American Chemical Society: Washington, D.C., 1999; MEDI 122.
6. Hu, L.-Y.; Ryder, T. R.; Millerman, E.; Rafferty, M. F.; Szoke, B. G.; Lotarski, S. M.; Taylor, C. P.; Rock, D. M. *J. Med. Chem.***1999**.
7. The preparation of N-benzyloxycarbonyl-phenylenediamine has been reported: Hasserodt, J.; Janda, K. D. *Tetrahedron* **1997**, *53*, 11237.
8. The preparation of 4-phenethylaniline has been reported: Baker, B. R.; Ho, B.-T.; Lourens, G. J. *J. Pharm Sci.* **1967**, *56*, 737.
9. All final products had analytical data consistent with proposed structures.
   Example: **20b**: APCI-MS: 591.3 (M+1 for $C_{36}H_{54}N_4O_3$); sticky solid; TLC: R_f 0.25 (3:2 hexane/EtOAc), ^{1}HNMR ($CDCl_3$) δ 0.84–0.89 (m, 9 H), 0.96 (d, 3 H, J = 6.3 Hz), 1.25–1.90 (m, 18 H), 2.59 (dd, 1 H, J = 22.5, 10.5 Hz), 2.98–3.06 (m, 3 H), 3.36–3.38 (m, 5 H), 3.98 (br, 1 H), 4.57 (t, 1 H, J = 14.4 Hz), 4.89 (br, 1 H), 4.97 (s, 2 H), 5.22 (dd, 1 H, J = 20.3, 8.5 Hz), 6.75–6.87 (m, 4 H), 7.26–7.41 (m, 5 H). Anal ($C_{36}H_{54}N_4O_3$) (calcd) C: 73.18, H: 9.21, N: 9.48 (found) C: 72.78, H: 9.24, N: 9.33.
10. IC_{50}'s for N-type calcium channel blockade were measured using the fluorescent Ca^{2+} indicator Indo-1 in IMR-32 human neuroblastoma cells in the presence of 5 μM nitrendipine to block L-type channels. PD 151307 was run in parallel as a standard in each assay.
11. (a) The protocol for audiogenic seizure studies using DBA/2 mice has been described: De Sarra, G. B; Meldrum, B. S.; Nistico, G. *Br. J. Pharmacol.* **1988**, *93*, 247. (b) The N-type calcium channel blocker ω-conotoxin GVIA is active in the DBA/2 model: Jackson, H. C.; Scheideler, M. A. *Psychopharmacology* **1996**, *126*, 85. (c) Na^+ channel blockers are active in the DBA/2 model: Meldrum, B. S. *Neurology* **1994**, *44*, S14.
12. Whole-cell voltage-clamp electrophysiology experiments evaluating drug actions on Ca^{2+} channels were performed 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.