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## SYNTHESIS AND BIOLOGICAL ACTIVITY OF SUBSTITUTED BIS-(4-HYDROXYPHENYL)METHANES AS N-TYPE CALCIUM CHANNEL BLOCKERS

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Abstract: Voltage activated calcium channel (VACC) blockers have been demonstrated to have utility in the treatment of stroke and pain. A series of aminomethyl substituted phenol derivatives has been identified with good functional activity and selectivity for N-type VACC's over sodium and potassium channels. The methods of synthesis and preliminary pharmacology are discussed herein. © 1999 Published by Elsevier Science Ltd. All rights reserved.

There are a number of different classes of voltage sensitive calcium channels (VSCC) that can be found in neurons.<sup>1</sup> These calcium channels regulate intracellular calcium concentrations, which in turn control a number of critical neuronal functions. These functions include hormone secretion, neurotransmitter release, metabolism, and cyctoskeletal function.<sup>2</sup> One of the secondary events following an ischemic event or other neuronal trauma, which has been well documented in the literature, is the influx of excessive amounts of calcium ion into the neurons. These pathologically high concentrations of calcium in neurons trigger a number of biochemical events that can ultimately lead to cell death. These events include the activation of proteases and phospholipases leading to damage to structural membrane proteins, activation of endonucleases leading to DNA damage, and NO synthetase leading to free radical damage of DNA and organelles.

 $\omega$ -Conotoxin MVIIA, a 25 amino acid-residue containing peptide with three disulfide bonds, is a selective N-type voltage-sensitive calcium channel blocker. The synthetic equivalent SNX-111<sup>3</sup> has demonstrated utility in a number of animal models of ischemia and pain.<sup>4</sup> SNX-111 is also currently in clinical trials for the treatment of ischemia-induced brain injury and chronic pain.<sup>5</sup> A New Drug Application for SNX-111 (ziconotide) has been filed for its use in the treatment of pain.



PD0032577 PD0157667 Because compounds that block N-type calcium channels may be useful in the treatment of stroke and other neuronal trauma, a number of companies are pursuing drug discovery efforts in this area.<sup>1a</sup> We began a

chemistry program in this area to develop small molecule replacements for SNX-111 that would selectively block N-type calcium channels. PD0032577 was identified as a chemical lead through the mass screening of our compound library. It possessed good activity ( $IC_{50} = 0.97 \mu M$ ) in blocking N-type calcium channels functionally in the IMR-32 human neuroblastoma cell assay.<sup>6</sup> However, it was not selective at blocking Ntype channels over L-type channels in GH-3 rat pituitary cells ( $IC_{50} = 2.0 \mu M$ ), nor Na<sup>+</sup> and K<sup>+</sup> channels in SCG neurons<sup>7</sup> (81% and 77% @ 10  $\mu M$ , respectively). Concurrent work in our laboratory<sup>8</sup> yielded PD0157667, which showed a vast improvement in activity in the IMR-32 assay ( $IC_{50} = 0.46 \mu M$ ) over the parent 6-hexamethyleneiminomethyl-5-hydroxyisoquinoline ( $IC_{50} = 35 \mu M$ ). Our goal was to improve upon PD0032577's functional activity and selectivity using the same approach that yielded PD0157667.

4,4-Bis-(4-hydroxyphenyl)pentanoic acid was converted to its methyl ester 2 by treatment with diazomethane in ether. The ester 2 was then treated with excess phenyl magnesium bromide to give the diphenyl alcohol 3 in 96% yield. The hydroxy group could be reduced off with 20% Pd/C in methanol to give the diphenylpropyl bis-phenol 4 in 85% yield. The carboxylic acid 1 was also treated with carbonyldiimadazole in refluxing THF, followed by dibenzyl amine in refluxing THF to give the amide 5 in 40% yield. The amide moiety was reduced to the amine using lithium aluminum hydride in refluxing THF to give the dibenzylamine 9 in 95% yield. The bis-phenols 3, 4, and 9, were treated with formaldehyde and hexamethyleneimine in ethanol at 50 °C to give the Mannich<sup>9</sup> products 6, 7, 8, and 10, respectively, in 20–35% yield. For bis-phenol 3, it was possible to isolate the mono Mannich product 6. This was the only example where the mono Mannich product was observed.

Octopamine hydrochloride was condensed with phenol in 6 N HCl at 100 °C to give the bis-phenol **11** in 68% yield. The amine could be acylated with either diphenylacetyl chloride or benzoyl chloride under Schotten–Baumann<sup>10</sup> conditions to give the corresponding amides in very good yield. These amides were treated with formaldehyde and hexamethyleneimine in refluxing ethanol to give the Mannich products **13** and **14**. 4-Hydroxynorephedrine hydrochloride was condensed with phenol in 6 N HCl at 100 °C to give the bis-phenol **15** in 58% yield. The amine was then acylated with benzoyl chloride under Schotten-Baumann conditions to give the corresponding amide in 77% yield. This amide was treated with formaldehyde and hexamethyleneimine in refluxing ethanol to give the Mannich product **7**, **8**, **10**, **13**, **14**, and **16** were all converted to their oxalate salts prior to testing.





These bis-phenols were evaluated in the IMR-32 assay for potencies in blocking N-type calcium channels. Those compounds showing good activity in the IMR-32 assay were then evaluated for selectivity of blocking N-type calcium channels over L-type channels (GH-3 rat pituitary cells), Na<sup>+</sup> and K<sup>+</sup> channels (rat SCG neurons). Compared to PD0032577, compounds **7**, **8**, and **10** all had similiar activity in the IMR-32 assay when the alkyl or amino alkyl side chain was introduced into the molecule. However, compound **6**, while having the diphenyl side chain, was less active in the IMR-32 assay because of a missing hexamethyleneimine methyl group. When no amino methyl groups were present, the analog **5** was completely

COMPOUND		IMR-32 IC <sub>50</sub> μΜ	GH-3 IC <sub>50</sub> μΜ	SCG-Na <sup>+</sup> (10 µM)	SCG-К⁺ (10 µМ)
PD 32577	CNDLCN	0.97	2.0	81%	77%
5	HO CH COH	-6%±11% @ 10 μΜ			
6	CN CH	1.8			
7	N HO N HO N Ph	0.49	0.9	78% ± 8.3	61% ± 6.7
8	HO N Ph Ph	0.74	9.1	5% ± 2.9	18% ±5.2
10	CN LL LL N NBn2	0.43	4.1	24% ± 5.8	28% ± 5.7
13	HO N N H H Ph	17.0			
14	$ \begin{array}{c} HO \\ N \\ H \\ H \\ H \\ Ph \end{array} \begin{array}{c} O \\ H \\ Ph \end{array} $	1.8			
16	$ ( \begin{array}{c} HO \\ N \end{array} ) ( \begin{array}{c} O \\ ) ( \begin{array}{c} O \\ N \end{array} ) ( \begin{array}{c}$	14.0			

inactive. Replacing the alkyl diphenyl or amino dibenzyl side chain with an amide side chain resulted in a loss of activity. While 13, 14, and 16 were all less active than PD0032577, 14 was tenfold more potent than either 13 or 16. This can be attributed to the presence of the two phenyl rings in the amide side chain compared to only one phenyl ring in the amide for 13 and 16.

Among compounds 7, 8, and 10, both 8 and 10 showed selectivity for N-type channels over L-type channels,  $Na^+$  and  $K^+$  channels. And while 8 and 10 were selective, 8 demonstrated better selectivity for N-type channels over other ion channels. Thus, a diphenyl alkyl side chain containing no functionality is slightly better than having an amino group in the side chain for N-type channel selectivity. Both of these side chains are better than a side chain containing a hydroxyl group for selectivity.

In summary, a series of bis-(4-hydroxyphenyl)-methane based N-type calcium channel blockers incorporating two amino methyl moieties and various substituted alkyl side chains was discovered. Some of these compounds were potent and in situ active in the IMR-32 assay. Compound **10**, possessing a dibenzylamine alkyl side chain, and compound **8**, possessing a diphenyl alkyl side chain, were of similar activity relative to PD0032577 in the IMR-32 assay. However, both **8** and **10** were selective for N-type calcium channels over other ion channels. Compound **8** demonstrated better selectivity for N-type calcium channels than did **10**. Thus, **8** and **10** are an improvement over PD0032577.

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## **References and Notes**

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- 7. Na<sup>+</sup> and K<sup>+</sup> channel currents were measured using the whole-cell voltage-clamp variation of the patch clamp technique using rat superior cervical ganglion (SCG) neurons isolated and cultured according to the methods outlined in Stoehr, S. J.; Campbell, G. W.; Rock, D. M. *Drug Dev. Res.* **1997**, *41*, 85.
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