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Design and Biological Evaluation of Non-Peptide Analogues of Omega-Conotoxin MVIIA

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Abstract—Omega-conotoxin MVIIA, a highly potent antagonist of the N-type voltage sensitive calcium channel, has shown utility in several models of pain and ischemia. We report a series of three alkylphenyl ether based analogues which mimic three key amino acids of the toxin. Two of the compounds have been found to exhibit IC_{50} values of 2.7 and 3.3 μM at the human N-type voltage sensitive calcium channel.

ω-Conotoxin MVIIA, a 25 amino acid peptide from the venom of the marine cone snail *Conus magus*,[1,2] is a highly potent and selective blocker of the N-type voltage sensitive calcium channel (VSCC).[3] It has a binding affinity of <10 nM for the N-type VSCC, compared to >1 μM for the L-type VSCC.[4] N-type VSCCs are found exclusively in neurons where they regulate intracellular calcium concentration, which affects various important neural functions such as neuronal excitability, neurotransmitter release and neurosecretory activity.[5–8] Antagonists of N-type VSCCs would, therefore, offer great potential for therapeutic intervention.[9] The synthetic version of ω-conotoxin MVIIA, Ziconotide®, has demonstrated efficacy in animal models of traumatic brain injury, focal cerebral ischemia and pain.[10–13] For severe neuropathic pain, it is more potent than morphine and chronic administration does not elicit tolerance or lead to addiction.[14] Ziconotide® is currently in clinical trials for the treatment of pain.

Encouraged by the clinical utility of ω-conotoxin MVIIA, we were interested in the possibility to produce low molecular weight mimetics of this peptide. Lead generation has been sought by a high volume screening strategy.[15–20] In this paper we disclose a ligand based approach, because the three-dimensional solution structure of the toxin is available from the Brookhaven Protein Data Bank.[21] Several of the key amino acids responsible for the binding of the toxin to the channel pores have been determined to be Lys-2, Arg-10, Leu-11, Tyr-13 and Arg-21.[22] As the middle segment of the peptide chain also plays a role in determining the selectivity for the N-type VSCC,[22,23] we focused our attention on the three central amino acids Nos. 10, 11 and 13 (Fig. 1).

Following our 'dendroid approach',[24] side chain mimetics of the three selected amino acids were attached to a dendritic scaffold, which is composed of aromatic branching units and adjustable linkers. Ether linkages were preferred over ester or amide bonds since they are expected to be more stable under physiological conditions. These molecules possess a high degree of conformational flexibility. In contrast to rigid molecules, the 'dendroid motif' allows each pharmacophoric group to find the preferred conformation for receptor binding independently. We argue that this facilitates the search for a global energy minimum of the receptor–ligand complex.

Our starting point for this design strategy was the previously reported analogue **1** (Fig. 1).[25] The activity of **1** at the N-type VSCC was evaluated in the IMR-32 assay.[26] This parent template had the necessary spatial requirements, but proved virtually inactive (19% inhibition at 10 μM, triplicate determination). In an attempt to reduce flexibility, we shortened the linkers between the branching units and the pharmacophoric groups. This modification led to the closely related analogues **2** and **3**,[27] the synthesis of which is outlined in Scheme 1.

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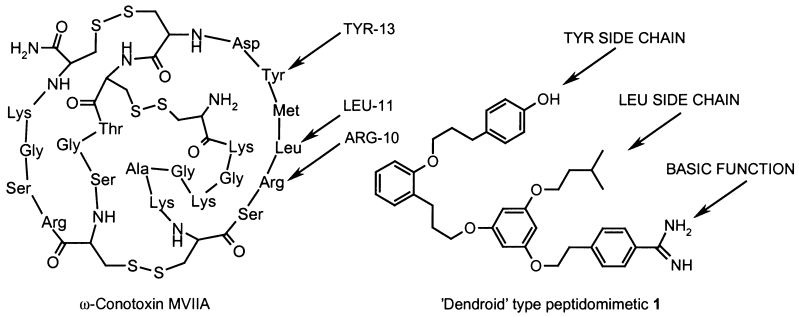


Figure 1. Peptidomimetic design rationale.

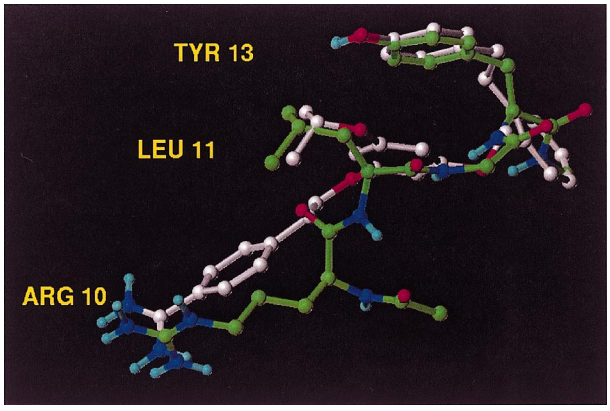
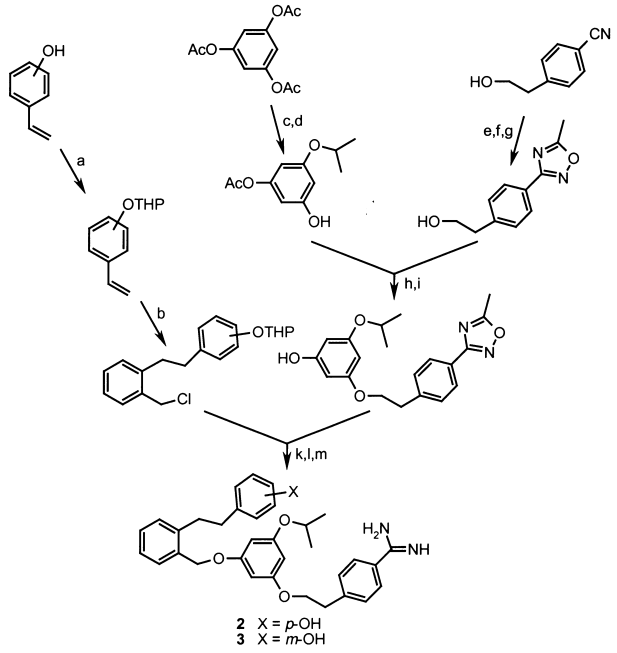


Figure 2. Overlay of **2** (grey) with three key amino acids of ω-conotoxin MVIIA (green).

Table 1. IC_{50} values for blocking N-type (IMR-32 assay) and L-type (A7r5-assay) VSCCs

Compounds	IMR-32 IC_{50} (μM)	A7r5 IC_{50} (μM)
1	19% at 10 μM	69% at 10 μM
2	3.3 ± 0.3 ($n = 3$)	4.2 ($n = 1$)
3	2.7 ± 0.9 ($n = 3$)	3.0 ($n = 1$)



Scheme 1. Reagents and conditions: (a) 3,4-Dihydro-2*H*-pyran/*p*-TsOH/EtOAc/0 °C-rt/1 h, 68–78%; (b) 9-BBN/THF, then 2-iodobenzyl chloride/K_3PO_4/Pd(PPh_3)$_4$/dioxane/60 °C/2 h, 46–70%; (c) 2-iodopropane/NaH/H_2O/DMF/0 °C-rt/15 h, 43%; (d) NaOMe/MeOH/rt/1 h, 60%; (e) NH_2OH/MeOH/rt/24 h, 57%; (f) Ac_2O/120 °C/4 h, 82%; (g) NaOMe/MeOH/rt/30 min, 94%; (h) PPh_3/DIAD/THF/0 °C-rt/2 h, 86%; (i) NaOMe/MeOH/rt/1 h, 85%; (k) NaH/DMF/0 °C-rt/2 h, 88–89%; (l) Dowex 50WX2-400/MeOH-PhMe 5:1/1 h, 88–93%; m) Ra-Ni/4 bar H_2/EtOH/rt/5 h, then EtOH:AcOH-H_2O 20:1:1 12 h, 40–46%.

In the IMR-32 assay, **2** and **3** were found to be approximately equipotent (IC_{50} 3.3 and 2.7 μM, respectively, see Table 1). Obviously, the position of the phenolic hydroxyl group is not crucial. Ongoing SAR studies will reveal if this site is amenable to further modification or even truncation.

The design of **2** is supported by molecular modelling. Figure 2 shows an overlay of **2** and the three key amino acid side chains of ω-conotoxin MVIIA. The overlay was generated with GASP 2.05 on a Silicon Graphics Octane workstation running IRIX 6.5.3.[28] Structure **2** was built and energetically optimised using the standard SYBYL 6.5 force field.[29] The middle segment of the toxin was used as a rigid template. As depicted in Figure 2, the pharmacophoric groups of **2** and the amino acid side chains of the toxin overlap with high stringency.

To assess the selectivity for the N-type VSCC, **1**–**3** were also tested for functional activity at the L-type VSCC in A7r5 rat vascular smooth muscle cells (Table 1).[30,31] Analogue **1** gave 69% inhibition at 10 μM; **2** and **3** were able to antagonise the L-channel with IC_{50} values of 4.2 and 3.0 μM, respectively.

In summary, a series of three small-molecule mimetics of ω-conotoxin MVIIA has been synthesised. Two compounds possess micromolar activity in blocking N-type VSCCs in the IMR-32 assay and L-type VSCCs in the A7r5-assay. They are interesting leads for the development of novel antinociceptive agents. Further evaluation of the pharmacophoric groups and the dendroid core architecture is in progress.

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27. Compounds **1** (see ref 12), **2** and **3** were fully characterised. Selected data: **2**×**AcOH**: ^{1}H NMR (400 MHz, CD_3OD) δ 1.34 (d, J = 6.1 Hz, 6 H, $CH(CH_3)_2$), 1.98 (s, 3H, CH_3COO^-), 2.88 (m, 2H, Ar-CH_2-CH_2-C_6H_4OH), 2.96 (m, 2 H, Ar-CH_2-CH_2-C_6H_4OH), 3.23 (t, J = 6.1 Hz, 2 H, Ar-O-CH_2-CH_2-Ar), 4.26 (t, J = 6.1 Hz, 2H, Ar-O-CH_2-CH_2-Ar), 4.58 (m, 1 H, $CH(CH_3)_2$), 4.95 (s, 2 H, Ar-CH_2-O-Ar), 6.13–6.21 (m, 3H, arom.), 6.70–7.83 (m, 12H, arom.). ^{13}C NMR (100 MHz, CD_3OD) δ 180 (CH_3COO^-), 168 ($C(NH_2)_2$), 162 (2 C), 161, 156 (C-OH), 147, 142, 136, 134, 131 (4 C), 130 (3 C), 129 (2 C), 127, 116 (3 C), 97 (2 C), 95, 71, 70, 69, 38, 36 (2 C), 24 (CH_3COO^-), 22 (2 C, $CH(CH_3)_2$). HRMS (FAB) calc. for $[M+H]^+$ 525.2753, found 525.2737. Analysis calcd for $C_{35}H_{40}O_6N_2$: C 71.90; H 6.90; N 4.79. Found: C 71.47; H 6.86; N 4.74. **3xAcOH**: ^{1}H NMR (400 Mhz, CD_3OD) δ 1.25 (d, J = 6.1 Hz, 6 H, $CH(CH_3)_2$), 1.90 (s, 3 H, CH_3COO^-), 2.83 (m, 2 H, Ar-CH_2-CH_2-C_6H_4OH), 2.93 (m, 2 H, Ar-CH_2-CH_2-C_6H_4OH), 3.15 (t, J = 6.1 Hz, 2 H, Ar-O-CH_2-CH_2-Ar), 4.18 (t, J = 6.1 Hz, 2 H, Ar-O-CH_2-CH_2-Ar), 4.48 (m, 1 H, $CH(CH_3)_2$), 4.87 (m, 2 H, Ar-CH_2-O-Ar), 6.04–6.12 (m, 3 H, arom), 6.56–7.74 (m, 12 H, arom). ^{13}C NMR (100 MHz, CD_3OD) δ 180 (CH_3COO^-), 168 ($C(NH_2)_2$), 162 (2 C), 161, 158 (C-OH), 148, 144, 142, 136, 131 (3 C), 130 (3 C), 129 (2 C), 128,127, 121, 116, 114, 97 (2 C), 95, 71, 70, 69, 38, 36 (2 C), 24 (CH_3COO^-), 22 (2 C, $CH(CH_3)_2$). HRMS (ES) calcd for $[M+H]^+$ 525.2753, found 525.2751. Analysis calcd for $C_{35}H_{40}O_6N_2 \cdot H_2O$: C 69.75; H 7.02; N 4.65. Found: C 69.73; H 6.95; N 4.63.
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