Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1813–1818

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

MULTIPLE PARALLEL SYNTHESIS OF *N,N*-DIALKYLDIPEPTIDYLAMINES AS N-TYPE CALCIUM CHANNEL BLOCKERS

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Received 17 March 1999; accepted 13 May 1999

Abstract: Selective N-type Voltage Sensitive Calcium Channel (VSCC) blockers have shown utility in several models of stroke and pain. A series of *N,N*-dialkyldipeptidylamines with potent functional activity at N-type VSCC's has been identified. Multiple parallel synthesis of a focused array of thirty compounds using polymer-supported quenching reagents and preliminary pharmacology are presented. Eighteen compounds were identified with an IC_{50} below 1 μM in an in vitro functional assay.

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 regulate the calcium flux involved in depolarization-evoked release of neurotransmitter, and thus 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 excessive influx of calcium following an ischemic or traumatic event is associated with neuronal injury.[1]

ω-Conotoxin MVIIA, a 25 amino acid-residue containing peptide found in the venom of a piscivorous marine snail (*Conus Magnus*), is a potent and selective N-type VSCC blocker.[2] The synthetic equivalent, SNX-111, has demonstrated utility in animal models of traumatic brain injury, focal cerebral ischemia, and pain.[2] SNX-111 is currently in clinical trials for the treatment of chronic pain.

As part of our efforts to develop small molecule N-type VSCC antagonists, PD 151307 was identified as a chemical lead by high-volume screening of the Parke-Davis compound library.[3] In the process of exploring the SAR of this compound, we discovered a novel series of *N,N*-dialkyldipeptidylamines, such as PD 173212, and found that they are active in vitro in the IMR-32 assay, and efficacious in vivo in an audiogenic seizure

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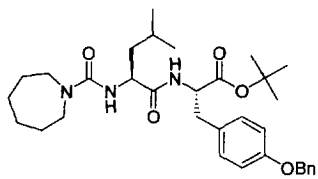
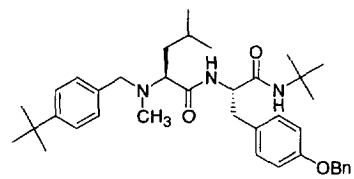
model using DBA/2 mice.[4] In this paper, we describe exploration of the SAR at both the N- and C-termini in the *N,N*-dialkyldipeptidylamine series through synthesis of a focused array of analogs using polymer-supported quenching reagents.



PD 151307

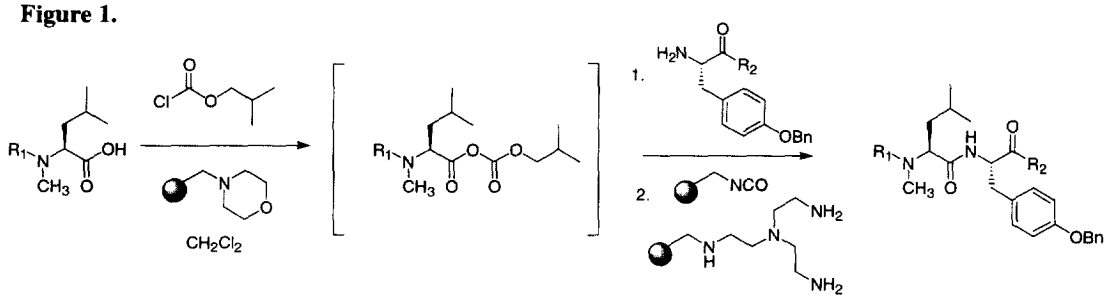


PD 173212

Chemistry

As shown in Figure 1, our synthetic strategy[5] involved coupling of an *N,N*-disubstituted leucine acid with a tyrosine amine.[6] Thus, the acid was dissolved in CH_2Cl_2 and activated with isobutyl chloroformate in the presence of a polymer-supported morpholine base,[7] followed by addition of the amine. After shaking the reaction in a capped vial, excess starting materials were captured by the addition of polymer-supported polyamine and isocyanate resins.[7] Filtration and evaporation of the mixture under a stream of dry N_2 afforded the final products in 60–95% yield and 80–98% purity.[8]

Figure 1.

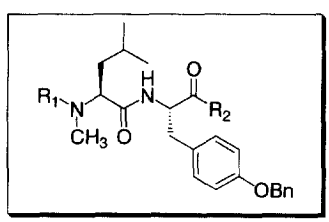


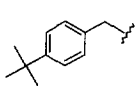
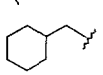
We chose five of the most potent N-substituents from our previous work[4] in this series to combine with five new C-terminal amide modifications. The C-terminal modifications were chosen for high potency or solubility based on the initial exploration of the SAR in the PD 151307 series.[3] R_1-groups chosen for the N-terminus included 4-*tert*-butylbenzyl (**1**), cyclohexylmethyl (**2**), 3-methylbutyl (**3**), cyclohexyl (**4**), and methyl (**5**). The C-terminal amide R_2-groups consisted of *tert*-butylamino (**a**), piperidinyl (**b**), *N*-benzylpiperazinyl (**c**), 4-amino-*N*-benzylpiperidinyl (**d**), 1-aminoethylpiperidinyl (**e**), and *N*-methylpiperazinyl (**f**). The *tert*-butyl amide analogs (**1a–5a**) were synthesized previously[4] by traditional synthetic procedures and were included in the array as a control.

Results

The IMR-32 in vitro results are shown in Table 1.[9,10] The most potent compound was the previously reported **1a**, substituted with a 4-*tert*-butylbenzyl group at the N-terminus and a *tert*-butyl amide at the C-terminus (IC_{50} = 0.04 μM), while the most potent novel analog was the corresponding C-terminal piperidinyl amide **1b** (IC_{50} = 0.09 μM). The least potent compound was **5f**, substituted with a methyl group at the N-terminus and an *N*-methylpiperazinyl amide at the C-terminus (IC_{50} = 13 μM). Compounds **1a**, **1c**, **1d**, **2a**, **3a**, **4a**, and **5a** were also prepared by traditional synthesis and purified to microanalytical purity as reported previously.[4] These lots were found to give identical IMR-32 IC_{50}'s when compared with the array compounds. The *N,N*-dialkyl leucine acid and tyrosine amine starting materials were tested in the IMR-32 assay for their N-type VSCC activity and found to be only weakly active.[10] In the array, the range of potencies between the most and least potent analogs was 325-fold. However, twenty three of the thirty analogs gave IC_{50}'s within the rather narrow 10-fold range of 0.2 μM to 2.0 μM. Despite the relatively flat SAR, trends were evident at both sites of variation in the array.

Table 1. IC_{50} in IMR-32 Assay (μM)



R_1	R_2 a.	b.	c.	d.	e.	f.
1. 	0.04	0.09	0.50	0.27	0.31	0.44
2. 	0.50	0.32	0.42	0.31	1.0	1.2
3. 	0.32	0.66	0. 59	0.88	1.1	1.2
4. 	0.20	0.38	0.76	1.3	0.88	5.6
5. H_3C	2.8	1.9	1.2	2.6	4.1	13

At the N-terminus, the overall rank of substituents in order of decreasing potency was: 4-*tert*-butylbenzyl > cyclohexyl ≅ 3-methylbutyl ≅ cyclohexylmethyl > methyl. The array demonstrated a general trend in which IMR-32 potency was correlated with the size of the alkyl group at this position. For example, the 4-*tert*-butylbenzyl group was both the largest and most potent substituent at the N-terminus, while the methyl group was the smallest and least potent substituent at this position. The cyclohexyl, 3-methylbutyl, and cyclohexylmethyl groups are of similar, more moderate size and exhibited similar potencies in between these two extreme cases. This trend was generally consistent across the different C-terminal modifications, with the exception of minor variations in relative potencies between the different amides.

At the C-terminus, the SAR trend was less well-defined and less consistent across the various N-terminal modifications. The *tert*-butyl and piperidinyl amide side chains both lack an ionizable nitrogen and were among the more potent substituents at this position. The other substituents all contain a basic nitrogen, and in these cases the potency correlated roughly with the size of the amide group. For example, the benzyl-substituted piperazinyl and 4-aminopiperidinyl amides were generally more potent than the smaller 1-aminoethylpiperidinyl and *N*-methylpiperazinyl amides.

Conclusions

In summary, we have prepared a focused array of thirty *N,N*-dialkyldipeptidylamine analogs using polymer-supported quenching reagents. Eighteen compounds had an IC_{50} below 1 μM in the IMR-32 assay for N-type calcium channel activity. The most potent analog in the array was the previously reported compound **1a**, while the most potent novel analog was **1b**. Our results suggest that replacement of the *tert*-butyl amide group at the C-terminus with other substituents is tolerated, and that the potency of analogs for a given C-terminal amide is generally correlated with the size of the alkyl group at the N-terminus.

Acknowledgements: We thank Wayne Yue for HPLC analyses, Don Johnson and Norm Colbry for assistance in preparation of the *N,N*-disubstituted leucine acid starting materials, and John Booth and Bruce Steinbaugh for polymer-supported quenching reagents and helpful discussions.

References and Notes

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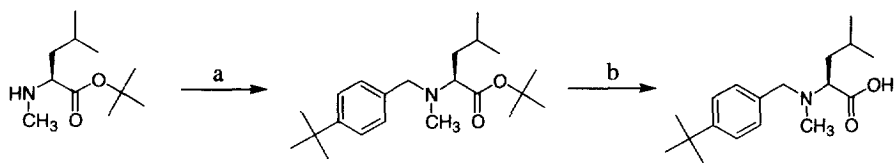
5. The *N,N*-dialkyl leucine acid (0.1 mmol) was placed in a vial and treated with CH_2Cl_2 (1.5 mL), polymer-supported morpholine resin (150 mg, 3.6 mmol/g), and isobutylchloroformate (19 μL, 0.14 mmol). The reaction was shaken for 30 min, then treated with a solution of the tyrosine amine (0.1 mmol) in CH_2Cl_2 (1 mL). The reaction was shaken for 2 h, then treated with polymer-supported amine resin (150 mg, 1.5 mmol/g) and polymer-supported isocyanate resin (150 mg, 1.5 mmol/g), and shaken for 3 h. The solids were filtered away through a plug of glass wool and rinsed with CH_2Cl_2. The solvent was evaporated under stream of dry N_2 to give the desired product.

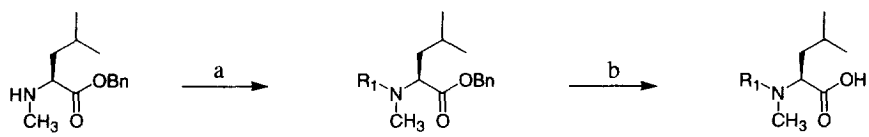
6. The tyrosine amine starting materials (**a–f**) were prepared as described previously.[4] The *N,N*-dialkyl leucine acids (**1–5**) were prepared according to the following procedures:

(I) For *N,N*-dialkyl leucine acid **1**:



(a) (i) 4-*tert*-buytlbenzaldehyde, CH_2Cl_2, (ii) $NaBH(OAc)_3$; (b) TFA, CH_2Cl_2

(II) For *N,N*-dialkyl leucine acids **2–4**:



(a) (i) R_1CHO, CH_2Cl_2, (ii) $NaBH(OAc)_3$; (b) H_2, Pd/C, THF

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8. (a) All final products were >80% pure by HPLC analysis. ^{1}HNMR and APCI–MS spectra were consistent with proposed structures.
Example: **3a**. APCI-MS: 524.0 (M+ for $C_{32}H_{49}N_3O_3$); sticky solid; TLC: R_f 0.53 (10% MeOH/CH_2Cl_2), HPLC: 92.46%, 6.172 min (C-18 column, linear gradient of 20% CH_3CN/80% pH 3 phosphate buffer to 70% CH_3CN/30% pH 3 phosphate buffer over 5 min, then isocratic elution).
^{1}HNMR ($CDCl_3$) ∂ 0.82 (d, 6 H, 5.9 Hz), 1.17 (s, 9 H), 1.21–1.29 (m, 3 H), 1.48 (p, 2 H, J = 6.3 Hz), 1.54 (s, 3 H), 1.60 (dd, 1 H, J = 12.9 6.1 Hz), 2.02 (s, 3 H), 2.33 (dd, 2 H, J = 13.2, 6.6 Hz), 2.86 (dd, 1 H, J = 13.9, 8.1 Hz), 2.94–3.00 (m, 2 H), 4.34 (dd, 1 H, J = 15.1, 7.6 Hz), 5.00 (s, 2 H), 5.46 (s, 1 H), 6.86 (d, 2 H, J = 8.3 Hz), 7.10 (d, 2 H, J = 8.3 Hz), 7.26–7.39 (m, 4 H), 7.49 (d, 1 H, J = 8.1 Hz).

9. 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.

10. In the IMR-32 assay, the *N,N*-dialkyl leucine acid starting materials (**1–5**) exhibited <25% blockade at 10 μM; the tyrosine amine starting materials (**a–f**) exhibited 11–67% blockade at 10 μM.