Bioorganic & Medicinal Chemistry Letters, Vol.3, No.6, pp. 1035-1040, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

STABLE ISOSTERES OF NEUROTENSIN C-TERMINAL PENTAPEPTIDES DERIVED BY MODIFICATION OF THE AMIDE FUNCTION

Thomas E. Christos,* Argyrios Arvanitis, Gary A. Cain, Alexander L. Johnson,
Richard S. Pottorf, S. William Tam , William K. Schmidt
The Dupont Merck Pharmaceutical Company, P.O. Box 80353,
Wilmington, Delaware 19880-0353

(*Received in USA* 13 *October* 1992)

Abstract: A series of amide bond modified neurotensin c-terminal pentapeptides has been prepared and tested for their *in vivo* analgesic properties. Reduced amide function and *trans* double bond isosteres did show analgesic activity.

Neurotensin (NT) is a thirteen amino acid peptide (pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu) originally isolated by Carraway and Leeman[1] from bovine hypothalami. It has since been shown to exhibit hypothermic,[2] psychotropic,[3] and analgesic[4-5] properties. As an analgesic, its potency is reported to be greater than that of morphine[6] but only when injected directly into the CNS (i.c.v.). The usefulness of neurotensin as a pharmaceutical agent is limited due to absorption and metabolic problems inherent to peptides.[7] Much of the research around this tridecapeptide has been directed towards overcoming these problems.

It is now known that the full thirteen amino acid sequence is not required for the biological activities observed.[5,8,9] Through systematic removal of amino acid residues from the N-terminal end of the peptide, Furata and Nicolaides were able to determine that only the pentapeptide NT (8-13) was necessary for analgesia. This pentapeptide, which retains essentially the same potency as NT, represents an important lead from which a new generation of analgesics could be developed. Our approach included systematic removal and replacement of native amino acid residues, incorporation of turn mimics and amide bond stabilization. The subject of this communication is isosteric amide bond replacements.

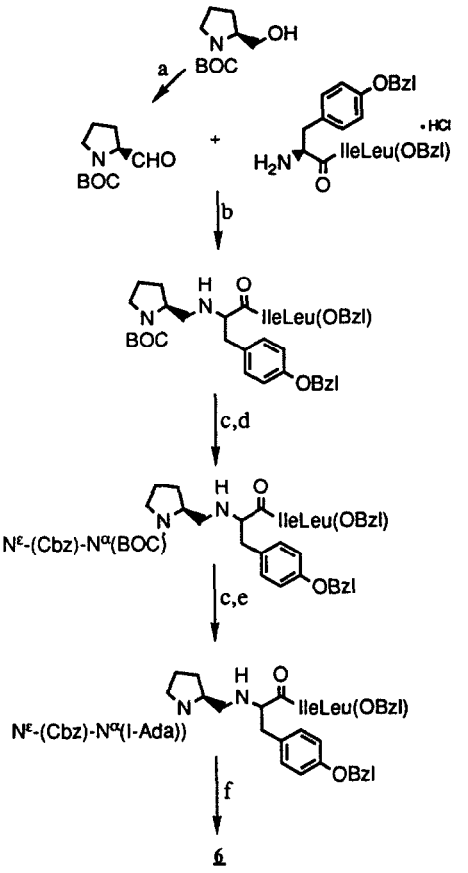
Through our early efforts, we were able to identify systemically active (i.v. administration) analogs of NT(9-13).[10] They included N^{α}(BOC)[Orn^{9}]NT(9-13) (**2**), N^{α}(Adamantanecarbonyl)[Lys^{9}]NT(9-13) (**3**), and N^{α}(BOC)[Lys^{9}]NT(9-13) (**4**), all of which showed an increased duration of action compared to [Lys^{9}]NT(9-13) (**1**), when administed i.c.v. These derivatives served as templates for bond stabilized peptides. We then directed our attention to the synthesis and pharmacological evaluation of reduced amide bond and trans double bond isosteres.

Reduced Amide Bond Isosteres

The general method used for preparing reduced amide analogs was through a reductive amination of a protected aminoaldehyde and the appropriately protected amino acid. The aminoaldehyde could be prepared from the corresponding chiral N-BOC-amino alcohol using the

complex as the oxidant[11] and immediate coupling of the crude aldehyde to the dipeptide H-IleLeu(OBzl). HPLC analysis of the resulting tripeptide showed no evidence of racemization. The target pentapeptides were subseqently prepared through standard solution phase methods[12] as exemplified by the synthesis of N^{α}(1-adamantanecarbonyl)LysProΨ[CH_2NH]TyrIleLeu (**6**), (scheme I).

Scheme I



(a) oxalyl chloride, dimethylsulfoxide, triethylamine, CH_2Cl_2, -78° to RT;
(b)2% AcOH/DMF, NaOAc, $NaBH_3CN$, RT; (c) 4 M HCl in dioxane, RT;
(d) N^{ε}-(Cbz)-N^{α}-BOC-Lys, isobutyl chloroformate, N-methylmorpholine, THF, DMF, -15° to RT; (e) 1-adamantanecarbonyl chloride, triethylamine, CH_2Cl_2, RT; (f) 20% $Pd(OH)_2$/C, cyclohexene, ethanol, AcOH, reflux.

***Trans* Double Bond Isostere**

Preparation of the pentapeptide with the amide bond between residues 9 and 10 replaced by a *trans* double bond was accomplished via a method described by Wang, *et al.*[13] starting from BOC protected l-proline (see scheme II). Grignard addition followed by reduction lead to the chiral vinyl alcohol **8** in 36% yield. Protection of the alcohol **8** as its tetrahydropyranyl ether **9** followed by ozonolysis furnished the aldehyde which upon treatment with trimethyl phosphonoacetate provided the *trans* -α,β-unsaturated ester **10**. Removal of the tetrahydropyran protecting group, followed by conversion of the resulting alcohol to the mesylate and exposure of the latter to p-benzyloxy-benzyl grignard yielded the fully protected dipeptide isostere **11**. Ester cleavage using lithium hydroxide in dioxane/water followed by mixed anhydride coupling with Ile-Leu(OBzl) provided the bond modified tetrapeptide **12** in 65% yield. Elaboration of the N- terminus was accomplished following BOC deprotection and DCC/HOBT coupling with N^{α}(BOC)N^{ε}(CBZ)Lys to give **13**. The desired bond modified pentapeptide **14** was prepared through final deprotection using 20% $Pd(OH)_2$ on carbon in ethanol, cyclohexene, and acetic acid. The fully elaborated pentapeptides were characterized using FAB-mass spectrometry and were shown to be homogeneous by both TLC and reverse-phase HPLC.

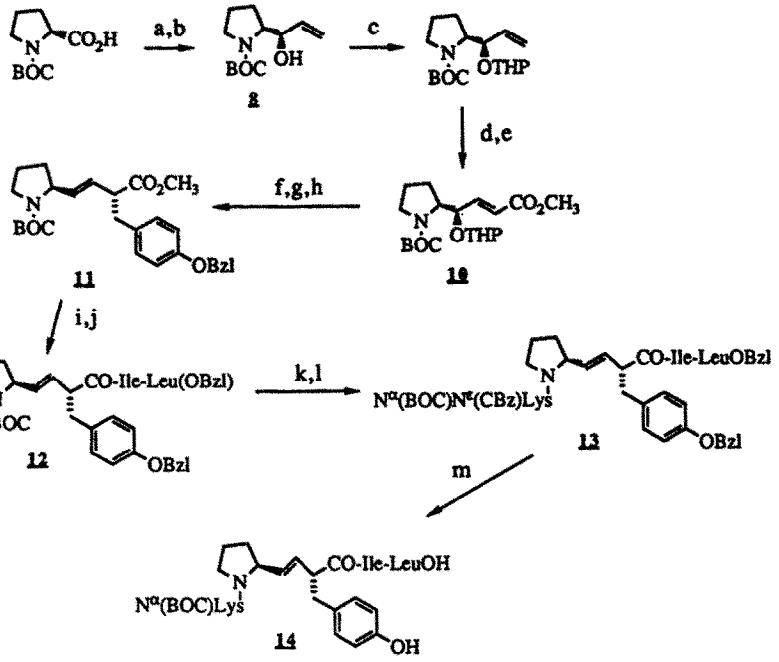
Pharmacology and Results

The compounds described in table 1 were evaluated in *in vitro* as well as *in vivo* assays. Neurotensin receptor binding affinity was determined by their ability to displace [^{3}H] neurotensin.[14] Antinociceptive efficacy was determined using the phenylquinone writhing assay[15] with initial i.c.v. administration. Those compounds showing sufficient potency were then retested using i.v. administration.

The modified pentapeptides were compared to the three model N^{α}acyl compounds **2,3** and **4** along with NT[Lys^9](9-13). Bond stabilization was restricted primarily to the Pro-Tyr and Tyr-Ile bonds. Example **15** is the only compound prepared with a modification of another bond.

An obvious conclusion is that NT binding is significantly reduced in all examples of bond modification. *In vivo* results were also affected adversely, however not as drastically. Compounds **6, 17** and **14**, while not as potent in the mouse PQW assay as their parent compounds (**3, 2** and **4** respectively), did show i.v. and i.c.v activity. When comparing compounds **6** with **7** and **17** with **9**, it can be concluded that replacement of the Pro-Tyr bond is better tolerated in terms of *in vivo* analgesia than replacement of the Tyr-Ile bond. Additionally, analgesic potency for the examples described in this work do not strictly follow binding affinities. This discrepancy could be due to increased stability toward peptidase for lower affinity modified peptides allowing more compound to reach the receptor. Similar bond repacement specificity has been observed in studies involving neurokinin A[16] and gastrin[17] with regard to their receptor binding affinities.

Scheme II



(a) nBuLi, THF, -78°; vinylMgBr -78° to 25°, (b) $NaBH_4$, $CeCl_3.7H_2O$, MeOH, -78° (36% yield); (c) THP, PPTS, CH_2Cl_2 ; (d) O_3, pyridine, CH_2Cl_2, -78°; Me_2S; (e) $(Me_2O)_2POCH_2CO_2Me$, NaH, THF, -78° to 0°; (f) MeOH, CSA, (g) MsCl, $(iPr)_2NEt$, CH_2Cl_2 0°; (h) p-BzlO$C_6H_4CH_2MgCl$, CuCN, THF, -40°; (i) LiOH dioxane/H_2O; (j) $ClCO_2iBu$, N-methylmorpholine; HCl Ile-LeuOBzl, THF/DMF, -10°; (k) Dioxane/H_2O, HCl (l) DCC, N-methylmorpholine, 1-hydroxybenzo-triazole, (N^{ε}BOC)(N^{ε}CBz)Lys.HCl, DMF; (m) 20%$Pd(OH)_2$/C 25%w/w, EtOH/cyclohexene 2.1, AcOH, reflux

Table1. Biological activiy of bond modified neurotensin pentapeptides

		NT binding	Mouse POW	
no.	Compound	Ki (nM)	i.c.v. ug/mouse	i.v. mg/kg
1	[Lys^9]NT(9-13)	688	5.9	>81
2	Nα(BOC)OrnProTyrIleLeu	82	0.00097	0.74
3	Nα(1-AdCO)LysProTyrIleLeu	117	0.15	2.2
4	Nα(BOC)LysProTyrIleLeu	63	0.018	3
6	Nα(1-AdCO)LysProΨ[CH_2NH]TyrIleLeu	1607	4.5	20
7	Nα(1-AdCO)LysProTyrΨ[CH_2NH]IleLeu	5958	>50	ND
17	Nα(BOC)OrnProΨ[CH_2NH]TyrIleLeu	2985	0.89	37
9	Nα(BOC)OrnProTyrΨ[CH_2NH]IleLeu	>10,000	29	ND
14	Nα(BOC)LysProΨ[trans CH=CH]TyrIleLeu	1626	22	>27
15	Nα(1-AdCO)LysΨ[CH_2NH]ProTyrIleLeu	3857	>50	ND

ND = No Data, 1-AdCO = 1-Adamantanecarbonyl

Although none of the compounds presented here showed oral bioavailability, it is obvious that some bond manipulation is possible while retaining analgesic properties. Continued changes to bond type and location will provide further insight into a general structure activity relationship of these analgesic pentapeptides.

Acknowledgment.

We would like to thank the Du Pont Merck Central Nervous System pharmacologists for providing the biological data. The author would also like to thank Dr's Parthasarathi Rajagopalan and Robert J. Chorvat for their help in the preparation of the manuscript.

References and Footnotes

(1) Carraway, R.; Leeman, S. The Isolation of a New Hypotensive Peptide, Neurotensin, from Bovine Hypothalami. *J. Biol. Chem.* **1973**, *248*, 6854-6861.

(2) Rivier, J.E.; Lazarus,L.H.; Perrin, M.H.; Brown, M.R. Neurotensin Analogs. Structure-Activity Relationships. *J. Med. Chem.* **1977**, *20*, 1409-1412.

(3) Nemeroff, C.B. The Interaction of Neurotensin with Dopaminergic Pathways in the Central Nervous System: Basic Neurobiology and Implications for the Pathogenesis and Treatment of Schizophrenia *Psychoneuroendochrinology* **1986**, *11*, 15-37.

(4) Clineschmidt, B.V.; McGuffin, J.C. Neurotensin: Antinociponsive Actions in Rodents. *Eur. J. Pharmacol.* **1979**, *54*, 129-139.

(5) Nicolaides, E.D.; Lunney, E.A.; Kaltenbronn, J.S.; Wiley, J.N.; Downs, D.A. Anti-writhing Activity of some Peptides Related to Neurotensin and Tuftsin. *Int. J. Peptide Protein Res.* **1985**, *25*, 435-441.

(6) Goedert, M. Neurotensin-a Staus Report. *Trends in Neurosciences* **1984**, *7*, 3-5.

(7) Lee, V.H.L. Enzymatic Barriers to Peptide and Protein Absorption and the use of Penetration Enhancers to Modify Absorption. In *Delivery Systems for Peptides Drugs*; Davis, S.S., Illum, L., Tomlinson, E., Eds.; Plenum Press: New York **1986**; pp 87-104.

(8) Carraway, R.; Leeman, S.E. Structural Requirements for Biological Activity of Neurotensin, a New Vasoactive Peptide. In *Peptides: Chemistry, Structure, and Biology*; Walter, R., Meienhofer, J., Eds.; Ann Arbor Science: Ann Arbor, MI, **1975**; pp 679-685.

(9) Furuta, S.; Kisara, K.; Sakurada, S.; Sakurada, T.; Sasaki, Y.; Suzuki, K. Structure-antinociceptive Activity Studies with Neurotensin. *Br. J. Pharmac.* **1984**, *83*, 43-48.

(10) Cain, Gary A. *et al.*, Manuscript submitted for publication in J. Med. Chem.

(11) Hamada, Y.; Shioiri, T. New Methods and Reagents in Organic Synthesis; A Practical Method for Preparation of Optically Active N-Protected α–Amino Aldehydes and Peptide Aldehydes. *Chem. Pharm. Bull.* **1982**, *30*, 1921-1924.

(12) Mixed anhydride coupling using isobutyl chloroformate of the appropriate BOC protected amine and benzyl protected -COOH amino acids; BOC deprotection using 4 M HCl in dioxane; benzyl deprotection hydrogenation.

(13) Wang, X.C., Kempf, D.J.; Poster 1990 ACS National meeting Washington D.C.

(14) Neurotensin binding assay procedure: Brain membranes were prepared according to Tam[a]. Neurotensin receptor binding was performed according to Goedert[b]. Radioactivity was determined by liquid scintillation spectrometry. IC_{50}s were calculated from log-logit plots. Apparent K_is were calculated from the equation $K_i=IC_{50}/[1+(L/K_d)]$, where L is the concentration of radioligand and K_d is it's dissociation constant. (a) Tam, S. W. Naloxone-inaccessible σ Receptor in Rat Central Nervous System. *Proc. Natl. Acad. Sci. USA* **1983**, *80*, 6703-6707. (b) Goedert, M.; Pittaway, K.; Williams, B.J.; Emson, P.C. Specific Binding of Tritiated Neurotensin to Rat Brain Membranes: Characterization and Regional Distribution. *Brain Research* **1984**, *304* 71-81.

(15) The phenylquinone writhing analgesic assay was modified from that of Siegmund[a]. Intracerebroventricular injections were made according to Haley and McCormick [b]. Test compounds for i.v. administration were suspended in an aqueous vehicle containing 2% by volume of Tween ® 80. I.V. doses were administered in a volume of 10 ml/kg body weight and are expressed as mg/kg doses. For i.c.v. administration, compounds were dissolved in 100% dimethylsulfoxide. Unilateral i.c.v. doses were injected in a volume of 5 microliters and are expressed as microgram/mouse doses. Test compounds were administered i.c.v. or i.v. to fasted (17-21 hours) male white mice (CF1), 5-15 animals per graded dose. After 5 minutes, aqueous 0.01% phenyl-p-benzoquinone, 0.125 mg/kg, was injected intraperitoneally. After the additional time indicated in the table, mice were observed for 10 minutes for the charateristic stretching or writhing syndrome which is indicative of pain produced by the phenylquinone. The effective analgesic dose in 50% of the mice (ED_{50}) was calculated by the moving average method of Thompson[c]. (a) Seigmund, E.; Cadumus, R.; Lu, G. A method for Evaluating both Non-Narcotic and Narcotic Analgesics. *Proc. Soc. Exp. Biol. Med.* **1957**, *95*, 729-731. (b) Haley, T.J.; McCormick, W.G. Pharmacological Effects Produced by Intracerebral Injection of Drugs in the Conscious Mouse. *Br. J. Pharmacol.* **1957**, *12*, 12-15. (c) Thompson, W. R. Use of Moving Averages and Interpolation to Estimate Median-Effective Dose. *Bac. Rev.* **1947**, *11*, 115-145

(16) Harbeson, S.L.; Shatzer, S.A.; Le, T.; Buck, S.H. A New Class of High Affinity Ligands for the Neurokinin A NK_2 Receptor: $\Psi(CH_2NR)$ Reduced Peptide Bond Analogues of Neurokinin A_{4-10}. *J. Med Chem.* **1992**, *35*,3949-3955.

(17) Martinez, J.; Bali, J.; Rodriguez, M.; Castro, B.; Magous, R.; Laur, J.; Lignon, M. Synthesis and Biological Activities of some Pseudo-Peptide Analogues of Tetragastrin: The Importance of the Peptide Backbone. *J. Med. Chem.* **1985**, *28*, 1874-1879.