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REDUCED AMIDE BOND NEUROTENSIN 8-13 MIMETICS WITH POTENT IN VIVO ACTIVITY.

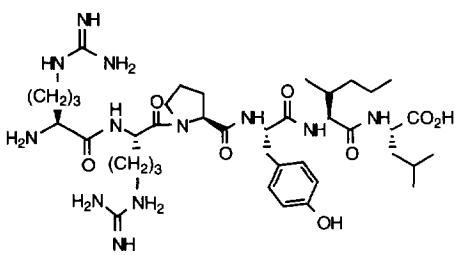
D.J. Wustrow*, M.D. Davis, H.C. Akunne, A.E. Corbin, J.N. Wiley, L.D. Wise, and T.G. Heffner.

Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, Ann Arbor, Michigan 48105

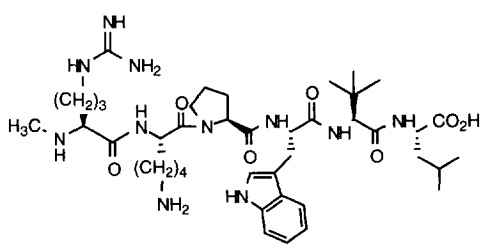
Abstract: Appropriately substituted 8-9 (ΨCH_2NH) isosteres of neurotensin (NT) 8-13 have been found which are active as NT agonists *in vitro* and *in vivo*. SAR studies suggest that preventing amide bond hydrolysis at the 8-9 and 11-12 positions of NT(8-13) mimetics is important for producing compounds with potent activity *in vivo*. Other simplified replacements for the Arg-Arg portion of NT(8-13) are reported.

Evidence gathered from biochemical,[1] behavioral[2] and clinical studies[3] suggest the tridecapeptide neurotensin (NT) may act as an endogenous neuroleptic, indirectly influencing dopaminergic neurotransmission and acting to restore or maintain homeostasis of the dopamine (DA) neurotransmission systems. Since the etiology of schizophrenia almost certainly involves hyperactivity of DA neurons, NT agonists which indirectly attenuate DA neurotransmission, would perhaps have utility as antipsychotic agents.[4] In addition NT has been shown to have antinociceptive effects[5] which do not appear to be modulated by opioid receptors.[6] Most of the biochemical and behavioral effects of neurotensin are also observed with the C-terminal hexapeptide fragment NT(8-13) **1**. However, due to their enzymatic instability,[7] both NT and the native hexapeptide NT(8-13) **1** must be administered directly into the CNS to observe activity.

Recently analogs of NT(8-13) have been prepared which both elucidate factors important for good receptor binding and have increased resistance to enzymatic degradation.[8-11] A modified hexapeptide analog of NT(8-13) **2** (NMeArg-Lys-Pro-Trp-Tle-Leu) good affinity (Ki = 0.64 nM) for the NT receptor, when administered peripherally has behavioral effects in rodents similar to NT administered directly into the ventricals of the brain (ICV).[8] Kitabgi, Martinez and co-workers have reported a Lys(ΨCH_2NH)Lys isostere of NT(8-13) **3** as having potent binding and functional activity at the NT receptor *in vitro* and analgesic activity when administered ICV.[10a] However we are not aware of any reports of **3** having *in vivo* activity on the central nervous system when administered peripherally; this is also consistent with observations made in these laboratories (Table 1).



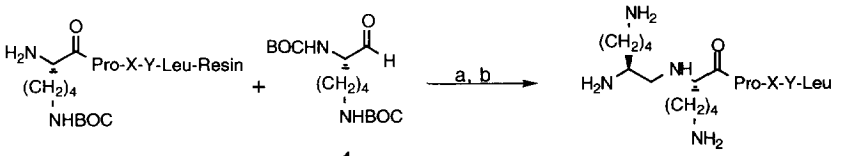
Arg-Arg-Pro-Tyr-Ile-Leu, NT(8-13) **1**



NMeArg-Lys-Pro-Trp-Tle Leu **2**

To better understand the structural requirements for *in vivo* activity, analogs of **3** were prepared with replacements for the amino acids tyrosine-11 and isoleucine-12. The syntheses of reduced amide bond isosteres **5-7** were carried out via reductive coupling of resin bound pentapeptides with bis-Boc-Lys aldehyde **4a** by an adaptation of the method of Sasaki and Coy[12] (Scheme 1). The pentapeptides were synthesized using standard Fmoc solid phase chemistry. After cleavage from the resin and side chain deprotection, the crude peptides were purified by preparative HPLC. All new target compounds were judged to be homogeneous by HPLC, capillary zone electrophoresis (CZE) and were characterized by NMR and EIMS.

Scheme 1



4a

3 X = Tyr, Y = Ile
5 X = Tyr, Y = Tle
6 X = Trp, Y = Ile
7 X = Trp, Y = Tle

a) $NaCNBH_3$, AcOH, NMP; b) TFA, anisole, thioanisole EDT

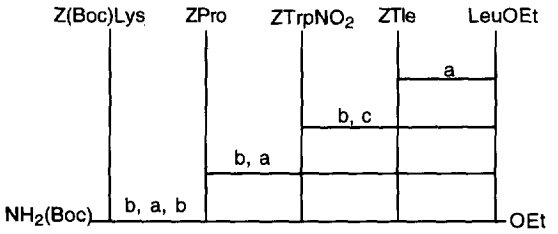
Compounds **5-7** were shown to bind to NT receptors[13] with an affinity comparable to that reported for compound **3**[10] (Table 1) and were able to mobilize calcium from HT-29 cells,[14] a functional assay for NT agonists. In addition to these *in vitro* assays the compounds were evaluated for their ability to inhibit spontaneous locomotor activity of mice when administered ip. This assay has previously been shown to be predictive of antipsychotic efficacy.[15] Compounds **5-7** were all active but compound **7** was clearly the most potent. A likely explanation is that replacement of the peptide bond at position 8-9 by a reduced bond isostere and stabilization of the 11-12 peptide bond with the unnatural amino acid tert-leucine resulted in compound **7** having sufficient resistance to enzymatic degradation to exert its effect on the central nervous system when given peripherally.

To explore the SAR 8-9 of reduced amide bond NT(8-13) mimetics, compounds **9**, **10**, **12** and **13** were prepared using solution phase synthetic methodology as outlined in Scheme 2. The arginine aldehyde derivative **11**, which was isolated as the aminol tautomer was prepared by an adaptation of a literature procedure.[16] The ethyl carboxylate esters **9** and **12** bound with approximately 25 fold less potency than the corresponding carboxylic acids **7** and **13**; however, they were at least equipotent at inhibiting locomotor activity. This data would suggest that **9** and **12** are readily cleaved to their corresponding carboxylic acids by nonspecific esterases *in vivo*. Similar observations have been made with hexapeptide neurotensin agonists.[17] The analgesic properties of compounds **9** and **12** were evaluated in the acetic acid induced stretching paradigm (Table 1).[18] Both inhibited the stretching induced by an ip injection of acetic acid with a potency greater than morphine. These effects appear to be centrally mediated since compound **12** is 500 times more potent (ED_{50} icv, 1.6 μg/kg).

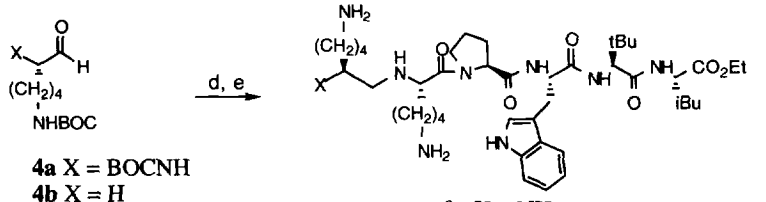
Table 1

Compound	Binding, Ki (nM)	Stim. Calcium Mob. in HT-29 cells EC50 (nM)	Inhibn. Locomotor Activity Mouse ED50 ip (mg/kg)	Inhibn of Acetic Acid Ind. Stretch. ED50 sc (mg/kg)
3	0.15[10a]		>10	
5	0.4	512	0.9	
6	0.06	110	6.3	
7	1.13	362	0.14	
9	31.2	4505	0.21	
10	27		0.3	0.05
12	437		0.15	
13	17.1		0.48	0.3
NT(8-13) **1**	0.52	4.21	>10	>10

Scheme 2

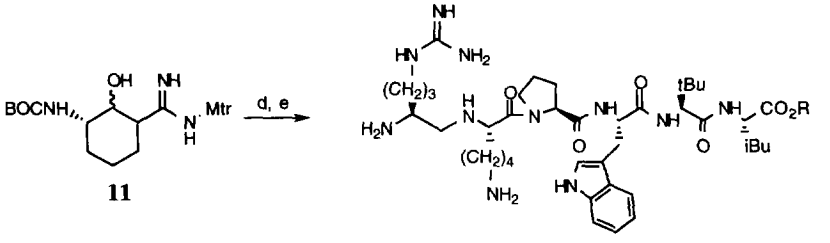


Preparation of Compound 8



4a X = BOCNH
4b X = H

9 X = NH_2
10 X = H



11

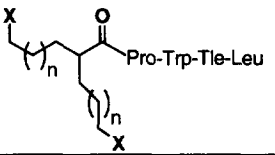
f → **12** R = Et
13 R = H

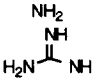
a) TBTU, NMM, DMF; b)H_2, Pd/C, EtOH; c)CH_2Cl_2, Et_3N; d) **8**, $NaCNBH_3$, MeOH; e) TFA, H_2O, anisole, thioanisole, EDT; f) NaOH

The analgesic effects of compound **12** could not be reversed by the opioid antagonist naloxone.

Compound **10** lacking an N-terminal amine had potency similar to **9** in both the binding assay and the locomotor activity test. This suggests that the terminal nitrogen does not play an important role in the binding of NT(8-13) mimetics. On the basis of this finding, we studied further simplifications of the dibasic portion of the NT(8-13). Symmetrically substituted amino alkyl acetic acid derivatives were prepared as outlined in Scheme 3. Bis alkylation of di-tertbutyl malonate with the phthalimido alkyl bromides gives diesters **14** and **19**. The phthalimide protecting groups were exchanged for benzoyloxycarbonyl (Z) protecting groups which were found to be more easily removed in the later stages of the syntheses. Similarly the guanidino group was introduced using *N,N'*-bis(benzoyloxycarbonyl)-*S*-methyl isothiourea.[20] The intermediates **17**, **18** and **20** were coupled to the tripeptide ester Tyr-Tle-LeuOEt, deprotected and purified to give NT(8-13) mimetics **21**, **22** and **23** (Table 2) which were analyzed as before.

Table 2

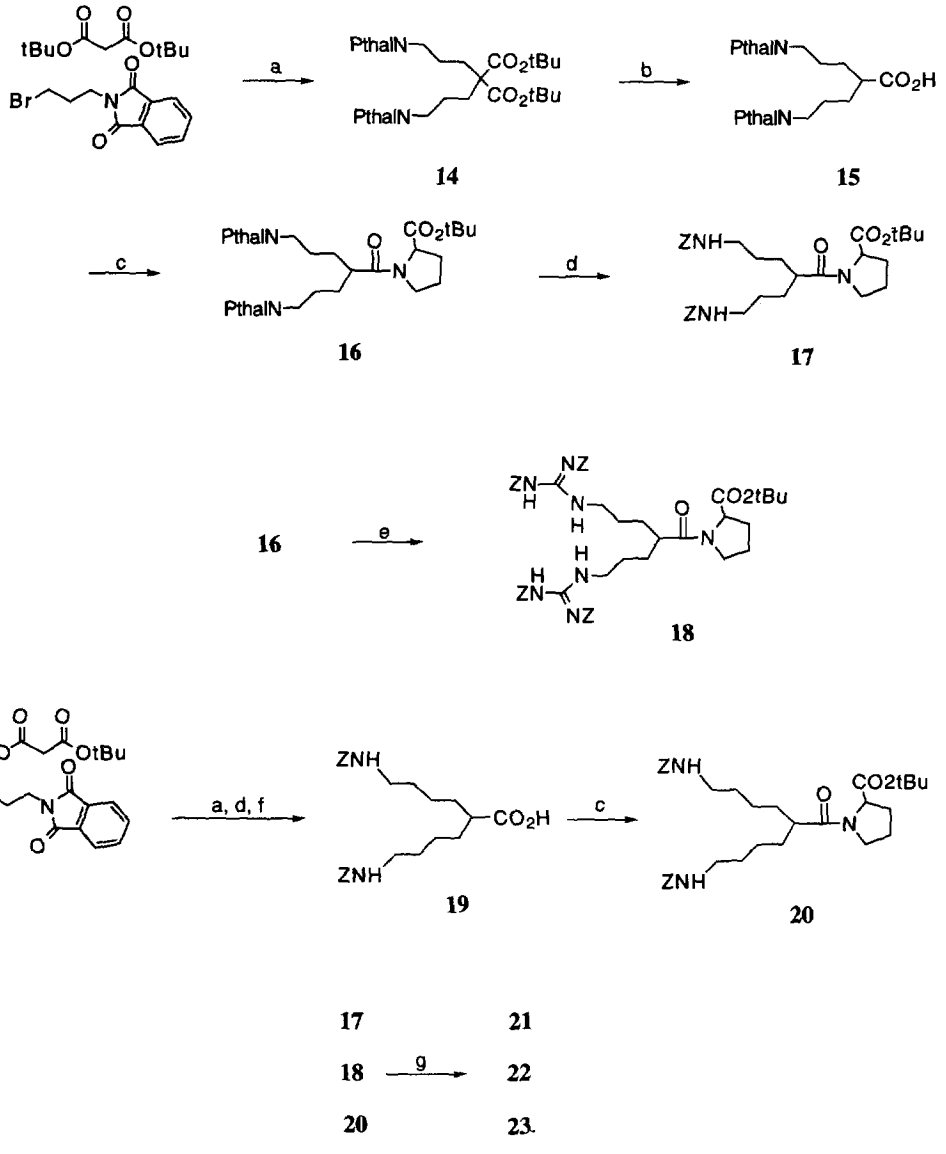


Compound	n	X	Binding, Ki (nM)	Inhibn. Locomotor Activity Mouse ED50 ip (mg/kg)
21	1	NH_2	17	0.5
22	1		0.10	1.5[19]
23	2	NH_2	3.8	0.6
Lys-Pro-Trp-Tle-Leu			60	>10

All three compounds had affinity for NT receptors; however it appeared longer chain length correlated with increased potency. The 4-carbon analog **23** was approximately 5 fold more potent at NT receptors than the corresponding 3-carbon analog. The bis guanidyl analog **22** had the most potent receptor binding of the group. Despite its greater potency at the NT receptor compound **22** (tested as the ethyl ester) was somewhat weaker in the *in vivo* assay. This may reflect decreased CNS bioavailablity due to the polar guanidine groups. These studies indicate that it is possible to replace the Arg-Arg portion of NT 8-13 with a simple dibasic acetic acid residue and still retain significant biological activity. A comparison of these compounds with the NT 9-13 analog Lys-Pro-Trp-Tle-Leu shows that the dibasic mimetics have increased binding potency and behavioral activity.

Appropriately substituted 8-9 (ΨCH_2NH) NT(8-13) isosteres can act as NT agonists *in vitro* and potently effect the central nervous system when given peripherally. Selected compounds have potential use as antipsychotic or analgesic agents. Bis-alkyl amino substituted carboxamides can function as effective replacements for the Arg-Arg portion of NT(8-13).

Scheme 3



a) NaH, THF; b) *i.* TsOH, toluene, reflux; ii. 200°C; c) Pro-OtBu; TBTU; d) i. NH_2NH_2, EtOH, reflux; ii. *N*-(benzoyloxycarbonyloxy)succinimide, $NaHCO_3$; e) i. NH_2NH_2, EtOH, reflux; ii. ZNH(ZN)CSMe (**18**) Et_3N; f) i. TFA, CH_2Cl_2; ii. 200°C; g) i. TFA, CH_2Cl_2; ii. Trp-Tle-Leu-OEt, TBTU, NMM, DMF; iii. H_2 Pd/C, EtOH; iv. NaOH

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