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TRIPEPTIDE GROWTH HORMONE SECRETAGOGUES

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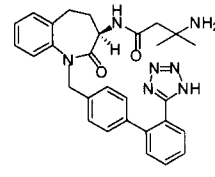
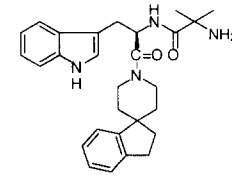
Abstract: A series of C-terminus capped dipeptides and tripeptides was synthesized as growth hormone (GH) secretagogues. Among them, tripeptide Aib-D-Trp-D-homoPhe-OEt showed low nanomolar activity in the rat pituitary assay. Thus, we have demonstrated that the GH secretagogue activity of the hexa- hepta-GH releasing peptides can be mimicked at the tripeptide level.

Introduction: While working with Met and Leu enkephaline analogues, Bowers and coworkers discovered a series of peptides that caused specific growth hormone (GH) release from rat pituitary.[1] These researchers found that the mechanism of action of these growth hormone releasing peptides (GHRP) is not mediated through the opiate receptors. The GH release mechanism is also different from that of the later discovered endogenous growth hormone releasing hormone (GHRH).[2] Interest in GHRPs grew as experiments in animals suggested that they could be used as alternatives for GH replacement therapy. More potent GHRPs were synthesized and they are represented by GHRP-6, GHRP-1, GHRP-2, and hexarelin.[3] Clinical studies with GHRP-6 and hexarelin have demonstrated that these GH secretagogues promote the release of GH in man. Extensive research efforts in identifying small molecular mimetics of these peptides have resulted in several classes of small molecule secretagogues,[4] the benzolactam biphenyl tetrazoles (e.g., L-692,429, **1**),[5] camphor derivatives,[6] 4-spiropiperidines (e.g., L-162,752, **2**),[7] and peptide amides and alcohols.[8] In this paper we report the identification of a series of potent tripeptide GH secretagogues as exemplified by L-164,080 (**3**).[9]

GHRP-6	His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2
GHRP-1	Ala-His-D-2-Nal-Ala-Trp-D-Phe-Lys-NH_2
GHRP-2	D-Ala-D-2-Nal-Ala-Trp-D-Phe-Lys-NH_2
hexarelin	His-D-2-MeTrp-Ala-Trp-D-Phe-Lys-NH_2
L-164,080	Aib-D-Trp-D-homoPhe-OEt (**3**)



L-692,429 (**1**)



L-162,752 (**2**)

Figure 1

The first nonpeptide GH secretagogue **1** was reported by Smith and coworkers.[5] A pharmacophore model was subsequently proposed by Schoen et al. based on the overlay between GHRP-6 and **1**.[10] This hypothesis was in agreement with the bioactive conformation of the peptides proposed by Momany and

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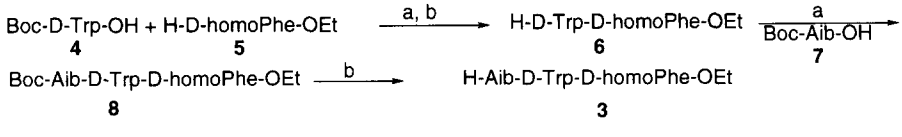
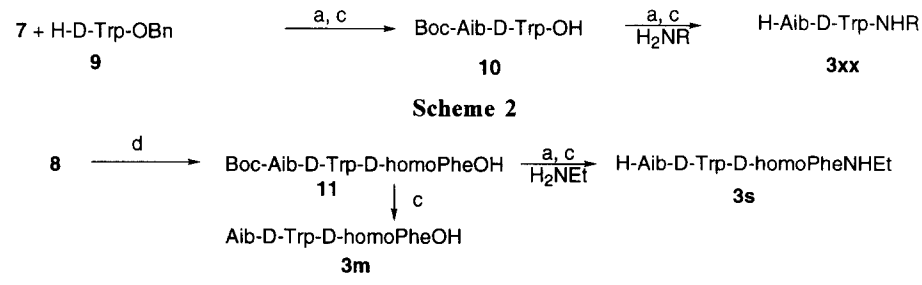
Bowers.[11] Molecular modeling studies with the second class of peptidal mimetic GH secretagogue L-162,752 (**2**) confirmed the initial overlay between the peptides and small molecules.[7,12] It was concluded that the middle phenyl in **1** and the piperidine in **2** act as a rigid spacer for the terminal aromatic rings to reach the receptor binding pockets.[13] To explore the rigid spiropiperidine's function in bioactivity, we sought to replace it with simple arylalkylamines.[14] With a floppy side chain, the resulting compound may suffer a loss in binding through entropic factors and, consequently, may have lower potency than **2**. On the other hand, it was not established whether the spiroindane represented the best fit in the receptor binding pocket; therefore, a more flexible tether on the phenyl ring might offer an enhancement in binding. Furthermore, restricted rotation may occur through the introduction of a side chain on the tether. We initiated a broad derivatization of the Aib-D-Trp dipeptide (Aib: α-aminoisobutyric acid) with different aromatic amines, which resulted in modestly potent secretagogues (entries **3a–g**, Table 1). Encouraged by these activities and on an unpublished lead,[15] we moved on to examine aromatic amino acids including Phe, homoPhe, and Nal. This approach turned out to be fruitful and resulted in the synthesis of highly potent tripeptide GH secretagogues.

Chemistry: The di- and tripeptidic GH secretagogues were prepared by stepwise peptide synthesis either from C to N for easy N-terminal modification, or from N to C for easy incorporation of the different amines and amino acid esters in the C-terminal. As shown in Scheme 1, Boc-D-Trp-OH (**4**) was coupled with D-homoPhe ethyl ester (**5**) using the standard EDC/HOBT coupling procedure to give **6**. Removal of the Boc group was accomplished by treatment with HCl in ethyl acetate. Coupling of the resulting amine with Boc-Aib-OH (**7**) followed by the same deprotection protocol as before yielded the desired tripeptide **3**. In the alternative approach, N-protected N-terminal dipeptides were prepared as a common intermediate for easy C-terminal amino acid modification. As shown in Scheme 2, coupling of Boc-Aib-OH with D-Trp benzyl ester (**9**) followed by hydrogenolysis of the benzyl group gave the protected dipeptide Boc-Aib-D-Trp-OH (**10**) as a common intermediate for C-terminal modification. Incorporation of the C-terminal units (i.e., amines, amino acid esters) such as D-homoPhe ethyl ester was done under standard coupling conditions. Modification of the ester was accomplished by preparation of the corresponding carboxylic acid **11** followed by appropriate coupling reactions. Scheme 3 illustrates the synthesis of an ethyl amide.



Scheme 1



Scheme 2

Scheme 3

Reagents: (a) EDC, HOBT, DIEA, DCM; (b) HCl(g)/EtOAc; (c) H_2, 1 atm, Pd/C, EtOH; (d) LiOH, MeOH-THF-H_2O.

Results and discussion: Growth hormone release in vitro was measured in rat pituitary cells as described previously.[16] Systematic modification of the lenghth of the tether between the aromatic ring and the amide moieties was carried out, which resulted in compounds with modest activity (Entries **3a–c**, Table 1). Noteworthy is that the optimal side chain length between the phenyl ring and the amine is three carbons, which is also found in the spiroindane series (Figure 2). The increased entropy of the phenyl ring is reflected in the over twenty fold drop in potency over the more rigid spiroindane L-162,752 (EC_{50} = 14 nM). Compounds with naphthalene (**3d–f**) as the aromatic replacement further substantiated the necessity of a three-atom linker to the "outer" phenyl ring. Substantial increases in potency were observed with **3f** (EC_{50} = 85 nM).

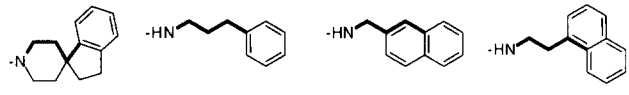
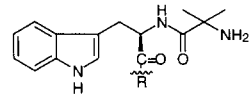


Figure 2

Encouraged by these results, we incorporated their corresponding amino acid esters into the Aib-D-Trp dipeptide scaffold. The Phe derivatives (**3g, 3g'**) were modestly active with similar potency for both D and L isomers. Dramatic potency enhancement were observed for D-homoPhe (**3**) with an EC_{50} of 3 nM, which is four times more potent than GHRP-6. Potency enhancement for the isomer L-homoPhe (**3'**, EC_{50} = 50 nM) was also observed over the parent compound, which suggest a possible role of the ester group, or an alternative binding mode to the receptor. Similar potency increases were observed for D-1-Nal (**3i**, EC_{50} = 40 nM). The derivative of 2-Nal (**3j**) was much less potent, which may be the result of the aromatic ring being extended too far with a four-carbon tether from the outer aromatic ring. Comparable explanation can be given to the compounds **3h, 3h'**. These observations are consistent with the modeling superposition of the GHRP's (*vide infra*).

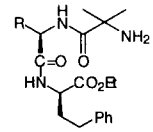


Entry	R	EC_{50} (nM)	Entry	R	EC_{50} (nM)
3a	-NH$(CH_2)_2$Ph	inactive	**3h'**	-L-phenylpropylGly-OEt	1140
3b	-NH$(CH_2)_3$Ph	390	**3i**	-D-1-Nal-OEt	40
3c	-NH$(CH_2)_4$Ph	700	**3j**	-D-2-Nal-OEt	2000
3d	-NHCH$_2$(1-naphthyl)	inactive	**3k**	-NMe-D-Phe-OEt	40
3e	-NH$(CH_2)_2$(1-naphthyl)	860	**3l**	-NMe-D-homoPheOEt	270
3f	-NHCH$_2$(2-naphthyl)	85	**3m**	-D-homoPhe-OH	>500
3g	-D-Phe-OEt	240	**3p**	-D-homoPhe-OMe	18
3g'	-L-Phe-OEt	350	**3q**	-D-homoPhe-O-iPr	4
3	-D-homoPhe-OEt	3	**3r**	-D-homoPhe-O-Bn	500
3'	-L-homoPhe-OEt	50	**3s**	-D-homoPhe-NHEt	246
3h	-D-phenylproplyGly-OEt	820	**3t**	-D-homoPhenylalanol	52

Table 1

We next turned our attention to *N*-methyl amino acid esters since it was recognized that the *N*-methylated peptides usually implicated a β-turn structure. While *N*-methylation of D-Phe-OEt (**3k**, EC_{50} = 40 nM) produced a sixfold improvement in potency over the parent **3g** (EC_{50} = 240 nM), a drop of 90-fold in activity was observed with D-homoPheOEt (**3l**, EC_{50} = 270 nM). These results suggest that the methylation may prevent the aromatic ring from adopting the optimal binding conformation. With D-homoPhe ethyl ester established as one of the best amino acids, additional SARs were carried out with compound **3** as the parent compound. Hydrolysis of the C-terminal ester afforded the carboxylic acid **3m**, which was found to be only weakly active. The effect of the size of ester on activity was clearly shown with the smaller methyl (**3p**, EC_{50} = 18 nM), slightly larger isopropyl (**3q**, EC_{50} = 4 nM), and large benzyl (**3r**, EC_{50} = 500 nM). Conversion of the ethyl ester to ethyl amide caused a dramatic drop in secretagogue activity (**3s**, EC_{50} = 500 nM). Reduction of the ester to the alcohol gave a fairly potent compound **3t** (EC_{50} = 52 nM)

Guided by research from the 4-spiropiperidine series of compounds,[17] analogues replacing the central amino acid D-Trp in **3** were prepared. Not surprisingly, the L-Trp derivative was inactive as a GH secretagogue. In contrast to earlier findings in the spiroindanes, replacement of the central amino acid D-Trp with D-homoPhe (**3v**, EC_{50} = 255 nM), D-5-phenyl-2-amino-pentanoic acid (**3w**, EC_{50} = 62 nM), and O-benzyl-D-Ser (**3x**, EC_{50} = 300 nM) caused a profound drop in the in vitro potency. Derivatives with D-1-Nal (**3y**) and D-2-Nal (**3z**), normally considered a surrogate of Trp, showed weak activity for GH release.



Entry	R (amino acid)	EC_{50} (nM)	Entry	R (amino acid)	EC_{50} (nM)
3	indolylmethyl (D-Trp)	3	**3x**	benzyloxymethyl (O-Bn-D-Ser)	300
3u	indolylmethyl (L-Trp)	inactive	**3y**	1-naphthylmethyl (D-1-Nal)	>50
3v	phenylethyl (D-homoPhe)	255	**3z**	2-naphthylmethyl (D-2-Nal)	inactive
3w	phenylpropyl (D)	62			

Table 2

Molecular Modeling: Modeling of GHRP-6 and L-692,429 was carried out using the MM2X force field and visualized with the graphics package C-View as described earlier.[10] To advance the structural model, additional conformational generation and minimization in accord with solution NMR studies of GHRP-6 and GHRP-2[18] were undertaken. Low energy structures which fit the measured constraints were retained and used in this work. Following an earlier hypothesis, the basic amino nitrogen of His in GHRP-6 and of D-Ala in GHRP-2 were aligned with the Aib amino group and the D-Trp of the peptides (or D-2-Nal in GHRP-2) were aligned to initiate the overlay process. Figure 3 shows the resulting optimal overlay of L-164,080 with GHRP-2. Although this comparison was made with the synthetic hexapeptides, which are not endogenous, we expect some of the insights obtained with respect to conformation, and the essentiality of aromatic residues will have some relevance to the presumed natural ligand.

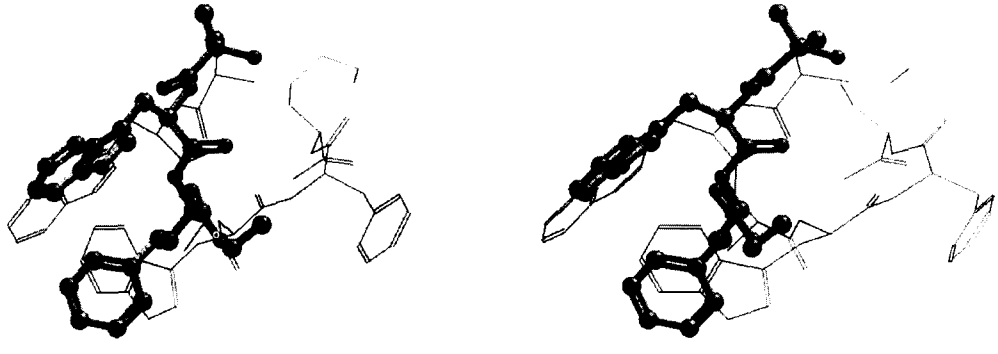


Figure 3. Stereo view of L-164,080 overlaying with GHRP-2 (wire).

Conclusion: We have shown that the GH secretagogue potency of the hexa- and heptapeptide GHRPs can be mimicked at the tripeptide level. The most potent compound Aib-D-Trp-D-homoPhe-OEt showed low nanomolar activity.

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