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ANALOGS OF THE ORALLY ACTIVE GROWTH HORMONE SECRETAGOGUE L-162,752

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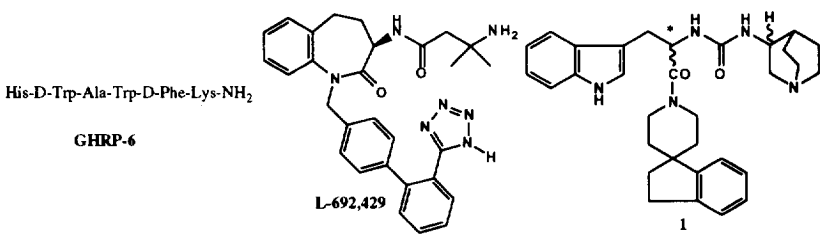
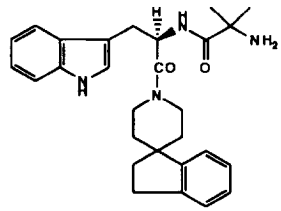
Abstract: A series of spiroindane growth hormone secretagogues that vary in their amino side chains is reported. Variations in these side chains markedly affect growth hormone release *in vitro* and *in vivo*. The best side chain in this series of secretagogues is α-methylalanine. **L-162,752** not only stimulated GH release from rat pituitary cells, but also induced GH release in dogs upon i.v. and p.o. administration.

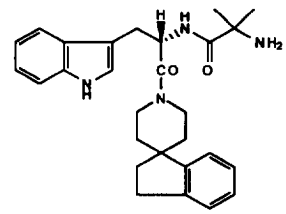
Recombinant growth hormone (GH) is currently used clinically to improve the growth of GH deficient children and to treat GH deficient adults and patients with Turner's syndrome. A number of additional uses are understudy which derive from its anabolic properties. However, the use of GH has limitations because it is a relatively large polypeptide (191 amino acids) which must be administered by injection. An alternative is to stimulate release of GH from the pituitary gland. Recently both peptide GH secretagogues including **GHRP-6**[1a] and hexarelin[1b] and a nonpeptide GH secretagogue **L-692,429**[2] have shown efficacy and specificity in the release of GH in clinical trials. Unfortunately these secretagogues have poor oral bioavailability. More recently our group has reported a highly potent, orally active GH secretagogue **L-163,191 (MK-0677)** which has been selected for clinical studies.[3] In this communication, we report the evaluation of a variety of amino side chains which formed the basis for using the aminoisobutyric acid side chain in **L-163,191**.



The design of our orally active secretagogues originated in a project to derivatize "privileged structures" as a strategy to discover leads for G-protein-linked receptors.[3] The lead compound, a spiroindanylpiperidine **1**, stimulated GH release in our rat pituitary cell assay[4] in a dose-dependent manner with an EC_{50} value of 50 nM. This intrinsic potency is especially remarkable since **1** is a mixture of four diastereomers. Compound **1** when given intravenously at a dose of 0.1 mg/kg to Beagle dogs[5] increased serum GH levels, however, it was poorly active when administrated orally.[6]



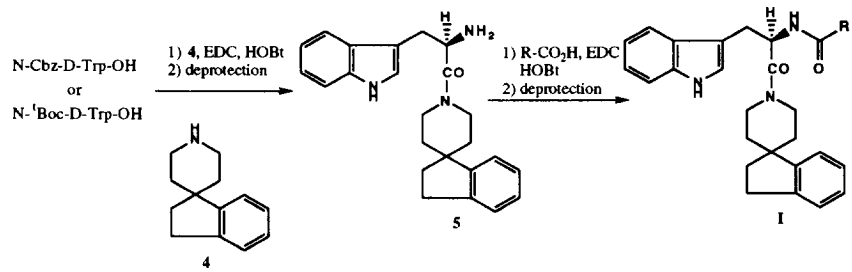
2, (L-162,752)



3

An extensive program was undertaken to replace the quinuclidinyl urea of **1** on the assumption that its polarity and strongly basic properties might hinder oral absorption. The highlight of that effort was the replacement of the quinuclidine urea of **1** with an α-methylalanine moiety with both (R)- and (S)- tryptophan to afford the individual antipodes **2** (**L-162,752**) and **3**. The more active of these compounds **L-162,752** was the first orally active GH secretagogue to be synthesized from the spiroindanylpiperidine lead **1** (see below). Herein we report the preparation and biological activities of **L-162,752** and its related analogs.

Scheme 1



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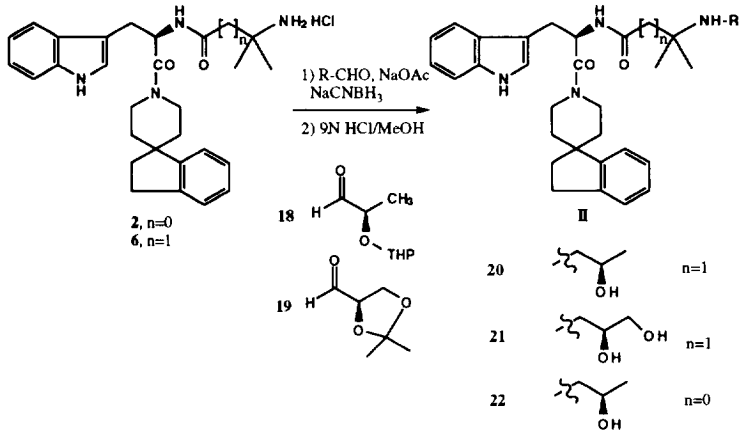
Chemistry

The synthesis of GH secretagogue analogs is illustrated in Schemes 1 and 2 and involved the coupling of Cbz or Boc protected D-tryptophan to the spiroindanylpiperidine **4**[8] using EDC and HOBt under standard peptide coupling conditions.[9] The protecting group was removed to give **5** which served as a convenient precursor for a variety of structurally diverse amino side chains which were added via a second peptide-type coupling reaction followed by a final deprotection of the coupled product **I** with HCl/Et_2O.

N-substituted derivatives **II** were prepared by a reductive amination of **2** and **6** with the appropriate aldehyde (Scheme II). For example, the synthesis of the hydroxypropyl derivatives **20** and **22** was accomplished using (R)-2-tetrahydropyranyloxypropanal **18**[10] and the dihydroxypropyl derivative **21** was prepared from (R)-2,3-O-

isopropylidene-glyceraldehyde **19**.[11] The GH secretagogues **20**, **21** and **22** were isolated after removing the tetrahydropyranyl or acetonide groups under acidic conditions.

Scheme 2

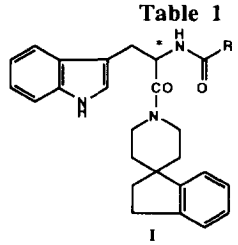


Results and Discussion

Growth hormone release *in vitro* was determined in rat pituitary cell assays.[4] Development of the lead compound **1** initially focused on the introduction of amino side chains which were known to work well in the benzolactam GH secretagogues.[7] One of the simplest and most active of these is aminoisobutyric acid and when it was used with (R)- and (S)-tryptophan the high activity of **L-162,752** established the importance of (R)- stereochemistry in the central amino acid. It is interesting that the more active benzolactam secretagogues also have an (R)-stereochemistry whose modeling suggested coincidence with the (D)-Trp of **GHRP-6**.[7] Subsequent analogs which are reported herein were all synthesized as (D)-Trp derivatives. Presented in Table 1 are results with some of the analogs that helped define the optimal amino acid side chain of these spiroindanylpiperidine GH secretagogues. Systematic modification of the α-methylalanine side chain confirmed the dependence of GH secretagogue activity on both chain length and substitution pattern. The homologated analog **6** shows a 5-fold loss in potency compared with **2**. The importance of the geminal dimethyl substitution flanking the basic amine is reflected in the poor activities of the alanine derivatives **7** and **8** (both D- and L-), and of the glycine derivative **9**. The spiro substituted glycine series (analogs **10-12**) revealed several important trends. Substitution with spirocyclopentane (analog **11**) and spirocyclohexane (analog **12**) groups reduces intrinsic activity significantly. These results suggest limited space in the receptor binding site adjacent to the carbonyl group. Evident from the poor activity of analog **10** (pKa 5.9)[12] is the requirement of a basic amine in achieving good GH secretagogue activity.[13] Comparison of the activities of the (S)-α-methylserine derivative **13** with that of the (R)-α-methylserine derivative **14** reveals tolerance for a polar substituent when it is in the R- configuration. As noted

above when the amino acid side is acyclic as in **L-162,752** (**2**) α- amino acids are preferred over β-amino acids as in **6**. When the amino acid side chain is cyclic as in **15-17**, the α-amino analog **15** is surprisingly much less active than the β- and γ-analogs, **16** and **17**, respectively.

Table 1



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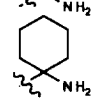
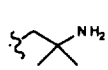
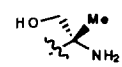
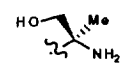
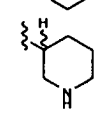
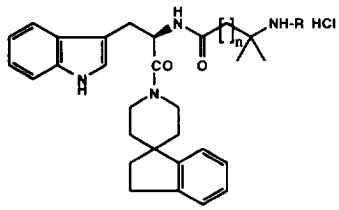
Compound	*	R	EC_{50} (nM)	Compound	*	R	EC_{50} (nM)#
L-162,752	R		14	11	R		2600
3	S		10,000	12	R		92% stimulation at 10 μM
6	R		76	13	R		600
7	R		1000	14	R		48
8	R		840	15	R		1900
9	R		2000	16	R		90
10	R		2100	17	R		100

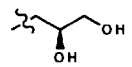
EC_{50} for half-maximal release of GH in the rat pituitary cell assay normalized for **L-692,429** as 60 nM.

Schoen and Ok[7] have reported that an additional hydroxy or dihydroxy alkyl group in the amino side chain of their benzolactam GH secretagogues dramatically increased GH releasing activity. We, therefore, tried the same changes in the current series of compounds as summarized in Table 2. In analogy with that work, N-alkylation of **6** with a (2R)-hydroxypropyl unit yielded compound **20** with an EC_{50} of 2.6 nM. This functionality enhanced

the potency of **6** by near 30 fold although the corresponding analog **22** of **2** (**L-162,752**) was significantly less active than **2**.

Table 2



Compound	n	R	EC_{50}(nM)#
20	1		2.6
21	1		16
22	0		700

EC_{50} for half-maximal release of GH in the rat pituitary cell assay normalized for **L-692,429** as 60 nM.

Biological Evaluations

Several studies support the hypothesis that **L-162,752** is a mimetic of the hexapeptide **GHRP-6**. For example, rat pituitary cells stimulated by maximal concentrations of either **GHRP-6** or **L-162,752** do not respond to further treatment with the other secretagogue, yet remain fully and synergistically responsive to GHRH. Furthermore, the **GHRP-6** antagonist His-D-Trp-D-Lys-Trp-D-Phe-Lys-NH_2 antagonizes the effect of **L-162,752** on GH release. The *in vitro* specificity of **L-162,752** was demonstrated in twenty-four G-protein-linked receptor assays in which IC_{50} values were >10 μM. Evaluation of **L-162,752** in dogs[5] by intravenous administration showed an increase in serum GH levels at a dose of 0.1 mg/kg. Significant increases of GH levels were observed upon oral dosing of **L-162,752** at 2.0 mg/kg (dogs, 2/2) and at 1.0 mg/kg (dogs 1/2). From a comparison of the plasma concentrations of **L-162,752** after oral and intravenous administration to rats, its oral bioavailability was estimated to be >40% (Dr. Kwan H. Leung and Dr. Shuet-Hing Lee Chiu, unpublished data). On the other hand, the highly active compound **20** was inactive orally in the dog assay at 1.0 mg/kg as was the more polar compound **21**. These results and the good potency of **L-162,752** helped establish the importance of the aminoisobutyric acid functionality in our spiroindanylpiperidine growth hormone secretagogues.

Acknowledgment

We thank Ms. Amy Bernick for providing mass spectrometry services, Dr. Shuet-Hing Lee Chiu and Dr. Kwan H. Leung for the rat bioavailability data and Dr. Karen Owens determined the pKa's of several compounds.

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5. Compounds were administered orally at the appropiate levels in water solution to male and female beagle 1.2-2.5 years old, weighing 8-16 kg. GH levels in plasma were determined by RIA at -5, 0 and +5 and every 10 minutes thereafter for 2 hours.
6. Compound **1** was effective i.v. at 0.1 mg/kg (1/1) but inactive at p.o. at 5.0 mg/kg (0/2).
7. (a) Schoen, W. R.; Pisano, J. M.; Prendergast, K.; Wyvratt, M. J.; Fisher, M. H.; Cheng, K.; Chan, W. W.-S.; Butler, B.; Smith, R. G.; Ball, R. G. *J. Med. Chem.* **1994**, *37*, 897. (b) Schoen W. R.; Ok, D.; DeVita, R. J.; Pisano, J. M.; Hodges, P.; Cheng, K.; Chan, W. W.-S.; Butler, B. S.; Smith, R. G.; Wyvratt, M. J.; Fisher, M. H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1117.
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12. pKa's were determined by direct titration in 50% aqueous methanol.
13. pKa value of **L-162,752** is 7.9.[12]

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