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PEPTIDOMIMETIC GROWTH HORMONE SECRETAGOGUES: SYNTHESIS AND BIOLOGICAL ACTIVITIES OF ANALOGS VARIED AT THE INDOLE NUCLEUS OF THE PROTOTYPICAL SPIROPIPERIDINE L-162,752

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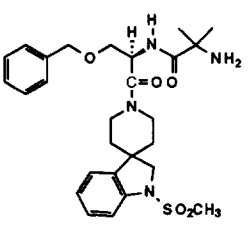
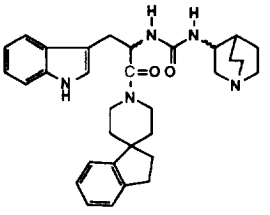
Abstract: SAR studies around the indole nucleus of the prototypical peptidomimetic L-162,752 revealed that the D-Trp could be replaced with 3-phenylpropyl-D-glycine and O-benzyl-D-serine to provide secretagogues with comparable intrinsic activity but with significantly better oral activity in dogs. Use of dimethyl β-alanine amino side-chains led to a considerable loss of activity in the D-homophenylalanine and O-benzyl-D-serine series.

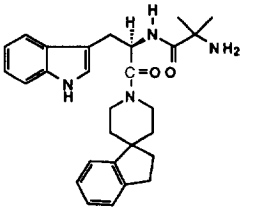
Peptidyl and peptidomimetic growth hormone (GH) secretagogues are currently being evaluated clinically as an alternative to GH replacement therapy. It is anticipated that the GH secretagogues will provide a more physiologically-relevant, pulsatile release of GH without the side effects associated with parenteral recombinant human GH therapy. The peptidyl secretagogue GHRP-6 (**1**; His-D-Trp-Ala-Trp-D-Phe-LysNH$_2$) is a synthetic peptide that was developed by Bowers, Momany and their colleagues.[1] It binds to a receptor that is distinct from the receptor for GH releasing hormone (GHRH; a hypothalamic peptide)[2] and causes GH release in a number of species, including man. Unfortunately, the oral bioavailabity of **1** in man has been reported to be less than 1%.[3]



2 (MK-0677)



3 (L-262,564)



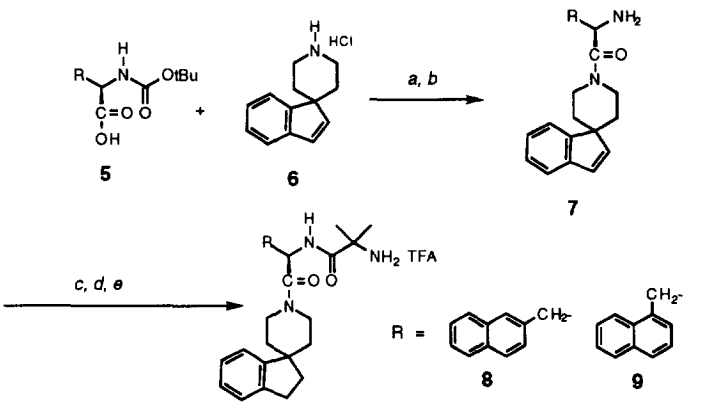
4 (L-162,752)

Attempts to develop peptidomimetics of **1** have culminated in the discovery of several structurally distinct GH secretagogues,[4] including our orally active clinical candidate **2** (MK-0677).[5] The design of **2** originated in a project to derivatize so called "privileged structures"[6] to find leads for G-protein coupled receptors. From this approach a lead compound **3**, a mixture of four diastereomers, was identified and optimization of it yielded the prototypical spiropiperidine GH secretagogue **4** (L-162,752).[7] This paper details some of our findings from structure-activity relationship studies that investigated variations and replacements for the indole nucleus of **4**.

Chemistry

The spiroindanylpiperidine compounds were prepared according to known procedures as described in Schemes 1, 2, and 3. The amino acids that were used for the synthesis of the spiropiperidine GH secretagogues were purchased from commercial sources or prepared according to literature methods. Compounds of formula **8** and **9** were prepared by coupling N-Boc D-amino acids **5** to spiroindenylpiperidine hydrochloride **6**[8] by using 1-hydroxybenztriazole (HOBT) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) followed by removal of the Boc protecting group under standard peptide synthesis conditions to give compounds of formula **7** as shown in Scheme 1. Analogs **8** and **9** were synthesized by carrying out a second peptide-type coupling reaction with N-CBZ aminoisobutyric acid (AIB) and the CBZ protecting group was removed by hydrogenation with concomitant reduction of the indene to give indanes **8** and **9** as their TFA salts.

SCHEME 1

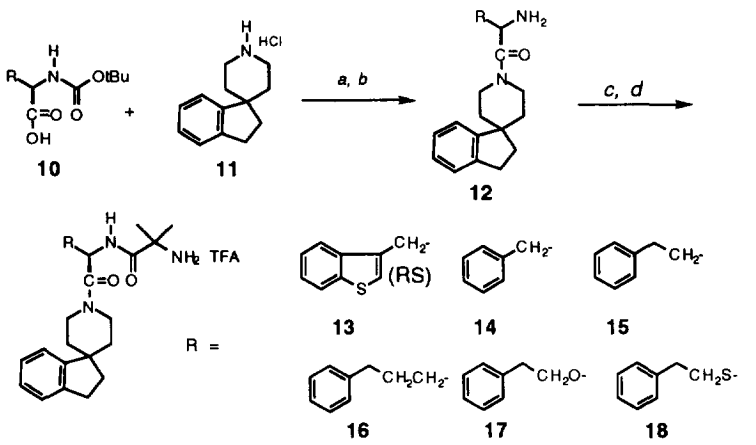


Reagents and conditions: (*a*) N-methylmorpholine, EDC, HOBT, CH_2Cl_2, 18 h; (*b*) (1:1) TFA-CH_2Cl_2, 30 min; basic work-up; (*c*) N-CBZ aminoisobutyric acid, EDC, HOBT, CH_2Cl_2, 18 h; (*d*) H_2, Pd/C, MeOH, TFA, 1 atmosphere.

As shown in Scheme 2, GH secretagogues **13-18** bearing indole replacements were synthesized by taking advantage of the peptide synthesis methods as described in Scheme 1. Spiroindanylpiperidine **11**[8] was used in place of **6** and all the final products were isolated as their TFA salts. The amino acid **10** (R = $PhCH_2CH_2CH_2$-) that was used for the synthesis of **16** was prepared according to the method of Williams et al.[9]

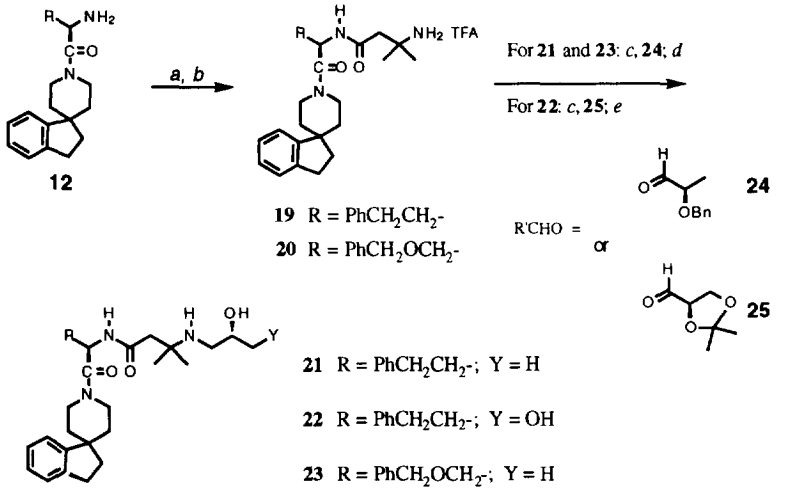
As shown in Scheme 3, dimethyl-β-alanine analogs **19** and **20** were synthesized from amine intermediate **12** and N-*t*-Boc protected dimethyl β-alanine[4b] by using the peptide-type synthesis methods as described in Scheme 1. N-terminal derivatization with hydroxyalkyl units (e.g. **21-23**) was achieved by well established reductive amination chemistry. For example, 2-hydroxypropylamine analogs **21** and **23** were synthesized by reductive amination of **19** and **20** with (R)-2-benzyloxypropionaldehyde **24**[10] followed by hydrogenolysis of the O-benzyl group. Similarly, use of D-glyceraldehyde acetonide **25**[11] in the reductive amination step followed by removal of the dimethylacetal protecting group with aqueous TFA gave dihydroxypropylamine analog **22**.

SCHEME 2



Reagents and conditions: *(a)* N-methylmorpholine, EDC, HOBT, CH_2Cl_2, 18 h; *(b)* (1:1) TFA-CH_2Cl_2, 30 min; basic work-up; *(c)* N-*t*-BOC aminoisobutyric acid, EDC, HOBT, CH_2Cl_2, 18 h; *(d)* TFA-CH_2Cl_2

SCHEME 3



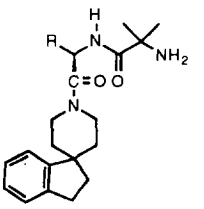
Reagents and conditions: *(a)* N-*t*-Boc β-dimethylalanine, EDC, HOBT, CH_2Cl_2, 18 h; *(b)* (1:1) TFA-CH_2Cl_2, 30 min; basic work-up; *(c)* R'CHO, $NaCNBH_3$, MeOH, NaOAc; *(d)* H_2, Pd/C, MeOH, 40 psi; *(e)* aqueous TFA.

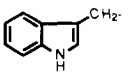
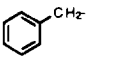
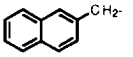
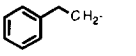
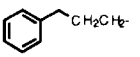
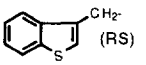
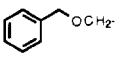
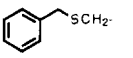
Results and Discussion

Guided somewhat by earlier structure-activity relationships that were established for the benzolactam GH secretagogues the spiropiperidine lead L-262,564 (**3**) was optimized to provide L-162,752 (**4**), an orally

bioavailable D-tryptophan-based GH secretagogue. Table I highlights the findings from SAR studies that were carried out around the indole nucleus of L-162,752 to identify GH secretogogues with improved intrinsic potency in the rat pituitary cell GH release assay[12] and enhanced oral activity in dogs. Varied aromatic replacements for the indole showed only very low potency as indicated by analogs **8, 9,** and **13** in Table I. Even the β-2-naphthylalanine derivative **8**, normally considered a close analog of Trp, but lacking the NH, showed only weak activity for the release of GH. Surprisingly, the indole could be replaced with a D-homophenylalanine derivative to provide GH secretagogue **15** with comparable GH releasing activity in vitro. The excellent intrinsic activity of **15** was unexpected especially in light of the relatively poor activity of the D-phenylalanine derivative **14.** Homologation of the phenylethyl side chain of **15** with a methylene group (**15**) or an oxygen unit (**17**) led to a modest increase in the intrinsic activity. Use of benzyl-(D)-cysteine in place of the D-homophenylalanine provided analog **18** with diminished GH releasing activity in vitro.

TABLE I



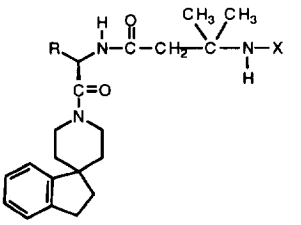
Compound	R	EC_{50} (μM)[a]	Compound	R	EC_{50} (μM)[a]
4 (L-162,752)		0.014	**14**		0.25
8		0.90	**15**		0.022
9		1.10	**16**		0.010
13	 (RS)	0.49	**17** (L-162,970)		0.017
			18		0.065

[a] Intrinsic GH secretory potency in the rat pituitary cell assay.[12] EC_{50} for half maximal release of GH normalized for L-692,429 at 60nM.

In their studies on benzolactam GH secretagogues Schoen et al.[13] have reported that the intrinsic activity of their benzolactam secretagogues can be increased by nearly 20-fold by appending either a (2R)-hydroxypropyl or a (2S)-dihydroxypropyl unit on the dimethyl β-alanine amino side-chain. Similar trends have been reported by Patchett and co-workers in the indole-based L-162,752 series.[4] Our results with SAR studies in the area of the amino side-chain of **15** and **17** are shown in Table II. In the D-homophenylalanine and O-benzyl-D-serine series use of the dimethyl β-alanine side-chain in place of the aminoisobutyric acid (AIB) side chain led to a considerable

loss in intrinsic activity (compounds **19** and **20**). N-terminal derivatization of **19** and **20** with a (2R)-hydroxy propyl unit improved their GH releasing activities somewhat although neither attained the potencies of the corresponding AIB analogs.

TABLE II



Compound	R	X	EC_{50} (μM)[a]
19	Ph-CH_2-CH_2-	H	>1.0
21	Ph-CH_2-CH_2-	-CH_2-CH(OH)-CH_3	0.20
22	Ph-CH_2-CH_2-	-CH_2-CH(OH)-CH_2OH	0.17
20	Ph-CH_2-O-CH_2-	H	>1.0
23	Ph-CH_2-O-CH_2-	-CH_2-CH(OH)-CH_3	0.24

[a] Intrinsic GH secretory potency in the rat pituitary cell assay.[12] EC_{50} for half maximal release of GH normalized for L-692,429 at 60nM.

In Vivo Evaluation in Beagles

The more potent spiropiperidines **15**, **16**, and **17** were tested orally in beagles (the protocol for the oral efficacy studies in dogs has been described in ref 4). Compound **15** was found to be active for releasing GH (five-fold elevations over basal levels were considered significant) at 5.0 mg/kg (n = 2; both animals responded) and 1.0 mg/kg (n = 2; 1/2 animals responded). The 3-phenylpropyl-D-glycine derivative **16** and the O-benzyl-D-serine compound **17** (L-162,970) were significantly more potent orally in dogs with 3/5 and 4/5 animals, respectively, responding at doses as low as 0.50 mg/kg. Hence, the oral potency of the D-homophenylalanine derivative **15** in dogs is comparable to that of L-162,752. Compounds **16** and **17** are approximately 4-fold more potent orally in dogs than L-162,752 and represent some of the most potent compounds for oral activity in dogs that were prepared in the spiroindanyl piperidine series.

Summary

A series of spiroindanyl piperidine analogs of the prototypical peptidomimetic GH secretagogue L-162,752 were prepared with indole variations and replacements. The D-tryptophan of L-162,752 could be

replaced with D-homophenylalanine, 3-phenylpropyl-D-glycine and O-benzyl-D-serine to provide secretagogues with comparable GH releasing activity in vitro . Peptidomimetics **16** and **17** (L-162,970) were approximately 4-fold more potent orally for releasing GH in beagles than their indole counterpart L-162,752. The discovery of the O-benzyl-D-serine replacement for the D-Trp as shown in compound **17** (L-162,970) formed the basis for its use in our clinical candidate MK-0677. Use of dimethyl-β-alanine side chains that have yielded highly active compounds in both the indole and benzolactam series led to a considerable loss of intrinsic potency in the D-homophenylalanine and O-benzyl-D-serine series.

Acknowledgement

We would like to thank Marcia Murphy for synthesizing compound **13** and Amy Bernick and Larry Colwell for mass spectrometry support.

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