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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF SPIROHETEROCYCLIC GROWTH HORMONE SECRETAGOGUES

Meng-Hsin Chen,* Patrick P. Pollard, and Arthur A. Patchett
Medicinal Chemistry Department, Merck Research Laboratories, Rahway, NJ, 07065, U.S.A.
Kang Cheng, Liente Wei, Wanda W.-S. Chan, Bridget Butler, Thomas M. Jacks and Roy G. Smith
Department of Biochemistry and Physiology, Merck Research Laboratories, Rahway, NJ 07065, U.S.A.

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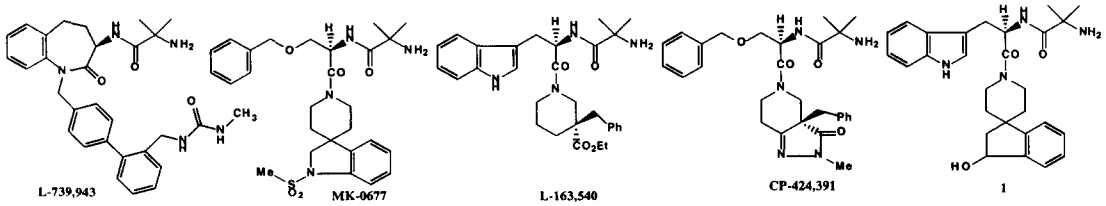
Abstract: The synthesis and biological activities of a series of spiroheterocyclic growth hormone secretagogues are reported. Modification of the spiroindane part-structure of the prototypal secretagogue **L-162,752** revealed that the spiroindane could be replaced with spirobenzodihydrothiophen derivatives to enhance not only *in vitro* potency but also oral activity. In this study non-aromatic D-2-amino-4-cyclohexylbutanoic analogs (**8a-8d**) were also identified to be active secretagogues.

Recombinant human growth hormone (rhGH) has been shown to have beneficial effects in the treatment of GH-deficient children,[1] in accelerating the healing of severe burns,[2] in Turner's Syndrome,[3] in reversing the catabolic effects of glucocorticoids,[4] in preventing osteoporosis[5] and in improving the exercise capacity of elderly individuals.[6] GH replacement therapy in the elderly has produced dramatic effects in reversing the bodily changes associated with aging including significant improvements in muscle tone, skin thickness and bone mass.[7] 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 method is to stimulate release of GH from the pituitary gland, because most cases of idiopathic GH deficiency appear to be due to inadequate hypothalamic function and not because the pituitary gland is unable to synthesize it. Recent reports have described both peptide[8] and peptidomimetic[9] growth hormone secretagogues.[10] The peptidomimetics include benzolactam (**L-739,943**),[9a] spiroindoline (**MK-0677**),[9b] 3,3-disubstituted piperidine (**L-163,540**)[9c] and pyrazolinone (**CP-424,391**)[9d] secretagogues.



As the Merck group has previously reported[9b,9e] incorporation of polar functionality into the benzylic position of the spiroindane secretagogues led to a large increase in potency *in vitro*. These more potent secretagogues also had greater oral activity in dogs. In this communication, we report a series of spiroheterocyclic compounds related to the spiro indanol secretagogue **1**[9e] (Table 1). High potency and good oral activity were obtained as exemplified by **5d**.

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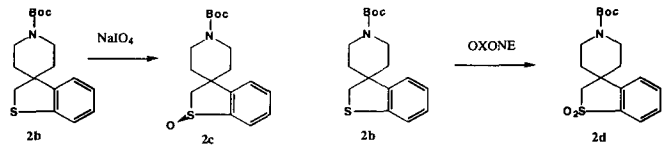
Chemistry

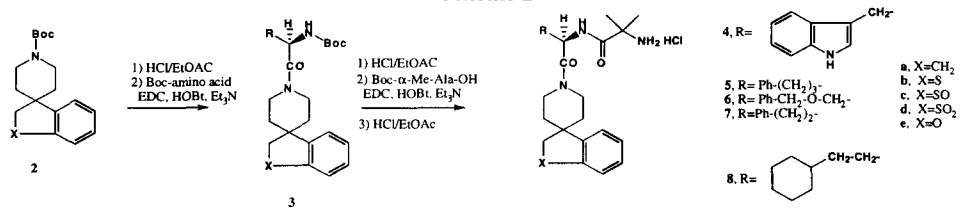
The spiroheterocyclic secretagogues were prepared according to known procedures as described in Schemes 1 and 2. Both the spirobenzodihydrothiophene **2b** and the spirobenzodihydrofuran **2e** were prepared by a radical cyclization method.[11] The spirobenzodihydrothiophene **2b** served as a convenient precursor for the preparation of the sulfoxide **2c** and sulfone **2d**. Oxidation of sulfide **2b** with $NaIO_4$[12] afforded sulfoxide **2c** while oxidation with OXONE[13] provided sulfone **2d** (Scheme 1).

Scheme 1



The BOC group of spiroheterocycles **2b~2e** was removed by treatment with a strong acid such as trifluoroacetic acid or hydrogen chloride in ethyl acetate and the resulting amine was then coupled with the desired BOC protected amino acid using a standard EDC and HOBt procedure[14] to give **3** (Scheme 2). After BOC removal from **3**, the resulting amine was subjected to a second peptide coupling with Boc-α-methylalanine. Final BOC deprotection using HCl/EtOAc provided the desired products (**4~8**).

Scheme 2



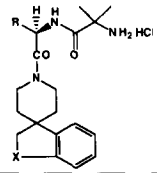
Results and Discussion

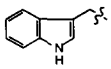
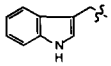
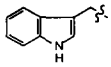
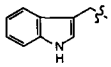
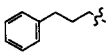
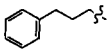
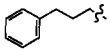
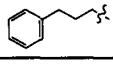
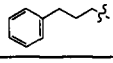
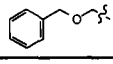
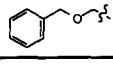
GH release *in vitro* was measured in the rat pituitary cell assay as previously described.[15] Guided by an earlier structure activity relationship (SAR) study that we reported for spiroindane GH secretagogues[9f] (**L-162,752**), this series of spiroheterocyclic compounds all incorporated α-methylalanine at the dipeptide N-terminus to optimize *in vitro* potency and *in vivo* oral activity. Table 1 highlights the SAR results arising from central amino acid and spiroheterocycle variations. Notably replacement of the spiroindane moiety of **5a** by the spirobenzodihydrothiophene moiety of **5b** and by the spirobenzodihydrofuran moiety of **5e** provided two to three fold increases in potency. This trend was also observed with D-Trp (**4a** and **4b**) and D-O-Bn-Ser (**6a**, **6b** and **6e**) analogs. The D-homoPhe analog **7b**, on the other hand, was three fold less active than **7a**, although earlier studies[9g] indicated that the D-homoPhe analog **7a** and the D-Trp analog **4a** showed comparable GH releasing activity in the spiroindane series. Surprisingly, the phenyl moiety of the D-homoPhe analogs **7a, b** and **d** could be replaced with a cyclohexyl unit. The resulting D-2-amino-4-cyclohexylbutanoic analogs **8a, b, c** and **d**

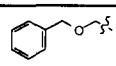
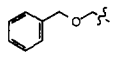
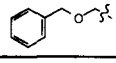
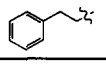
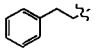
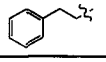
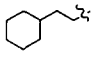
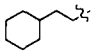
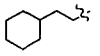
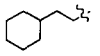
showed comparable GH *in vitro* activity to the D-Trp analogs, **4a**, **b** and **d**, and to the D-2-amino-5-phenylpentanoic analogs, **5a**, **b**, **c** and **d**. These findings are of particular interest because initial lead selections for the GH secretagogue program at Merck had been based on the importance of aromatic amino acids in GHRP-6. The comparable intrinsic potency of **8b** to **4b**, **5b** and **6b** indicates that lipophilicity not π stacking by the residue plays an important role in binding to the receptor.

Incorporation of sulfoxide or sulfone group into the spiroheterocyclic secretagogues increased intrinsic potency. Comparing the sulfone analogs with the spiroindane analogs (**5d** vs **5a** and **8d** vs **8a**) shows a ten fold increase in potency. A sulfoxide group was also beneficial, again providing an increase in potency over the spiroindane analogs (**5c** vs **5a** and **8c** vs **8a**). These results are consistent with the earlier finding[9e] that a polar functional group is preferred in the spiroindane benzylic position (**1** vs **L-162,752**).

Table 1



Compound	R	X	EC_{50}#(nM)
1		CH-OH	0.6
4a; L-162,752		CH_2	14
4b		S	5.6
4d		SO_2	2.1
5a		CH_2	10
5b		S	4.8
5c		SO	2
5d		SO_2	1.1
5e		O	2.7
6a		CH_2	17
6b		S	10

6c		SO	8.5
6d		SO_2	5
6e		O	8.5
7a		CH_2	22
7b		S	57
7d		SO_2	6
8a		CH_2	21
8b		S	8.1
8c		SO	3.4
8d		SO_2	1.4

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

Oral Evaluation in Beagles

The more potent secretagogues (**5c**, **5d**, **5e** and **8d**) were evaluated for oral activity in beagles.[9b] The minimum effective doses that caused at least a five fold elevation of GH over basal levels were determined. Although the spirobenzodihydrofuran analog **5e** was four fold more potent in the rat pituitary cell assay than the spiroindane analog **5a**, the oral activity in dogs of **5e** was only comparable to **5a** at 0.5 mg/kg at which dose only one of two animals responded. However, both the sulfoxide analog **5c** and the sulfone analogs **5d** and **8d** were found to be much more orally active. For example, compound **5c** gave a positive response in two dogs at 0.25 mg/kg (2/2) and the D-2-amino-4-cyclohexylbutanoic derivative **8d** also responded at the same doses (0.25 mg/kg, 2/2). The most orally active analog in this series is compound **5d** which showed consistently strong growth hormone release after oral dosing at 0.125 mg/kg; **5d** is at least four fold more potent orally in dogs than the spiroindane analog **5a**.

Summary

A series of compounds was prepared in which the spiroindane of the prototypical peptidomimetic GH secretagogue **L-162,752** was replaced by a heterocycle. In general, these spiroheterocyclic analogs were more potent than the corresponding spiroindanes. In the series of sulfoxide and sulfone analogs, some were shown to

increase *in vitro* potency by ten fold and these GH secretagogues also demonstrated increased oral activity in the beagle dogs relative to the lead spiroindane compounds. Further SAR studies of the central aromatic amino acid of these GH secretagogues revealed that this residue could be replaced by a non-aromatic amino acid such as D-2-amino-4-cyclohexylbutanoic acid to provide secretagogues (**8a~8d**) with good intrinsic activity and potent oral activity.

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