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THE SYNTHESIS AND ACTIVITY OF SPIROINDANE GROWTH HORMONE SECRETAGOGUES

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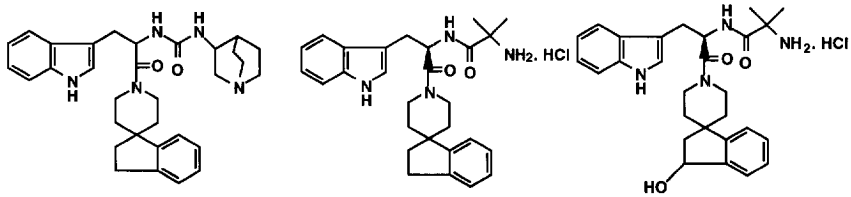
Abstract: The synthesis and activities of a series of spiroindane growth hormone secretagogues is reported. Modification of the benzylic position of the spiroindane has resulted in a dramatic increase in potency resulting in subnanomolar peptidomimetic growth hormone secretagogues. In vivo data demonstrating the good oral activity of these analogs is reported. © 1997 Elsevier Science Ltd. All rights reserved.

Currently growth hormone (GH) replacement therapy is used clinically for the treatment of growth hormone deficient children and recent reports suggest that it may be beneficial the treatment of Turner's syndrome,[1] in promoting more rapid healing in burn victims,[2] preventing osteoporosis,[3] reducing the catabolic side effects in patients treated with prednisone,[4] and reversing bodily decline due to aging.[5] Newer investigational methods of treatment involve the release of endogenous growth hormone by administration of the hypothalamic hormone, growth hormone releasing hormone,[6] or its analogs.[7] Recent reports have described both peptide[8,9] and peptidomimetic[10-13] GH secretagogues.[14] GH secretagogues may have advantages over bolus GH therapy in that they more closely mimic the natural pulsatile release of GH.[15]

As previously reported replacement of the urea unit of the spiroindane lead **1** with aminoisobutric acid produced the more potent spiroindane secretagogue **2**.[11,12] In this paper we describe modification of the spiroindane moiety of these secretagogues. Incorporation of polar functionality into the benzylic position of the spiroindane led to a breakthrough in in vitro potency and good oral activity as exemplified by **3** (**L-163,021**).

Scheme 1

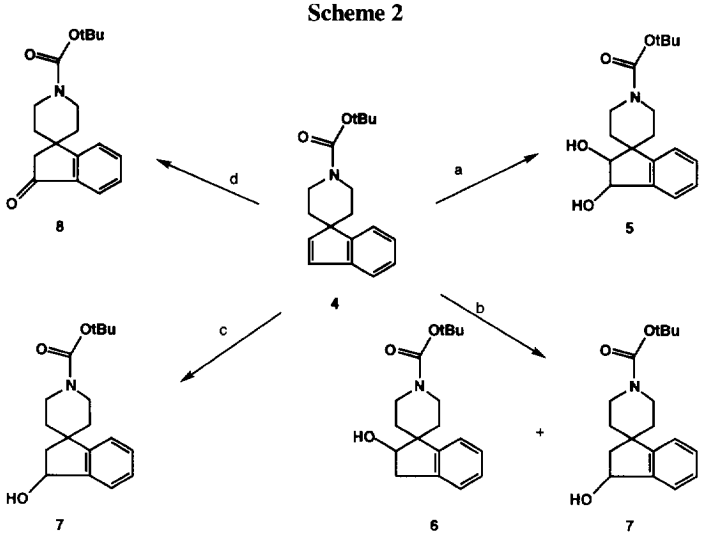


1 (L-262,564) **2** (L-162,752) **3** (L-163,021)

CHEMISTRY

Spiroindene **4**[16] served as a convenient precursor for the preparation of the oxygenated spiroindanes. Hydroxylation of **4** with osmium tetroxide and N-methylmorpholine-N-oxide gave diol **5** in good yield. Hydroboration of **4** with borane tetrahydrofuran complex followed by an oxidative workup provided a 1:1 mixture of alcohols **6** and **7**. Alternatively, **7** could be cleanly prepared by hydroboration with 9-borabicyclo[3.3.1]nonane (9-BBN) followed by an oxidative workup. The ketone **8** could be prepared in good yield by the direct oxidation of the indene/9-BBN adduct with pyridinium chlorochromate.

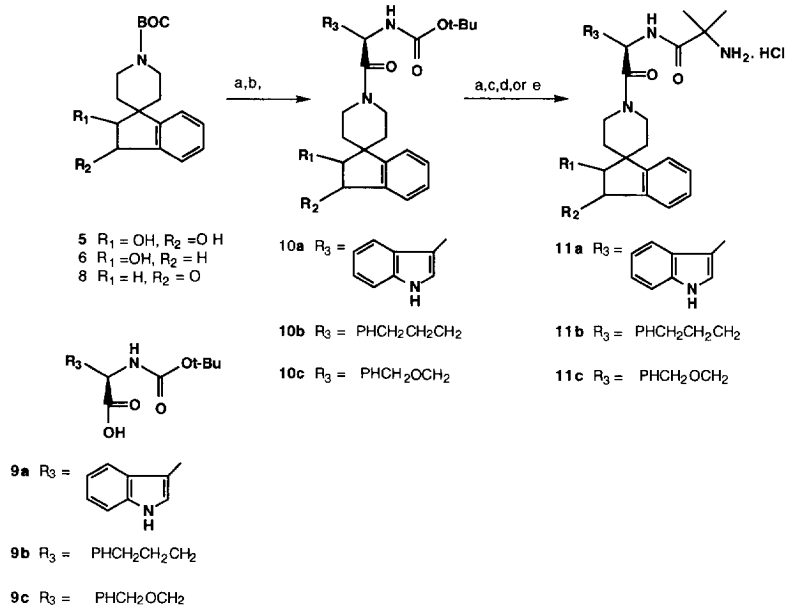
Scheme 2



(a) OsO_4, NMO; (b) BH_3/THF,H_2O_2, NaOH; (c) 9-BBN, H_2O_2, NaOH; (d) 9-BBN, PCC.

The BOC group was removed by treatment with strong acid, such as trifluoroacetic acid, and the resulting amine was then coupled to the desired BOC protected amino acid using standard coupling techniques. Once again the BOC was removed and the resulting amine was coupled to BOC-α–methylalanine. Final treatment with acid produced the desired compounds.

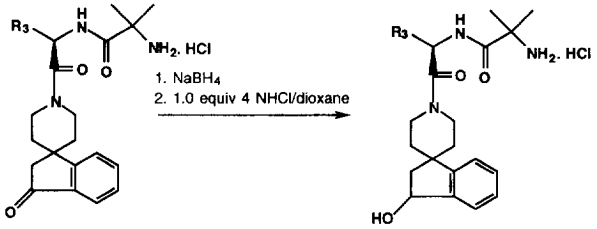
Scheme 3



(a) TFA/CH_2Cl_2; (b) **9a**,**b**,**c**, EDC, HOBT, NMM; (c) Boc-α-methylalanine, EDC, HOBT, NMM; (d) TFA/CH_2Cl_2; (e) 4 N HCl/dioxane.

Not unexpectedly the benzylic hydroxyl group was found to be unstable to the acid deprotection conditions; therefore, the ketone was carried along until the final step where it was then reduced to the alcohol with sodium borohydride.

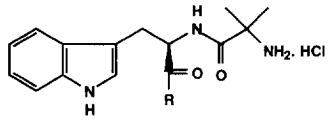
Scheme 4

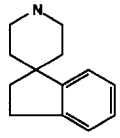
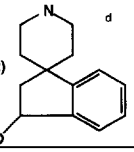
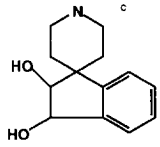
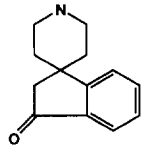
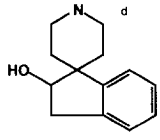


BIOLOGY

The compounds were initially evaluated for their ability to release growth hormone in the rat pituitary cell assay.[17] Incorporation of the diol unit into the spiroindane had little effect on potency (Table 1). However, the monohydroxy compound **14** was much less active than the unsubstituted analog **12** suggesting that the benzylic hydroxyl group contributes to the potency of **13**. In fact, incorporation of only the benzylic hydroxyl group into the spiroindane increased potency by more than a factor of ten. A keto group in the benzylic position was almost as beneficial, again showing a large increase in potency over the unsubstituted compound **12**.

Table 1

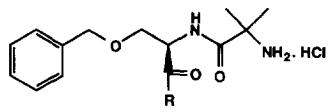


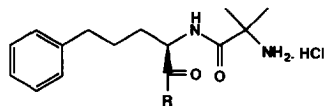
Compound	R	EC_{50} nM[a,b]	Compound	R	EC_{50} nM[a,b]
12		14	**15 (L-163,022)**		0.6
13		30	16		1.2
14		65			

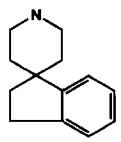
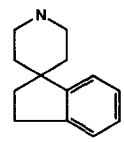
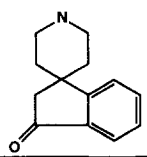
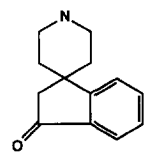
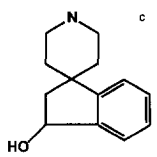
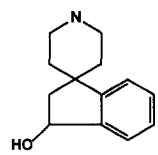
(a) Data from the rat pituitary cell assay;[17] (b) All EC_{50} are normalized against standards, either **1** (**L-692,429**) (60 nM)[11] or **L-692,585** (3.0 nM)[18] (c) 1:1 mixture of *cis* diastereomers; (d) 1:1 mixture of diastereomers.

Substitution of the D-tryptophan with either O-benzyl-D-serine or D-2-amimo-5-phenylpentanoic acid has been shown to give potent orally active secretagogues.[11,19] The benzylic hydroxyl and keto analogs were prepared in these series. In the O-benzyl-D-serine series the gain in potency was approximately a factor of four for both the hydroxy and keto compounds (Table 2). In the D-2-amimo-5-phenylpentanoic acid case as in the D-tryptophan case there was a greater than 10-fold gain in potency for both the keto and hydroxyl analogs when compared to the unsubstituted spiroindane.

Table 2



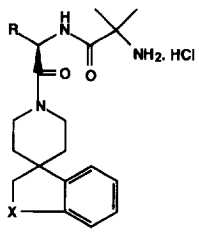


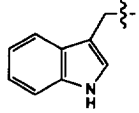
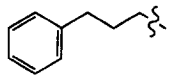
Compound	R	EC_{50} nM[a,b]	Compound	R	EC_{50} nM[a,b]
17		17	20		10
18		4.8	21		0.8
19	 [c]	4.0	22	 [c]	0.72

(a) Data from the rat pituitary cell assay;[17] (b) All EC_{50} are normalized against standards, either **1** (**L-692,429**)[11] or **L-692,585**;[18] (c) 1:1 mixture of diastereomers.

The improvements in in vitro potency led to higher potencies in vivo following either intravenous (iv) or oral (po) administration to beagle dogs[20] as highlighted in Table 3. The minimum effective dose that caused at least a four fold increase in serum GH levels was considered a positive responce and was used to compare in vivo potencies. For example, compounds **15** and **16** both had a positive responce in two dogs (2/2) while **12** was only active in one out of two dogs (1/2) when dosed at 1.0 mpk po in the beagles. The activities in the D-2-amimo-5-phenylpentanoic acid series were even more dramatic. The keto compound **21** is approximately four times more active, both iv and po, in the beagle dogs, than is the unsubstituted spiroindane analog **20**.

Table 3



Compound	R	X	iv (mpk)	Response[a,b]	po (mpk)	Response[a,b]
12		CH_2	0.05	(0/1)	2.0	(2/2)
			0.1	(1/1)	1.0	(1/2)
15		C=O	0.05	(1/1)	1.0	(2/2)
					0.5	(1/2)
16		CH-OH	0.05	(1/1)	1.0	(2/2)
					0.5	(1/2)
20		CH_2	0.1	(0/1)	1.0	(2/2)
					0.5	(1/2)
21		C=O	0.025	(1/1)	0.125	(1/2)
					0.25	(2/2)

(a) Data from the beagle dog model;[20] (b) A fourfold increase in GH levels above basal is considered a positive response.

SUMMARY

The incorporation of polar functionality (specifically hydroxyl and keto groups) into the benzylic position of spiroindane secretagogues has been shown to increase in vitro potency by an order of magnitude providing sub-nanomolar peptidomimetic GH secretagogues (**15**, **21**, and **22**). These compounds also demonstrated an increase in both iv and po activity in the beagle dog model as compared to the lead compounds (**12** and **20**). Further modifications of the benzylic position of the spiroindanes are under way and will be the subject of future papers.

ACKNOWLEDEMENTS

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20. Compounds were administered orally at the appropriate levels in water to male and female beagles weighing 8-16 kg. Dogs were bled at the jugular vein at -20, 0, 15, 30, 45, 60, 90, 120, 180, 240, and 480 minutes after dosing. The sera was analyzed for GH. For more details see ref 12.

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