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ACYCLIC STRUCTURAL VARIANTS OF GROWTH HORMONE SECRETAGOGUE L-692,429

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Abstract: Systematic investigation of acyclic analogs of L-692,429, the prototype benzolactam growth hormone secretagogue, has helped to further define the structural requirements for the release of growth hormone from rat pituitary cells for this class of secretagogues. © 1999 Elsevier Science Ltd. All rights reserved.

Introduction: In recent years human growth hormone (GH) has been used clinically in the treatment of GH deficient children and adults, in patients with Turner's syndrome, in reversing the catabolic effects of glucocorticoid treatment and AIDS, in accelerating wound healing of burn patients, in facilitating the regeneration of bone tissue in bone fracture patients and also in improving the exercise capacity of elderly subjects.³ Unfortunately, the widespread use of GH has been limited by its high cost and lack of oral bioavailability.

Recent reports have highlighted nonpeptidyl benzolactam GH secretagogue compounds that mimic the growth hormone releasing ability of the peptidyl secretagogue GHRP-6.⁴ For example, the benzolactam compound L-692,429 has been shown to promote the release of endogenous GH in humans when administered intravenously.⁵ Studies on the structure–activity relationships within the benzolactam secretagogue series have been reviewed.⁶ Recently, a new class of GH secretagogues (e.g., MK-0677) has been reported by Patchett et al.⁷ In this Letter the design, synthesis, and biological activity of acyclic structural variants of the benzolactam secretagogue L-692,429 is reported.



Contemporaneously with the work on benzolactam GH secretagogues, a second area of research focused on a number of acyclic variants that formally may be seen to be based on the benzolactam growth hormone secretagogue structure L-692,429. The study on acyclic GH secretagogues began with two acyclic variants of L-692,429 that may be considered hypothetically to be derived by scission of a bond in the lactam ring [either (a) and (b)] and thus leading to structures 1 and 2 respectively (see Figure 1). Further systematic variation of substituents around the acyclic structure in the areas highlighted led to the generalized molecule 3.



Figure 1. Design of Acyclic Variants of L-692,429



Synthesis: The synthesis⁸ of the various compounds reported in this letter was straightforward. Compound 10 was prepared from (R)-2-aminobutyric acid 4 as shown in Scheme 1. The amino group of the starting material 4 was first protected with a BOC group and this derived intermediate coupled with aniline using EDC to give

Scheme 1

amide 5. N-Alkylation of the amide nitrogen with bromide 6 gave adduct 7, which was subsequently deprotected to give the tetrazole amine 8. Amide formation with activated ester 9 followed by acidic removal of the BOC-protecting group gave compound 10. The D-homo-phenylalanine (D-homo-Phe) derived compound 15d was synthesized from BOC-protected D-homo-Phe 12d according to Scheme 2. The biphenyl tetrazole amine 11 was coupled to acid derivative 12d using a standard EDC coupling⁹ and the trityl and BOC protecting groups sequentially removed by hydrogenation and acid treatment to give amine derivative 13d. The CBZ-protected 3-amino-3-methylbutanoate side chain portion was attached to the amino group of 13d via its N-hydroxysuccinimide ester 14. Removal of the CBZ protecting group by hydrogenolysis afforded acyclic D-homo-Phe derived secretagogue 15d.

Scheme 2



 $\mathbf{a} = \mathbf{H}$; $\mathbf{b} = \mathbf{Me}$; $\mathbf{c} = \mathbf{CH}_2\mathbf{Ph}$; $\mathbf{d} = \mathbf{CH}_2\mathbf{CH}_2\mathbf{Ph}$; $\mathbf{e} = 3$ -indolyICH₂-; $\mathbf{f} = \mathbf{PhCH}_2\mathbf{OCH}_2$ -

Using this synthetic sequence five additional D-amino acid compounds were prepared with different Rgroups (see Table 1). In one case where the amino acid was D-tryptophan [12e, R = 3-indolylmethyl] the reaction sequence was slightly modified. The indole nitrogen of D-tryptophan was protected as its formyl derivative for the entire sequence shown in Scheme 2 and the formyl group removed last with hydrochloric acid in methanol.



The effects of N-alkylation of the amide nitrogen were also explored as well as that of the length of the amine side-chain attached to the 2-position. A synthetic route to three D-homo-Phe analogs is shown in Scheme 3 below. The acid **16a** was coupled with amine **17a** using BOP¹⁰ to give the adduct **18a**. In a similar manner the two amides **18b,c** were obtained from acid **16b** and the two amines **17b,c**. Removal of the trityl and

CBZ groups by hydrogenation from amide 18a and the trityl and BOC groups from amides 18b,c using acidic conditions led to the three acyclic GH analogs 19a-c.

Compounds 16b and 20 served as versatile intermediates for preparing new derivatives with the 2'tetrazole moiety (Scheme 4). A 2'-cyano substituted biphenyl methylamine 21 was coupled with acid derivatives 16b and 20 to give adducts 22a,b and then the cyano group was converted into a tetrazole using trimethyltin azide. The final products 23a,b were isolated after removal of the protecting group using dilute hydrochloric acid in methanol.





N-Methyl biphenylamide compound 30 was also synthesized from the FMOC-D-homo-Phe 24 according to Scheme 5. The acid 24 was converted into the acid chloride¹¹ and immediately coupled with N-methyl biphenylamine 25 in the presence of triethylamine. The FMOC-protecting group was removed using 4-(methylamino)-piperidine 26. The resulting primary amine 27 was then coupled with side-chain carboxylic acid 28 and the nitrile 29 was converted into a tetrazole moiety using trimethyltin azide. Acidic work up removed both the trimethyltin and the BOC group to provide the desired analog 30.



Results and Discussion: The in vitro GH release assay ED_{50} results for rat pituitary cells were obtained as previously described¹² and reported in Table 1. From these results obtained for "acyclic" compounds 10 and 15d it became apparent that lactam ring scission at (b) (see Figure 1) leading to D-homo-Phe analog 15d gave more potent compounds than analog 10. The acyclic analog 15d was still less active than benzolactam L-692,429, therefore, optimization around the structure 15d seemed warranted in order to improve potency.

The first series of analogs with general structure **15**, shown in Table 1 attempted to optimize the Damino acid for GH release. From Table 1, the following inferences may be drawn from the data. Firstly, nonaromatic D-amino acids were essentially inactive and secondly D-homo-Phe and D-Trp appear to be optimal. These results follow a similar observed trend shown in a study¹³ recently reported for GH secretagogue MK-0677.

	Compound	R	R'	ED ₅₀ "
	L-692,429			0.06
	(10)	Et	Ph	11.0
R'_N Ö	(15a)	H	H	inactive
N=N	(15b)	Me	Н	inactive
N NH	(15c)	PhCH ₂ -	Н	11
	(15d)	PhCH ₂ CH ₂ -	Н	0.2
	(15e)	3-indolyl-CH2-	н	0.4
	(15f)	PhCH ₂ OCH ₂ -	н	>1
15				

^a μM; rat pituitary cell assay

Table 1 Variation of the D-Amino Acid Side Chain and ED₅₀ Values for GH Release

Ph	Compound	R	n	x	ED ₅₀ ^a	
$H (CH_2)_{n} NH_2$ $R (CH_2)_{n} NH_2$ $(CH_2)_{x} NH_{x} NH_{x}$	(15d)	н	0	1	0.2	
	(23a)	Me	0	1	0.03	
	(19b)	Et	0	1	>0.5	
	(19c)	iPr	0	1	>0.5	
	(30)	Me	0	0	0.3	
	(23b)	Me	1	1	>0.3	
31	(19a)	Me	2	1	>0.3	
	^a μM; rat pitu	litary cell	assay			

Table 2 Variation of the Amide Substituents on the D-Amino Acid and ED₅₀ Values for GH Release

The GH release data from optimizing the substituents around the tertiary amide showed a distinct preference for R (see Table 2) to be methyl (23a) for analogs of 31 over hydrogen (15d), ethyl (19b), or isopropyl (19c). Also, a biphenylyl methyl amide (23a) (x = 1) appears to be preferred over that of the biphenyl amide 30 (x = 0), since the latter was approximately tenfold less active. The activities of three D-homo-Phe biphenyl tetrazole analogs (Table 2, 23a, 23b, and 19a) which differed only in the length of the amino acid side-

chain showed that the short amine side-chain with n = 0, (23a) was the most active compound and exceeded that of D-homo-Phe analog 15d by sixfold and over GH secretagogue L-692,429 by twofold. Overall, the acyclic GH secretagogues reported in this letter did not tolerate much variation from the prototype L-692,429 benzolactam structure.

Summary: Starting with L-692,429 as a design template, several new acyclic growth hormone secretagogues were synthesized and evaluated for their activity for in vitro growth hormone release. The N-phenyl amide resulting from scission of the lactam ring of L-692,429 resulted in inactive compounds. Aromatic amino acid derivatives were found to be active in the GH assay with the D-homo-Phe analog being the most potent in vitro. Preparation of N-alkyl D-homo-Phe derivatives resulted in the discovery of one acyclic GH secretagogue, compound **23a**, that had comparable growth hormone releasing properties with the parent benzolactam secretagogue L-692,429.

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References

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- (a) Strobl, J. S.; Thomas, M. J. Pharmacol. Rev. 1994, 46, 1. (b) Bowers, C.Y. J. Pediatr. Endocrinol. 1993, 6, 21.
- (a) Momany, F. A.; Bowers, C. Y.; Reynolds, G. A.; Hong, A.; Newlander, K. Endocrinology 1984, 114, 1531. (b) Smith, R. G.; Cheng, K.; Schoen, W. R.; Pong, S. -S.; Hickey, G.; Jacks, T.; Butler, B.; Chan, W.W. -S.; Chaung, L. -Y. P.; Judith, F.; Taylor, J.; Wyvratt, M. J.; Fisher, M. H. Science 1993, 260, 1640. (c) Schoen, W. R.; Pisano, J. M.; Wyvratt, M. J.; Fisher, M. H.; Prendergast, K.; Cheng, K.; Chan, W. W. -S.; Butler, B.; Smith, R. G.; Ball, R. G. J. Med. Chem. 1994, 37, 897.
- 5. Gertz, B. J.; Barrett, J. S.; Eisenhandler, R.; Krupa, D. A.; Wittreich, J. M.; Seibold, J. R.; Schneider, S.H. J. Clin. Endocrinol. Metab. 1993, 77, 1393.
- (a) DeVita, R. J.; Wyvratt, M. J. Drugs Future 1996, 21, 273. (b) Patchett, A. A.; Smith, R. G.; Wyvratt, M. J. In Pharmaceutical and Biotechnology; Borchardt, R., Ed; Plenum: New York, 1998; Vol. 11 (Integration of Pharmaceutical Discovery and Development: Case Studies), 525-554.
- Patchett, A. A.; Nargund, R. P.; Tata, J. R.; Chen, M. -H.; Barakat, K. J.; Johnston, D. B. R.; Cheng, K.; Chan, W. W. -S.; Butler, B.; Hickey, G.; Jacks, T.; Schleim, K.; Pong, S. -S.; Chaung, L. -Y. P; Chen, H. Y.; Frazier, E.; Leung, K. H.; Chiu, S. - H. L.; Smith, R. G. Proc. Natl. Acad. Sci. U.S.A. 1995, 92, 7001.
- 8. For full experimental details see Lin, P.; Schoen, W. R.; Pisano, J. M., Wyvratt, M. J., U.S. Patent 5,726,319, 1998. Chem. Abstr. 1995, 122, 240444d.
- 9. Bodanszky, M; Bodanszky, A. The Practice of Peptide Synthesis; Springer-Verlag: New York, 1984; pp144-146.
- 10. Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. Tetrahedron Lett. 1975, 1219.
- (a) Carpino, L. A.; Cohen, B. J.; Stephens Jr. K. E.; Yahya Sadat-Aalaee, S; Tien, Jien-Heh; Langridge, D. C. J. Org. Chem. 1986, 51, 3734. (b) Beyermann, M.; Bienert, M.; Niedrich, H. J. Org. Chem. 1990, 55, 721.
- 12. Cheng, K.; Chan, W. W. -S.; Barreto, A.; Convey, E. M.; Smith, R. G. Endocrinology 1989, 124, 2791.
- 13. Yang, Lihu; Morriello, G.; Patchett, A. A.; Leung, K., Jacks, T.; Cheng, K.; Schleim, K. S.; Feeney, W.; Chan, W. W. -S.; Chiu, Shuet-Hing L.; Smith, R. G. J. Med. Chem. **1998**, 41, 2439.