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STRUCTURE-ACTIVITY RELATIONSHIPS IN THE AMINO ACID SIDECHAIN OF L-692,429

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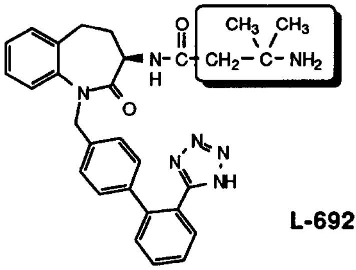
Abstract: Development of L-692,429, the prototype compound of a novel class of growth hormone (GH) secretagogues[1], focused on defining the structure-activity relationships in the amino acid sidechain. Modification of the dimethyl-β-alanine group revealed the basic amine as an essential pharmacophore for GH releasing activity. Evaluation of a variety of amino-substituted derivatives led to the identification of analogs with improved potency.

His-*D*-Trp-Ala-Trp-*D*-Phe-Lys-NH$_2$

GHRP-6

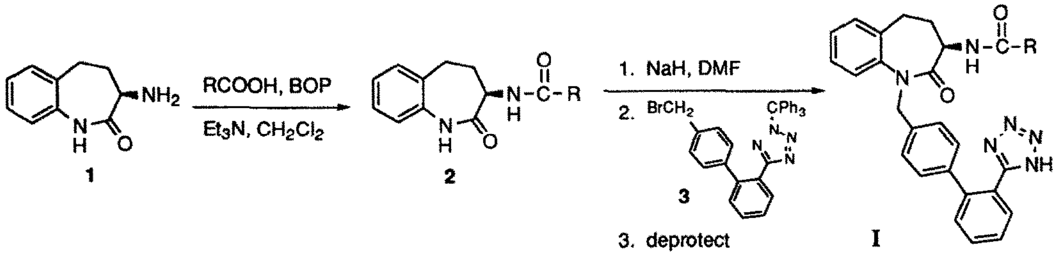


L-692,429

Introduction: Historically, treatment of growth hormone deficiency involved administration of either cadaver-derived or, more recently, recombinant human GH (rhGH). The inherent cost and inconvenience of rhGH has sparked interest in identifying alternative treatments. The strategy of stimulating endogenous GH release through the action of a GH secretagogue has emerged as an attractive therapeutic option to treatment with rhGH.[2] Among the more extensively studied GH secretagogues have been the growth hormone releasing peptides (GHRP's) identified by Bowers.[3] One of these compounds, GHRP-6, shows excellent activity and selectivity for GH release *in vitro* and *in vivo*. Consideration of the proposed bioactive conformations[4] of GHRP-6 provided a working hypothesis for the structural elements required in a non-peptidyl mimic. Directed screening of a variety of structures led to the discovery of the benzolactam class of GH secretagogues, of which L-692,429 is the prototype. Initial development of L-692,429, which focused on variation of the lactam ring, established the seven-membered benzazepinone skeleton as a preferred template.[5] Subsequent modification of the structure has concentrated on the amino acid sidechain. The amine of L-692,429 is an attractive focal point for chemical modification since it can serve as a precursor for a variety of structurally diverse analogs relevant to defining structure-activity relationships. In addition to optimizing GH releasing activity, modification of this portion of the molecule also provides an opportunity to manipulate physico-chemical properties such as basicity, solubility or lipophilicity. As shown in Tables 1-3, an assortment of analogs of L-692,429 have been studied that elucidate the structure-activity relationships of this essential pharmacophore.

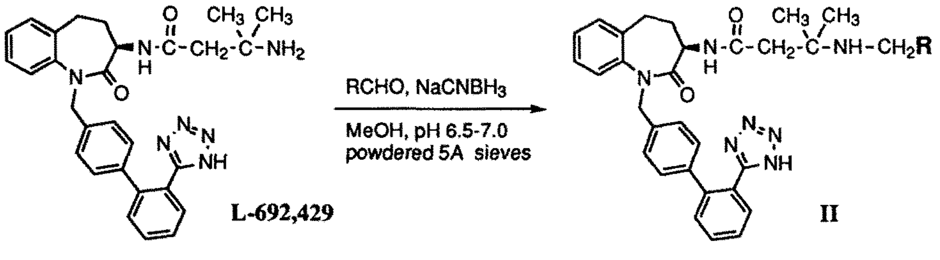
Synthesis: GH secretagogue analogs were constructed by stepwise attachment of amino acid and 2'-substituted biphenyl sidechains to a 3-aminobenzolactam nucleus. As shown in SCHEME I, the 3(*R*)-amino benzolactam **1**[5] was coupled with the desired carboxylic acid (or acid chloride[6]) in the presence of benzotriazol-1-yloxy*tris*(dimethylamino)phosphonium hexafluorophosphate ("BOP"). Compounds containing the dimethyl-β-alanine sidechain were prepared from 3-*t*-butoxycarbonylamino-3-methylbutanoic acid as described for the synthesis of L-692,429.[5] *N*-*t*-Butoxycarbonyl (BOC) or carbobenzyloxy (CBz) protected derivatives of amino acid sidechains were employed in the synthesis of compounds **9**-**17**. Alkylation of lactam **2** was carried out in dry DMF using 1.3 equivalents of sodium hydride followed by addition of bromide **3**[7] (1.3 eq) in DMF. The use of a slight excess of sodium hydride in the alkylation step is preferable, yet does not lead to *bis*-alkylation. Removal of trityl and BOC protecting groups by treatment with trifluoroacetic acid in methylene chloride or 6N hydrochloric acid in methanol, followed by reverse phase chromatography (C8) afforded product **I** in good overall yield. Removal of CBz groups was achieved by hydrogenolysis of a methanol solution of the substrate in the presence of 10% $Pd(OH)_2$ (30% w/w, 1 atm, RT, 1-3 h).

SCHEME I



N-Substituted derivatives **18**-**24** were prepared by reductive alkylation of L-692,429 with the appropriate aldehyde as shown in SCHEME II. In general, efficient conversion to the *N*-alkylated product **II** was achieved using a 5-fold excess of the aldehyde in methanol and carefully adjusting the pH to 6.5-7.0 *prior* to addition of sodium cyanoborohydride. Purification of the crude product mixture by reverse phase chromatography (C8) affords the products in 50-80% yield. The chiral hydroxypropyl derivatives **23** and **24** were synthesized by reductive alkylation of L-692,429 with (*R*)- or (*S*)-2-benzyloxypropanal, prepared from ethyl D- or L-lactate according to the procedure of Hanessian.[9]

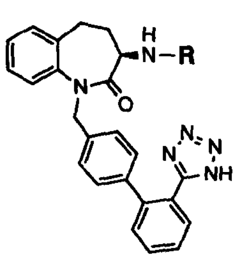
SCHEME II

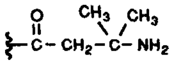
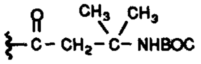
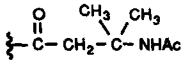
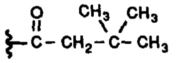
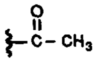


Results and Discussion

Growth hormone release *in vitro* was determined in rat pituitary cells as previously described.[8] Our initial efforts to elucidate the structure-activity relationships in the amino acid sidechain of L-692,429 focused on defining the role of the amine group. Presented in Table 1 are the results of several simple compounds that reveal fundamental structure-activity relationships of the amino acid sidechain.

Table 1



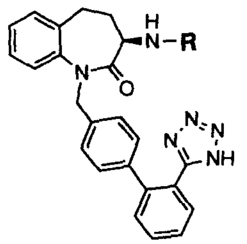
Compound	R	ED_{50} (μM)[a]
L-692,429	 $-C(=O)-CH_2-C(CH_3)_2-NH_2$	0.06
4	 $-C(=O)-CH_2-C(CH_3)_2-NHBOC$	inactive[b]
5	 $-C(=O)-CH_2-C(CH_3)_2-NHAc$	inactive[b]
6	 $-C(=O)-CH_2-C(CH_3)_2-CH_3$	inactive[b]
7	$-H$	9
8	 $-C(=O)-CH_3$	inactive[b]

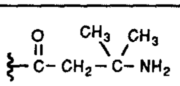
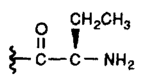
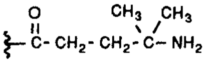
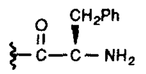
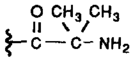
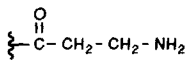
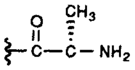
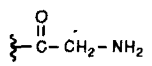
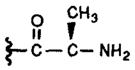
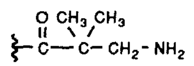
[a] Rat pituitary cell assay. [b] 20 μg/mL

Evident from the activities of compounds **4**-**8** is the absolute requirement of a basic amine element in maintaining GH releasing activity. Masking the amine as the *t*-butoxycarbonyl (BOC) derivative or by *N*-acetylation affords inactive analogs **4** and **5**, respectively. Similarly, isosteric replacement of the amino group by a methyl group, as in compound **6**, eliminates secretagogue activity. The weak activity exhibited by the truncated derivative **7** supports the hypothesis that a basic amine is an essential pharmacophore. The observed 150-fold difference in activity of **7** compared to L-692,429 also suggests that location of the amine with respect to the benzolactam nucleus is an important parameter in optimizing GH releasing activity. Presented in Table 2 are the results of a variety of analogs that help define the optimal amino acid sidechain of the benzolactam GH secretagogues.

Systematic modification of the dimethyl-β-alanine sidechain confirms that GH secretagogue activity is dependent on both chain length and substitution pattern. Examination of compounds **9** and **10** highlights the strong dependency on chain length. Although less basic (pK_a 7.9)[10] than L-692,429 (pK_a 9.3), a modest two-fold increase in potency is seen in the shortened α-methylalanine derivative **10**. In contrast, the homologated analog **9** shows a 50-fold loss in activity! Similar sensitivity to amine location is also observed in the corresponding six- and eight-membered lactam analogs of L-692,429, where secretagogue activities are reduced ten- and 100-fold, respectively.[5] Within the substituted alanine series (compounds **11**-**14**) several important structure-activity trends emerge. Comparison of the activities of the L-Ala derivative **11** with that of the D-Ala conjugate **12** reveals a preference for methyl substitution in the *pro-R* configuration. The observed ten-fold difference in activities is suggestive of differential binding affinity of the two diastereotopic methyl groups. An alternative explanation may be that methyl group occupying the *pro-R* position induces a conformational bias to more efficiently position the basic amine for receptor binding. Substitution with an ethyl group (compound **13**) reduces activity slightly while replacement with a benzyl group (**14**) results in a greater than 30-fold loss of potency. The results with compounds **12**, **13** and **14** seem to imply that the substituent adjacent to the carbonyl is, in fact, occupying a receptor binding site of limited volume. The importance of geminal dimethyl substitution flanking the basic amine is reflected by the poor activities of the unsubstituted β-alanine and glycine derivatives **15** and **16**, and the transposed isomer **17**.

Table 2



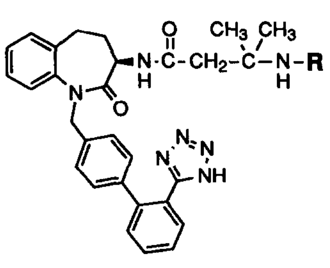
Compound	R	ED_{50} (μM)[a]	Compound	R	ED_{50} (μM)[a]
L-692,429	 $-C(=O)-CH_2-C(CH_3)_2-NH_2$	0.06	**13**	 $-C(=O)-CH(CH_2CH_3)-NH_2$	0.3
9	 $-C(=O)-CH_2-CH_2-C(CH_3)_2-NH_2$	3	**14**	 $-C(=O)-CH(CH_2Ph)-NH_2$	2
10	 $-C(=O)-C(CH_3)_2-NH_2$	0.03	**15**	 $-C(=O)-CH_2-CH_2-NH_2$	2
11	 $-C(=O)-CH(CH_3)-NH_2$	1.4	**16**	 $-C(=O)-CH_2-NH_2$	9
12	 $-C(=O)-CH(CH_3)-NH_2$	0.1	**17**	 $-C(=O)-C(CH_3)_2-CH_2-NH_2$	3

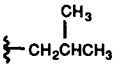
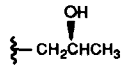
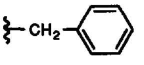
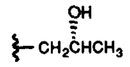
[a] Rat pituitary cell assay.

Effect of Amine Substitution

To further explore the structure-activity relationships in the amino acid sidechain of the benzolactam GH secretagogues, a series of analogs substituted on the amino group was examined. As illustrated in Table 3, substitution with simple alkyl groups, either linear (**18**) or branched (**19**), gives rise to analogs with potency comparable to the parent. The benzyl derivative **20** is equipotent to L-692,429 indicating that some steric bulk is tolerated in this region of the molecule. The insensitivity to alkyl substitution may also be exploited to append functionality that alters physico-chemical properties of the molecule without significantly affecting intrinsic secretagogue activity. Incorporation of an additional basic site in the structure, as in the aminoethyl derivative **21**, leads to a significant loss of activity. In contrast, addition of a hydroxyethyl group (**22**) produces a two-fold increase in potency. Subsequent development of this hydroxyethyl lead resulted in the identification of a series of highly potent hydroxyalkyl derivatives.[11] Among the more extensively studied analogs in this series are the hydroxypropyl derivatives **23** and **24**. The 3 nM ED_{50} of compound **24** (L-692,585) is significant in that it eclipses the activity of the peptidyl secretagogue, GHRP-6 (ED_{50} 10 nM).[8] The mechanism by which hydroxypropyl modification results in improved potency is unclear. The basicity of the amino group is practically unchanged; the pK_a of **24** is 9.1 as compared to 9.3 for L-692,429. The hydroxypropyl group may introduce a favorable conformational change to the secretagogue or may access an additional binding site on the receptor, or a combination of both phenomena. Although the (*R*)-hydroxypropyl moiety has proven to be a general potency-enhancing modification in combination with the β-alanine sidechains, an analogous transformation in the alanine series produces opposite results! For example, the (*R*)-hydroxypropyl derivative of compound **10** shows almost a ten-fold loss in activity, with an ED_{50} of 200 nM.[11] Work on the hydroxyalkyl series continues with the goal of resolving some of the paradoxes in the structure-activity relationships.

Table 3



Compound	R	ED_{50} (nM)[a]	Compound	R	ED_{50} (nM)[a]
L-692,429	$-H$	60	21	$-CH_2CH_2NH_2$	230
18	$-CH_2CH_2CH_3$	50	22	$-CH_2CH_2OH$	30
19	 $-CH_2\overset{CH_3}{C}HCH_3$	85	23	 $-CH_2\overset{OH}{C}HCH_3$	7
20		65	24	 $-CH_2\overset{OH}{C}HCH_3$	3

[a] Rat pituitary cell assay.

Summary

A variety of analogs of the GH secretagogue L-692,429 have been evaluated to define the structure-activity relationships in the amino acid sidechain. Systematic modification of functional groups permitted identification of the basic amine as an essential pharmacophore. Both chain length and substitution pattern of the amino acid sidechain were shown to be key factors in modulating secretagogue activity. Substitution on the amino group provided an opportunity to manipulate collateral physical properties without sacrificing secretagogue activity. Hydroxyalkyl substitution on the amino group of L-692,429 leads to significant improvement in potency *in vitro*. Additional details of the structure-activity work on this novel class of GH secretagogues will be presented in due course.

Acknowledgment

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References and Notes

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