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STRUCTURE-ACTIVITY RELATIONSHIPS OF THE NON-PEPTIDYL GROWTH HORMONE SECRETAGOGUE L-692,429

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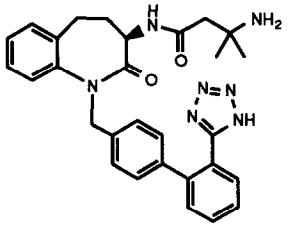
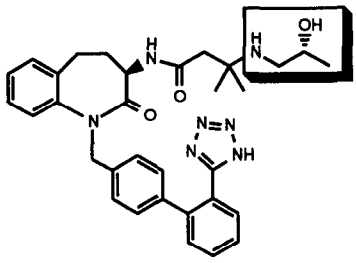
Abstract: Systematic investigation of the amino acid sidechain of L-692,429, the prototype of a novel class of benzolactam growth hormone (GH) secretagogues, has led to the preparation of L-692,585, a 2(R)-hydroxypropyl amino analog, which is twenty times more potent *in vitro* than L-692,429. Additional amino modifications reported here further define the structure-activity profile for L-692,429.

Potential clinical applications for growth hormone (GH) have expanded dramatically over the last few years with the availability of recombinant human growth hormone (rhGH). In addition to the treatment of GH deficient children and adults, rhGH has been shown to produce positive results in the treatment of burn patients, in patients with Turner's syndrome, in reversing the catabolic effects of glucocorticoid treatment and even in improving the exercise capacity of elderly patients.[1] Growth Hormone Releasing Peptide (GHRP-6) is a recently described hexapeptide that stimulates the secretion of GH in animals and in humans via a mechanism different from the natural secretagogue - growth hormone releasing factor (GRF).[2,3] Recent reports from these laboratories[4-6] have described the discovery of L-692,429 as a novel non-peptidyl mimic of the hexapeptide GHRP-6. In clinical studies to date, L-692,429 has been a highly effective and selective GH secretagogue.[7] Preliminary structure-activity studies on the amino acid sidechain of L-692,429 have led to the identification of the 2(R)-hydroxypropyl substituent found in L-692,585 as a potency enhancing functionality.[8,9] This communication describes additional amino modifications that further define the structure-activity profile of the amino substituent in L-692,585.



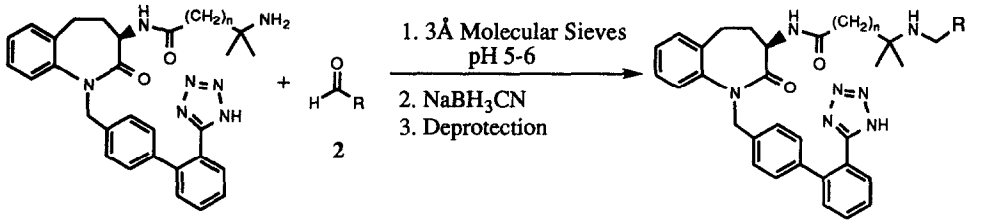
L-692,429



L-692,585

Synthesis: All compounds studied here were prepared by modification of the terminal amino group of L-692,429 [6] (or its shortened α-methylalanine derivative **18** [8]) utilizing a reductive amination method as the key bond forming reaction. The general synthetic approach is outlined in **SCHEME 1** and the general reaction procedure is as follows: To a solution of L-692,429 (**1**, n=1) or its α-methylalanine derivative (**1**, n=0) and an appropriate aldehyde **2** (3-5 eq.) in dry methanol was added 3Å molecular sieves (3/1 w/w sieves/amine) and the pH of the resulting mixture was carefully adjusted to 5-6 with acetic acid. After the mixture was stirred for 30 min, sodium cyanoborohydride (6 eq.) in THF was added and stirring was continued for an additional 3 h. The reaction mixture was filtered, the filtrate quenched with acetic acid, concentrated and purified by reverse phase (C8) chromatography (MPLC) to give the protected alcohol. The protecting group was removed to afford the alkylated product **3** either by hydrogenolysis (10 % Pd/C, H_2, 40 psi) for a benzyl group (**6-8**, **10-13**, **19-22**) or by hydrolysis for such groups as ester (**5**), acetonide (**14**), silyl (**15**) or tetrahydropyranyl (**16**).

SCHEME 1

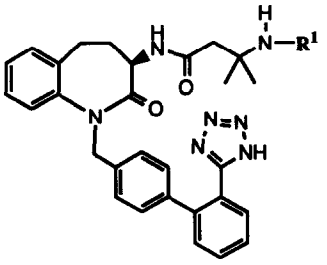


Glyoxylic acid ethyl ester was used for the preparation of compounds **4** and **5**. The synthesis of the diastereomers **6** and **7** was accomplished by using (S)- or (R)-2-benzyloxypropanal[10,11]. The chiral polyhydroxyl derivatives **14** and **15** were synthesized with (R)-2,3-O-isopropylideneglyceraldehyde[12] or 4-O-(*tert*-butyldimethylsilyl)-2,3-O-isopropylidene-D-threose[13], respectively. Compound **26** was obtained by the treatment of an 1:1 diasteromeric mixture of **6** and **7** with excess diethylamino sulfur trifluoride (DAST) in HF•Pyridine (-78°C→rt) for two days. Reverse-phase chromatography was used for the separation and purification of these compounds (C8, CH_3OH/0.1% aqueous CF_3COOH). Final compounds were obtained as the trifluoroacetate salts in 40-70% overall yield from **1**.

Results and Discussion

Growth hormone release *in vitro* was determined in rat pituitary cells as previously described.[14] Tables **1-3** summarize additional structure-activity relationships established for the potency enhancing 2(R)-hydroxypropyl amino substituent found in L-692,585. The insensitivity of amine substitution to simple

Table 1



Compound	R^1	ED_{50} (nM)[a]	Compound	R^1	ED_{50} (nM)[a]
L-692,429	-H	60	**11**	-CH_2CH(OH)$CH(CH_3)_2$	1000
4	-CH_2COOH	weakly[b] active	**12**	-CH_2CH(OH)$CH(CH_3)_2$	200
5	-$CH_2CO_2CH_2CH_3$	56	**13**	-CH_2CH(OH)-Ph	1000
6	-CH_2CH(OH)CH_3	7	**14**	-CH_2CH(OH)CH_2OH	10
7 (L-692,585)	-CH_2CH(OH)CH_3	3	**15**	-CH_2CH(OH)·CH(OH)CH_2OH	40
8	-CH_2CH_2OH	30	**16**	-CH_2CH_2CH(OH)CH_3	10
9	-$CH_2CH_2OCH_2Ph$	weakly[b] active	**17**	-CH_2CH_2CH(OH)CH_3	weakly[b] active
10	-CH_2C(OH)$(CH_3)_2$	10			

[a] Rat pituitary cell assay
[b] At 1 μM drug concentration

alkyl substituents (e.g., N-benzyl/N-propyl are equipotent with the unsubstituted analog L-692,429) has been demonstrated previously.[8] Addition of functional groups to this N-alkyl sidechain has been found to have dramatic effects on GH releasing activity. While carboxylic acid substitution, **4**, is detrimental to bioactivity, its corresponding ethyl ester **5** is equipotent with L-692,429. In contrast, hydroxy substitution of this alkyl chain has been shown to be potency enhancing.[8] The 2-hydroxypropyl amino sidechain (e.g., in **6** and **7**) yields

compounds 10-20 fold more potent than L-692,429. The critical contribution of the methyl group in the 2-hydroxypropyl sidechain in **6** and **7** is revealed by comparison to the 2-hydroxyethyl derivative **8** which is 5 to 10-fold less active. Although the absence of a strong stereochemical preference for one stereoisomer over the other is surprising, it is supported by the nearly equipotent activity of the *gem* dimethyl analog **10.** Larger substituents than methyl (e.g., isopropyl analogs **11, 12** and phenyl analog **13**) lead to analogs that are significantly less active indicating a steric component in this region (also suggested by benzyl ether derivative **9**). Polyhydroxylation of this N-alkyl sidechain, as exemplified by diol **14** and triol **15,** resulted in a modest decrease in GH releasing activity. Homologation of the potent 2-hydroxypropyl analogs **6** and **7** afforded the 3-hydroxybutyl derivatives **16** and **17.** In contrast to the diastereomeric analogs **6** and **7,** a surprising disparity in GH releasing activity is observed in the 3-hydroxybutyl series. The (R) isomer **17** was found to be only weakly active at 1 μM whereas the (S) isomer **16** is nearly equipotent to **6** and **7.**

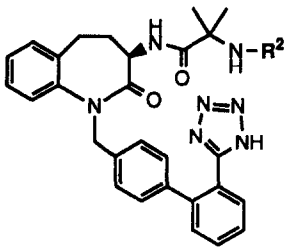


Table 2

Compound	R^2	ED_{50} (nM)[a]
18	-H	30
19	-CH$_2$C(OH)HCH$_3$	300
20	-CH$_2$C(OH)HCH$_3$	700
21	-CH$_2$CH$_2$C(OH)HCH$_3$	100
22	-CH$_2$CH$_2$C(OH)HCH$_3$	50

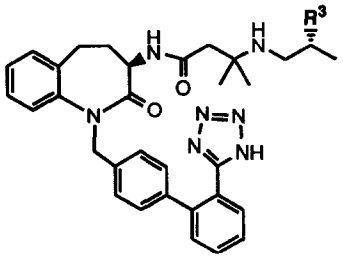
[a] Rat pituitary cell assay

It has been reported that the shorter α-methylalanine analog **18** was two-fold more potent than L-692,429 in GH releasing activity.[8] Table 2 summarizes efforts to incorporate the potency-enhancing hydroxyalkyl sidechains into the more potent α-methylalanine series. Attachment of the preferred 2-hydroxypropyl sidechains into **18** afforded the diastereomers **19** and **20** which exhibited significantly less GH releasing activity than **18.** Since analogs **19** and **20** may not be able to position the hydroxy group optimally because of their shorter α-methylalanine sidechain, the longer 3-hydroxybutyl derivatives were prepared. While the 3-hydroxybutyl analogs **21** and **22** are more potent than the 2-hydroxypropyl derivatives **19** and **20,** they are still less active than the parent **18** and significantly less active than the L-692,429 analogs **6** and **7** in spite of

their identical chain length. Apparently, the α-methylalanine sidechain cannot align the hydroxyalkyl substituent into its optimal position on the receptor.

The mechanism through which the 2(R)-hydroxy function in L-692,585 exerts its potency enhancing effects is not clear and is particularly difficult to address without the availability of a receptor binding assay. However, structural modifications directly on L-692,585 (Table 3) have yielded some insights. Formation of methyl ether **23** resulted in a 7-fold decrease in GH releasing activity while the benzyl ether analog **24** is essentially inactive, consistent with its being too sterically demanding (cf. **9**). Likewise, acetate **25** and fluoro analog **26** are less active. The data are consistent with the hydroxy function in L-692,585 being responsible, presumably through the formation of a hydrogen bond with the receptor, for its greater GH releasing activity relative to L-692,429 or the N-propyl analog **27** [8].

Table 3



Compound	R^3	ED_{50} (nM)[a]
L-692,585	-OH	3
23	$-OCH_3$	20
24	$-OCH_2Ph$	weakly[b] active
25	$-OCOCH_3$	200
26	-F	90[c]
27	-H	50

[a] Rat pituitary cell assay [b] At 1 μM concentration
[c] Mixture of diastereomers

Summary

The structure-activity relationship for the potency enhancing 2(R)-hydroxypropyl amino substituent in L-692,585 has been further investigated. Through systematic modifications of chain length, substituents, and stereochemical orientation, the 2(R)-hydroxypropyl amino substituent has been identified as the preferred amino substituent for the prototype non-peptidyl GH secretagogue L-692,429. The structure-activity relationships established for the 2(R)-hydroxypropyl group are consistent with the hydroxyl function in L-692,585 being responsible for its greater GH releasing activity. Attempts to adapt this potency enhancing group or its variants to the more potent, α-methylalanine analog **18**, resulted in a decrease in GH releasing activity.

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