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BENZOLACTAM GROWTH HORMONE SECRETAGOGUES: REPLACEMENT OF THE C-3 AMIDE BOND IN L-692,429

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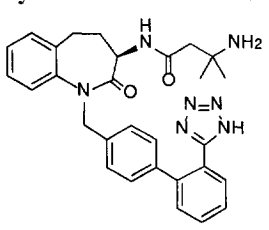
Abstract: The synthesis and structure-activity relationships of various C-3 amide bond modifications in the novel nonpeptidyl growth hormone secretagogue L-692,429 are described. Several C-3 amide surrogates were prepared and the urea moiety was found to exhibit growth hormone releasing activity similar to that observed with L-692,429.

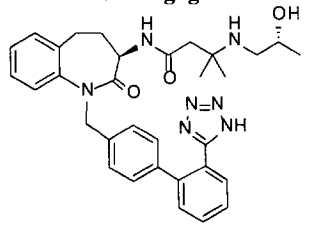
During the past decade, the availability of recombinant human growth hormone (rhGH)[2] and the identification of a growth hormone-releasing factor (GRF)[3] has resulted in renewed interest in potential therapeutic applications of growth hormone (GH).[4] In addition, a series of growth hormone releasing peptides (GHRPs), which are mechanistically distinct from GRF but also specifically release GH from the pituitary, has been discovered.[5] Extensive studies on these peptides have resulted in the identification of the potent growth hormone releasing hexapeptide, GHRP-6 (His-*D*- Trp-Ala-Trp-*D*-Phe-Lys-NH_2) and related congeners.[6]

A novel, nonpeptidyl benzolactam class of growth hormone secretagogues, which mimic the hexapeptide GHRP-6, was first reported in 1993.[7] L-692,429, a prototype secretagogue of this class, stimulates GH release in a dose-dependent manner in vitro and synergizes with the naturally occurring GRF, but acts through an alternative signal transduction pathway. Although the clinical results to date with L-692,429 were very promising,[8] its low oral bioavailability and modest potency in animal models prompted us to further investigate the structure-activity relationships associated with this benzolactam lead. Since the C-3 amide bond might be a contributing factor to the low bioavailability, alternative C-3 amide bond surrogates (**1**) were investigated and reported in this paper.

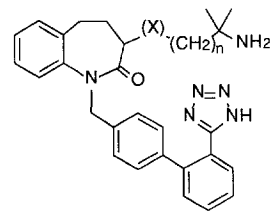
Nonpeptidyl Benzolactam Growth Hormone Secretagogues



L-692,429
ED_{50} 0.06 μM



L-692,585
ED_{50} 0.003 μM

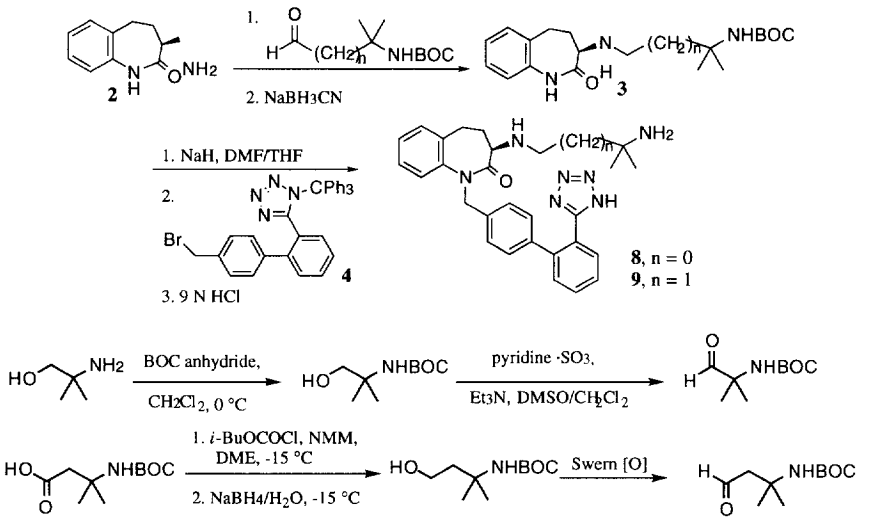


1

Chemistry: The syntheses of the benzolactam growth hormone secretagogues L-692,429 and L-692,585 have already been reported.[9,10] Analogs of L-158,432 (racemic) and L-692,429 with amide bond modifications at the C-3 position were prepared from known intermediates as follows.

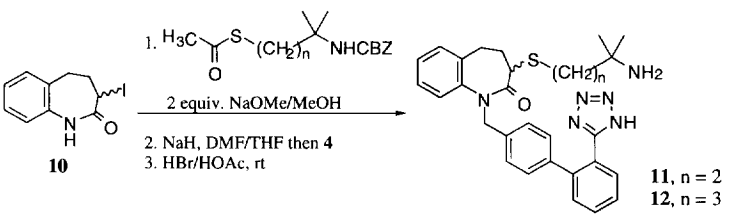
Scheme I illustrates the synthesis of amine derivatives that were prepared by utilizing a reductive amination as the key step. Chiral 3-(R)-aminobenzolactam **2**[11] and the requisite aldehyde prepared from the corresponding amino alcohol were reacted in dry methanol with 3 Å molecular sieves to give the imine which was then reduced with sodium cyanoborohydride in THF. Alkylation of benzolactam **3** at N-1 was carried out in dry DMF using a slight excess of sodium hydride followed by addition of bromide **4** in DMF. Removal of the trityl and BOC protecting groups was accomplished by treatment with 9 N hydrochloric acid to afford compounds **8** and **9**.

Scheme I



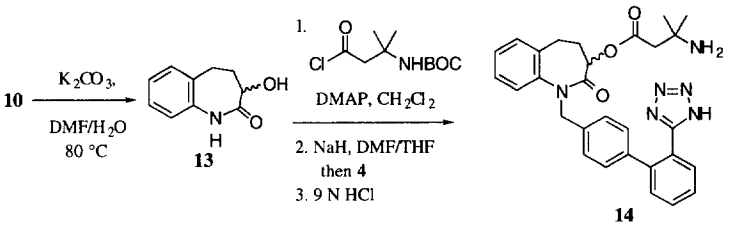
For the corresponding thioether analogs, 3-iodobenzolactam **10**[11] was treated with sodium thioates prepared in situ followed by the alkylation procedure described above (Scheme II). Removal of the terminal amine CBZ and trityl protecting group with HBr/AcOH yielded the final compounds **11** and **12**.

Scheme II



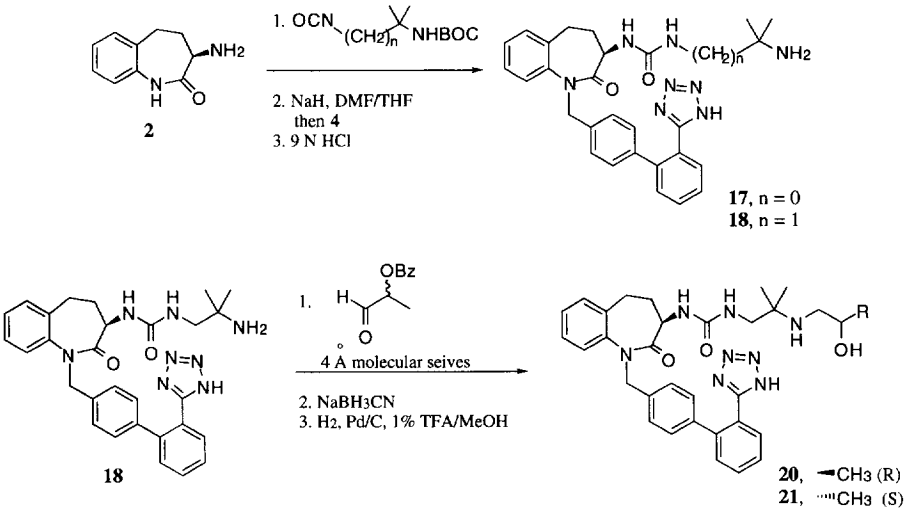
The ester analog **14** was also prepared from 3-iodobenzolactam **10** as shown in Scheme III. Treatment of **10** with potassium carbonate in wet DMF gave 3-hydroxybenzolactam **13**, which was coupled with 3-*t*-butoxycarbonylamino-3-methylbutanoyl chloride to give the intermediate ester which was unmasked in the usual fashion to give the final compound **14**.

Scheme III



The preparation of C-3 urea analogs is shown in Scheme IV. The required isocyanates were freshly prepared by treatment of the corresponding amines with triphosgene in the presence of triethylamine.[12] 3-(R)-Aminobenzolactam **2** was reacted with these isocyanates and the resulting ureas were alkylated and deprotected as above to give the desired products **17** and **18**. The two diastereomeric 2-hydroxypropyl derivatives **20** and **21** were prepared by subsequent reductive alkylation of the terminal amine of **18** with either (R)- or (S)-2-benzyloxypropanal, respectively, followed by hydrogenolysis of the benzyloxy group.[10]

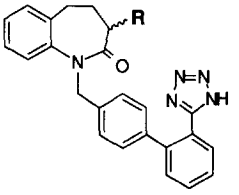
Scheme IV

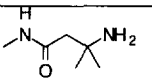
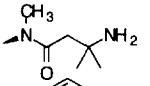
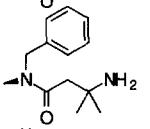
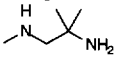
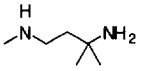
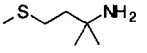
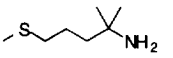
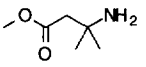


Results and Discussion: Growth hormone release in vitro was measured using rat pituitary cells as previously described.[7] Table 1 illustrates the critical nature of the C-3 amide bond for this class of GH secretagogues. Alkylation of the C-3 amide bond (**6** and **7**)[13] leads to a substantial decrease in GH releasing activity. Steric and conformational factors caused by the lack of N-H bonding to the receptor may contribute to

this attenuation of biological activity. Removal of the amide carbonyl (e.g., **8** and **9**) also resulted in loss of GH releasing activity as did replacement with the thioether linkage (**11** and **12**). In addition, the ester replacement **14** showed a drastic reduction in activity. These results identified the -NHCO- group as a critical pharmacophore for receptor activity.

Table 1

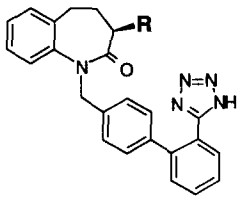


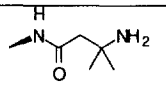
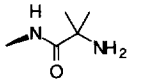
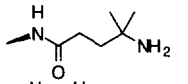
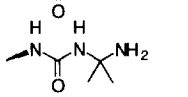
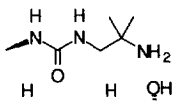
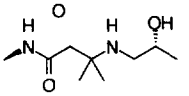
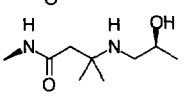
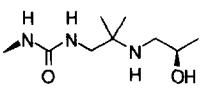
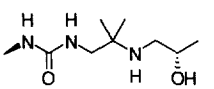
Compound	R	ED_{50} (μM)[a]
L-158,432		0.12
6		7
7		Inactive[b]
8		1
9		Weakly Active[c]
11		6
12		7
14		Weakly Active[c]

[a] Rat pituitary cell assay [b]At 10 μM [c]At 1 μM

Based on the above findings, a series of C-3 sidechain urea derivatives was proposed that would attempt to maintain the hydrogen bonding capabilities of the C-3 amide bond (Table 2). The 2-amino-2-methylpropylurea analog **18** was found to exhibit comparable GH releasing activity with the parent amide analog L-692,429, although the conformational constraints of the urea requires the longer amino alkyl chain to maintain equivalent biological activity (cf, L-692,429 vs. **17** and **16** vs. **18**). The functional data presented here strongly suggest that the C-3 -NHCO- bond in this class of GH secretagogues forms a critical hydrogen bond with its receptor. Thus, the urea moiety, which still maintains the critical N-H for high receptor affinity but might have enhanced pharmacological properties, was further investigated.

Table 2



Compound	R	ED_{50} (μM)[a]
L-692,429		0.06
15		0.03
16		3
17		Weakly Active[b]
18		0.1
L-692,585		0.003
19		0.007
20		Weakly Active[b]
21		2.5

[a]Rat Pitutary cell assay [b]At 10 μM

It has previously been reported[10] that substitution on the amino group of L-692,429 with the 2-hydroxypropyl substituent significantly enhanced the potency of L-692,429, the R-isomer (L-692,585) being twice as effective as the S-isomer **19**. Therefore, we incorporated this potency-enhancing substituent into our most promising analog **18**. As shown in Table 2, the attachment of either of the chiral 2-hydroxypropyl groups resulted in a significant decrease in GH releasing activity and only the S-isomer **21** shows modest activity. This is most likely due to subtle changes in binding interactions with the secretagogue receptor for the longer urea sidechain relative to the shorter amide sidechain found in L-692,429 and L-692,585.

In summary, the structure–activity relationships of the amide bond at C-3 of the benzolactam nucleus in L-692,429 have been examined. Amide bond modifications (e.g., $-NHCH_2-$, $-SCH_2-$, -OC(O)- and alkylation of the amide nitrogen) significantly attenuated the GH releasing activity in the rat pituitary cell assay. These results illustrate the critical role that the amide N-H hydrogen bond plays in the biological activity of L-692,429 and led to the identification of the urea derivative **18** as an effective amide bond replacement at the C-3 position of the benzolactam nucleus. However, unlike with L-692,429, the addition of the potency enhancing 2-hydroxypropyl group to the terminal amine as in the urea derivative **21** resulted instead in a significant decrease in GH releasing activity.

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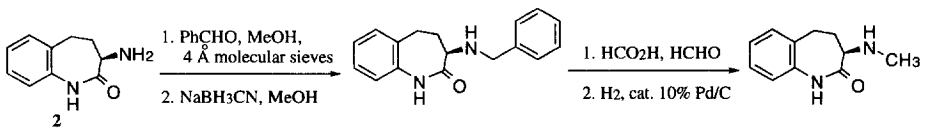
References and Notes

1. Current Address: Institute For Chemistry, Bayer Corp., 400 Morgan Lane B24, West Haven, Ct 06516
2. Strobl, J. S.; Thomas, M. J. *Pharmacological Reviews* **1994**, *46*, 1.
3. Thorner, M. O. *Acta Paediatr. Suppl.* **1993**, *388*, 2.
4. Schoen, W. R.; Wyvratt, M. J.; Smith, R. G. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed; Academic: San Diego, 1993; Vol. 28, Chapter 19.
5. Low, L. C. K. *Neuroendocrinology* **1991**, *53*, 37.
6. Bowers, C. Y. *J. Pedatr. Endocrinol.* **1993**, *6*, 21.
7. 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.
8. 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.
9. 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. For a review see: DeVita, R. J.; Wyvratt, M. J. *Drugs of the Future*, **1996**, *21*, 273.
10. Ok, D.; Schoen, W. R.; Hodges, P.; DeVita, R. J.; Brown, J. E.; Cheng, K.; Chan W. W. -S.; Butler, B.; Smith, R. G.; Fisher, M. H.; Wyvratt, M. J. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2709.
11. Armstrong, J. D. III; Eng, K. K.; Keller, J. L.; Purick, R. M.; Hartner, F. W., Jr.; Choi, W. B.; Askin, D.; Volante, R. P. *Tetrahedron Lett.* **1994**, *35*, 3239.
12. Majer, P.; Randad, R. S. *J. Org. Chem.* **1994**, *59*, 1937.
13. Compounds **6** and **7** were prepared by the same protocol reported in reference 9 except for using N-3 alkylated benzolactams prepared from **2** by utilizing a reductive amination method with the requisite aldehyde.



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