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Benzolactam Growth Hormone Secretagogues: Replacements for the 2'-Tetrazole Moiety of L-692,429

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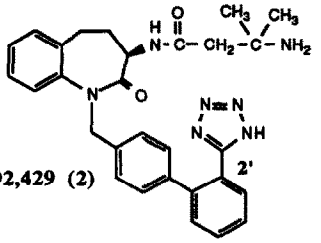
Abstract: A variety of 2'-biphenyl substituents was investigated as replacements for the 2'-tetrazole moiety of the non-peptidyl growth hormone secretagogue L-692,429. The 2'-carboxamide **22** and N-2-hydroxypropyl tetrazoles **7a,b** were identified as neutral pharmacophores with similar potency to that of the acidic 2'-tetrazole. N-Alkyl tetrazoles, sulfonamides and N-acyl sulfonamides were generally less potent 2'-replacements.

His-*D*-Trp-Ala-Trp-*D*-Phe-Lys-NH_2

GHRP-6 (1)

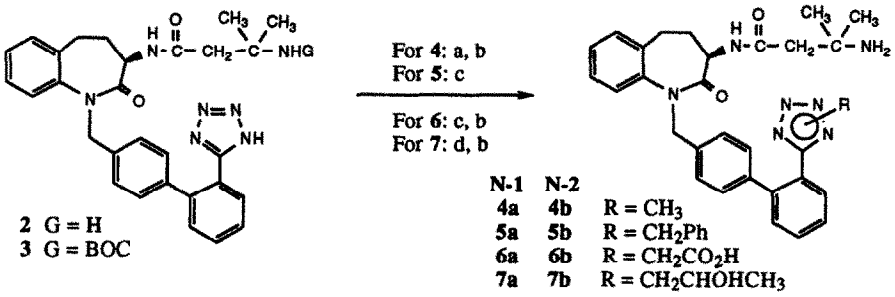


L-692,429 (2)

Introduction: The current clinical treatment for growth hormone (GH) deficiency in children is *intramuscular* or *subcutaneous* administration of recombinant human growth hormone (rhGH) or its endogenous releasing factor (GRF).[1] Several growth hormone releasing peptides, the most interesting of these being GHRP-6 (**1**), promote the release of endogenous GH from the pituitary via a novel mechanism.[2] A non-peptidyl mimic of GHRP-6, L-692,429 (**2**), has recently been reported[3] and shown to release growth hormone in humans.[4] Both GHRP-6 and L-692,429 provide a potential alternative therapy to direct treatment with rhGH. In order to manipulate the physico-chemical properties and study the structure-activity relationships of L-692,429, we sought to modify the zwitterionic nature of the molecule. Since the structure-activity relationships of the benzolactam template[5] and the amino acid sidechain[6] of L-692,429 have been explored and the amine sidechain was found to be essential for biological activity, a suitable functionalization or replacement of the acidic 2'-tetrazole pharmacophore was studied. This Letter reports the investigation of modifications at the 2'-biphenyl position of L-692,429 and the identification of the carboxamide pharmacophore as an equipotent replacement for the tetrazole moiety of L-692,429.

Synthesis: The preparation of the benzolactam growth hormone secretagogues from known intermediates is outlined in Schemes I and II.[7] The alkylated tetrazoles were prepared from L-692,429 (**2**)[5] or **3** by treatment with diazomethane or an alkyl halide and triethylamine[8] in dichloromethane then deprotection to give the products **4** and **5** as a mixture of N-1 and N-2 isomers. These were easily separable by flash chromatography or reverse phase medium pressure liquid chromatography (RP MPLC) on C-8 (Scheme I). The tetrazole acetic acids **6** were prepared from compound **2** by treatment with t-butyl bromoacetate followed by deprotection with trifluoroacetic acid and anisole in dichloromethane to afford the separable isomers **6a** and **6b**. Treatment of the BOC-protected L-692,429 (**3**) with propylene oxide followed by acidic deprotection gave the 2-hydroxypropyl tetrazole isomers **7a** and **7b**.

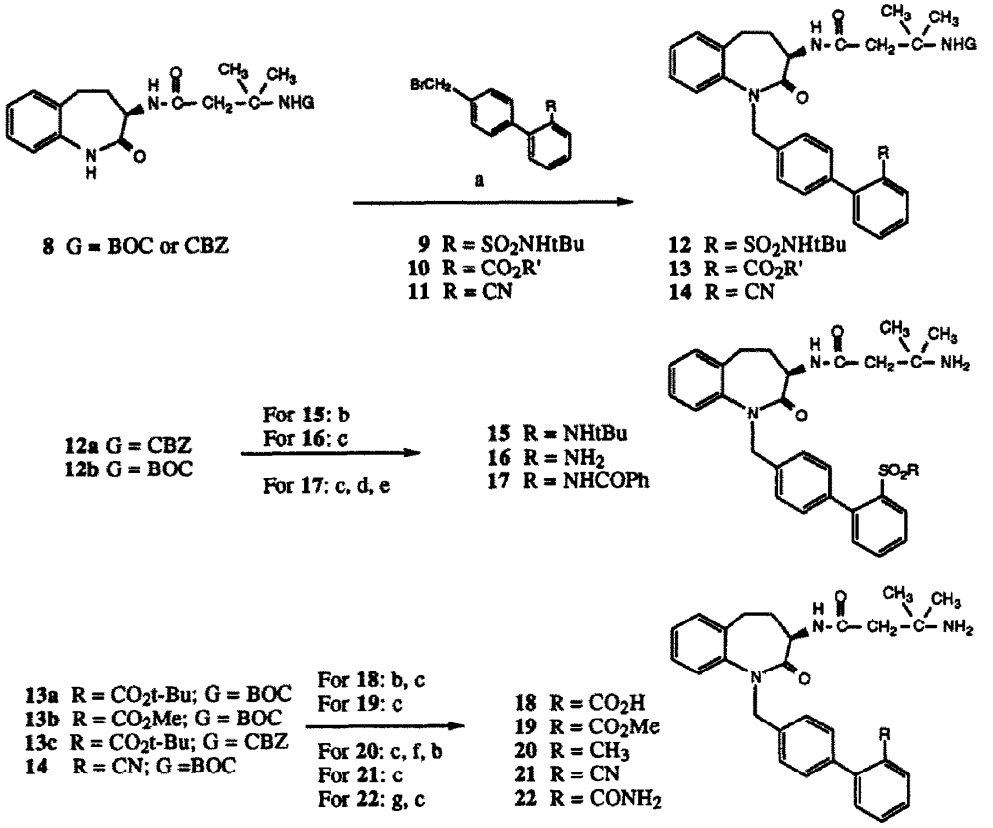
Scheme I



Reagents: (a) CH_2N_2; (b) TFA, anisole, CH_2Cl_2; (c) TEA, RX; (d) Propylene oxide, MeOH, 24hr.

Analogs of L-692,429 with other functional groups at the 2'-position were prepared from the protected benzolactam intermediates **8** (G = BOC)[5] and **8** (G = CBZ) containing the dimethyl β-alanine sidechain as shown in Scheme II. Deprotonation of the benzolactam nitrogen with sodium hydride in anhydrous dimethylformamide followed by addition of the known 4-bromomethyl biphenyl 2'-t-butyl sulfonamide **9** (R = SO_2NHtBu),[9a] 2'-esters **10** (R = CO_2t-Bu[5] or CO_2Me), or 2'-nitrile **11** (R = CN)[9b] afforded the intermediates **12**, **13** and **14** in high yield. The t-butyl sulfonamide derivative **15** was prepared from intermediate **12a** by deprotection of the CBZ-group. The unsubstituted sulfonamide **16** was prepared from the BOC-protected intermediate **12b** by treatment with trifluoroacetic acid. The acyl sulfonamide **17** was prepared by treatment of intermediate **12a** with TFA, acylation of the resulting primary sulfonamide with the acyl imidazolide of benzoic acid[9c] followed by removal of the CBZ-group. The 2'-carboxylic acid analog **18** and the methyl ester **19** were prepared by TFA removal of the BOC-group and t-butyl ester of intermediate **13a** (R' = t-Bu, G = BOC) or just the BOC-group of intermediate **13b** (R' = Me, G = BOC), respectively. The 2'-methyl analog was prepared from the t-butyl ester intermediate **13c** (R'= t-Bu, G = CBZ) by TFA ester cleavage, reduction of the mixed anhydride of the resulting acid followed by hydrogenolysis of the CBZ-group with concomitant benzylic alcohol reduction. The nitrile analog **21** was prepared from the BOC-protected nitrile **14** by treatment with TFA while the carboxamide **22** was prepared by initial hydrolysis of the nitrile to the amide with basic hydrogen peroxide[10] followed by amine deprotection.

Scheme II



Reagents: (a) NaH, DMF, RT, 1 hr; (b) $H_2/Pd(OH)_2$, MeOH, 1 hr; (c) TFA, anisole, CH_2Cl_2, 1 hr; (d) $PhCO_2H$, CDI, DBU, THF; (e) HBr, HOAc; (f) N-methyl morpholine, isobutyl chloroformate, DME, 0°C; then $NaBH_4$; (g) 30% aq. H_2O_2 , cat. K_2CO_3, DMSO, RT; 24 hr.

Results and Discussion

Growth hormone release *in vitro* was measured in rat pituitary cells as previously described.[11] Initial efforts to change the zwitterionic nature of L-692,429 centered on modifications of its 2'-tetrazole substituent. Simple alkylation of the tetrazole ring replaces the acidic hydrogen of zwitterionic L-692,429. It was hoped that such a modification would alter the physico-chemical parameters of the molecule while retaining the GH releasing activity. Results are shown in Table 1. Simple methylated analogs of L-692,429, the N-1 and N-2 tetrazole regioisomers **4a** and **4b**, were respectively 8-fold and 5-fold less potent *in vitro* while the more sterically demanding N-benzyl analogs **5a** and **5b** were even less effective for GH release. The results suggested the necessity of the acidic character or the hydrogen bonding capacity of the tetrazole. The tetrazole acetic acid analogs **6a** and **6b** were also less potent, although the N-2 isomer was at least 5-fold more potent that the N-1

isomer. In contrast, the 2-hydroxypropyltetrazole analogs **7a** and **7b** were found to be approximately equipotent to the parent tetrazole L-692,429 although these analogs are no longer zwitterionic. It is possible that the recovery in potency is due to a new interaction of the 2-hydroxyl group at the receptor since the simple alkyl analogs (e.g. **4**, **5**) were much less potent secretagogues in comparison.[12]

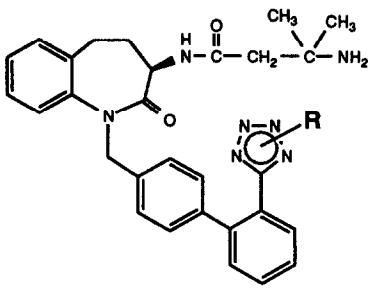


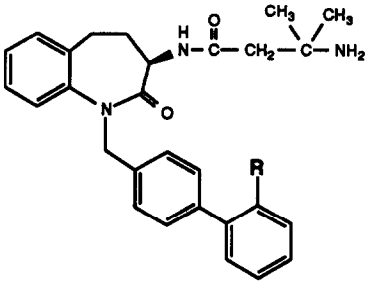
Table 1

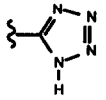
Compound	Isomer	R	ED_{50} (μM)[a]
L-692,429 (2)		H	0.06
4a	N-1	CH_3	0.5
4b	N-2	CH_3	0.3
5a	N-1	CH_2Ph	0.7
5b	N-2	CH_2Ph	>1
6a	N-1	CH_2CO_2H	>1
6b	N-2	CH_2CO_2H	0.2
7a	N-1	$CH_2CHOHCH_3$	0.05
7b	N-2	$CH_2CHOHCH_3$	0.05

[a] Rat pituitary cell assay.

Table 2 shows the results of a different approach that focused on replacing the 2'-tetrazole moiety of L-692,429 with a non-heterocyclic pharmacophore. A series of different 2'-substituents was prepared and tested for *in vitro* potency. The initial compounds explored were the 2'-sulfonamides which had been utilized successfully as tetrazole replacements in structurally similar AII antagonists.[9a] The t-butyl sulfonamide **15** and the primary sulfonamide **16** were much less potent secretagogues *in vitro*. Even the more acidic N-acyl sulfonamide **17** has reduced potency. Other functional groups were also tested as shown in Table 2. The 2'-carboxylic acid **18**,[3] the neutral 2'-methyl ester **19**, the 2'-methyl analog **20** and the 2'-nitrile **21** all exhibited weak secretagogue activity relative to the 2'-tetrazole analog. The fact that acyl sulfonamide **17** and the carboxylic acid **18** are much less potent than the tetrazole **2** in spite of their similarity in pKa's and the decreased potency of the alkylated tetrazoles led us to believe that the key binding interaction of the 2'-pharmacophore involves the hydrogen bonding capacity of the tetrazole. To test this hypothesis, the 2'-carboxamide **22** was prepared and was found to be approximately equipotent to L-692,429. The net charge of this secretagogue structure has now changed from zwitterionic in the case of L-692,429 to cationic in the case of the protonated form of carboxamide **22**. Thus, the 2'-carboxamide substituent, which possesses the ability to donate or accept a hydrogen bond, has been identified as a suitable, neutral surrogate for the 2'-tetrazole of L-692,429.[12]

Table 2



Compound	R	ED_{50} (μM)[a]
L-692,429 (2)		0.06
15	SO_2NHtBu	4
16	SO_2NH_2	3
17	$SO_2NHCOPh$	3
18	COOH	3
19	$COOCH_3$	2[b]
20	CH_3	1
21	CN	5[b]
22	$CONH_2$	0.08

[a] Rat pituitary cell assay. [b] racemic

Summary

Modifications of the 2'-tetrazole of the benzolactam growth hormone secretagogue L-692,429 have been investigated. Alkylation of the 2'-tetrazole ring leads to a large decrease in potency except for the unique N-(2-hydroxypropyl)tetrazole analogs **7a** and **7b**. Many other 2'-substituents have been investigated as replacements for the 2'-tetrazole moiety of L-692,429 including the carboxylic acid and the acyl sulfonamide, both of which have comparable acidity to that of the tetrazole, yet are significantly less potent pharmacophores. The 2'-carboxamide analog **22** has been identified as an equipotent neutral replacement for the 2'-tetrazole which eliminates the zwitterionic nature of the parent. Further studies of 2'-tetrazole replacements for the benzolactam series of growth hormone secretagogues including the structure-activity relationships of the 2'-carboxamide series[12] will be reported later.

Acknowledgment

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9. (a) For preparation of the sulfonamide intermediate **9** see Naylor, E.M.; Chakravarty, P.K.; Costello, C.A.; Chang, R.S.; Chen, T.-B.; Faust, K.A.; Lotti, V.J.; Kivlighn, S.D.; Zingaro, G.J.; Siegl, P.K.S.; Wong, P.C.; Carini, D.J.; Wexler, R.R.; Patchett, A.A.; Greenlee, W.J. *BioMed. Chem. Lett.* **1994**, *4*, 69-74. (b) For preparation of nitrile **11** see U.S. Patent 5,260,235, **1993** and E.P.O 324,377, **1990**. (c) Drummond, J.T.; Johnson, G. *Tetrahedron Lett.* **1988**, *29*, 1653.

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