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INVESTIGATION OF BIOISOSTERS OF THE GROWTH HORMONE SECRETAGOGUE L-692,429.

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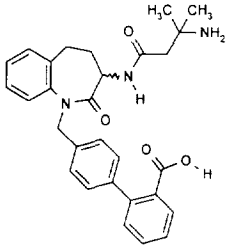
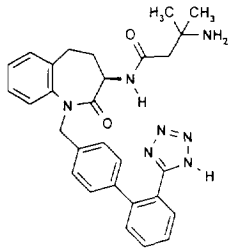
Abstract: The synthesis and structure-activity relationships of several analogs of L-692,429 with modifications in the biaryl and tetrazole moieties are described and several derivatives were found to be equipotent or slightly more potent than L-692,429. © 1997 Elsevier Science Ltd.

Introduction: Human growth hormone (hGH) has been used in the treatment of growth hormone deficient children for decades. However, following the introduction of recombinant hGH, a number of studies now point to new potential clinical applications of rhGH. In addition to the classical indications, rhGH has been shown effective in the treatment of burns, in patients with Turners syndrome, to reverse catabolic conditions and in reversing some of the effects of age. Instead of direct hGH replacement therapy it appears attractive to use hGH-secretagogues, in particular if an oral route of administration is possible. Short peptide sequences (GHRP's) that release GH *via* a distinct mechanism are known and recently a receptor with affinity to GHRP's has been cloned.[1] The prototype GHRP-6[2] is a hexapeptide, with the amino acid sequence His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2. Recently, a nonpeptide GH Secretagogue L-692,429[3] (optimized from the lead compound L-158,077)[4] have shown efficacy and specificity in the release of GH in clinical trials.



1 (L-158,077)



2 (L-692,429)

An increase in potency was observed when carboxylic acid **1** (EC_{50} = 2500 nM) was replaced by tetrazole (**2**; EC_{50} = 125 nM). Consequently we established a program to investigate a variety of bioisosters of the biarylic

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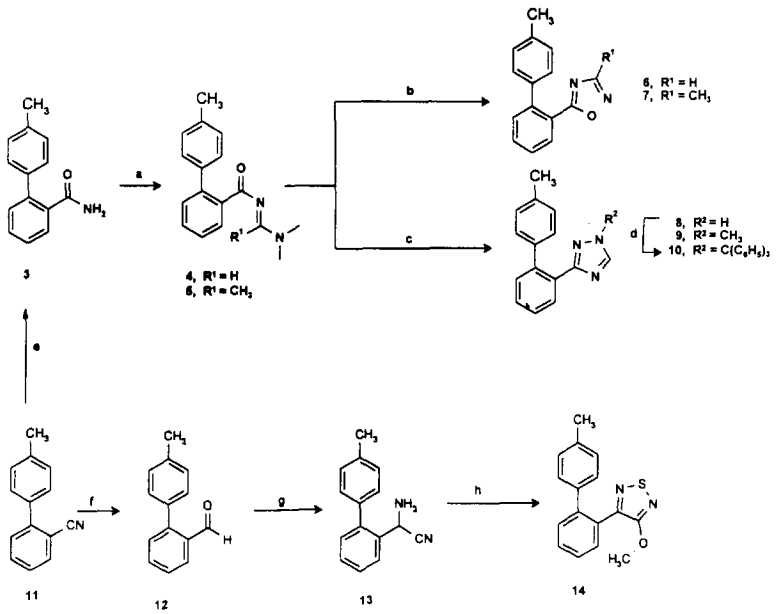
part of the molecule.[5,6,7] From this program we now report two series of compounds. One series in which the tetrazole moiety of **2** is replaced by other 5-membered ring systems and one series in which the outer phenyl moiety is replaced by heteroaromatic moieties.

Chemistry: The schemes 1 through 5 illustrate various procedures for the preparation of bioisosteric analogs of L-692,429. According to scheme 1 the incorporation of triazoles could be achived *via* the *N*'-acyl-*N,N*-dimethylamidines **4** and **5** prepared by reactions of 2-(4-methylphenyl)benzamide **3** with *N,N*-dimethylformamide dimethyl acetal or *N,N*-dimethylacetamide dimethyl acetal.[8] The obtained acylamidines reacted readily with hydroxylamine or hydrazines in a mixture of 5 N sodium hydroxide, acetic acid and dioxane and cyclized upon heating to the 1,2,4-oxadiazoles **6** and **7** or 1,2,4-triazoles **8**, **9** and **10**.

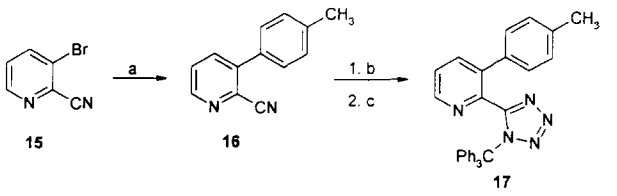
Scheme 1



Reagents: a) $(CH_3)_2NC(R^1)(OCH_3)_2$, Δ ; b) $NH_2OH{\cdot}HCl$, 5 N NaOH, CH_3COOH, Dioxane; c) $NH_2NHR^2{\cdot}$ HCl, 5N NaOH, CH_3COOH, Dioxane; d) $(C_6H_5)_3CCl$, Et_3N, Acetone; e) H_2SO_4, Δ ; f) DIBAH; g) NH_3/NaCN; h) S_2Cl_2 then MeONa, MeOH

In order to synthesize the 1,2,5-thiadiazole derivative **14** (scheme 1), **11** was reduced with diisobutylaluminum hydride to the corresponding aldehyde **12**, which was subjected to a Strecker synthesis to form the cyanoamine **13**. Reaction with sulfur monochloride[9,10] and subsequent treatment with sodium methoxide in methanol furnished the 3-methoxy-1,2,5-thiadiazole **14**. The pyridine derivative **17** was synthesized starting with 3-bromo-2-cyanopyridine **15**,[11] which was reacted with 4-tolueneboronic acid and tetrakis triphenylphosphinepalladium as catalyst to give the biaryl **16**.[12,13] Reaction with trimethyltin azide, followed by protection with tritylchloride furnished the tetrazole **17**.

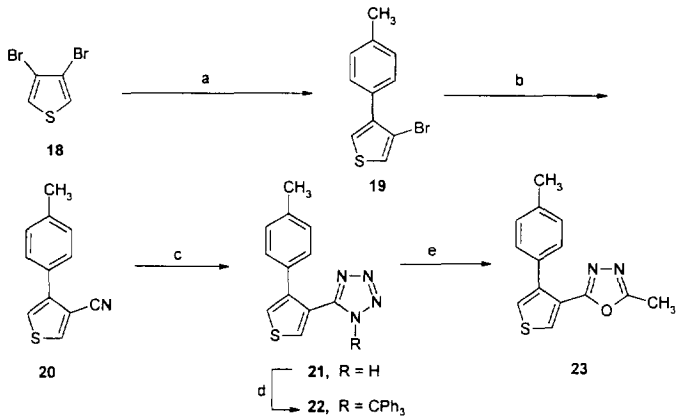
Scheme 2



Reagents: a) 4-toluneboronic acid, $Pd(PPh_3)_4$, b) $(CH_3)_3SnN_3$, c) CPh_3Cl, Et_3N

The synthesis of the 3,4-disubstituted thiophene analog **21** proceeded from 3,4-dibromothiophene **18** *via* a Suzuki coupling followed by substitution with copper(I)cyanide and subsequent cycloaddition with trimethyltin azide to **21** or to the more soluble tritylprotected **22**.[14] Trimethyltin azide proved to be the reagent of choice for the formation of the tetrazole ring system. The unsubstituted tetrazole **21** gave the oxadiazole **23** in excellent yield by reaction with acetic anhydride - a reaction which probably has a nitrilimine as intermediate.[15]

Scheme 3



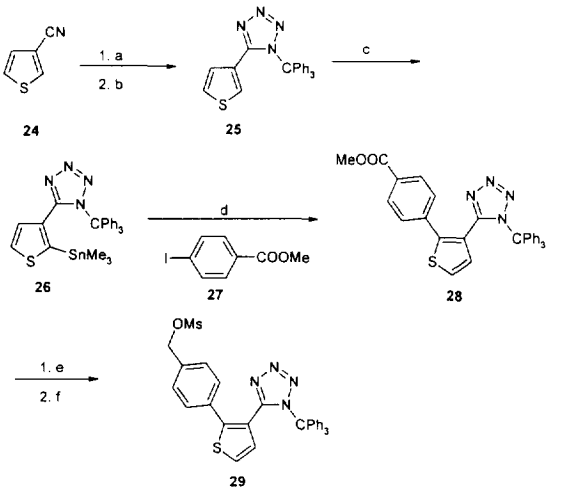
Reagents: a) 4-toluneboronic acid, $Pd(PPh_3)_4$, Toluene, b) CuCN, quinidine, c) Me_3SnN_3 then HCl, d) CPh_3Cl, Et_3N, e) Ac_2O

The 2,3-disubstituted thiophene isomer **29** was prepared from 3-cyanothiophene **24** *via* a cycloaddition with trimethyltin azide and subsequently metalated with BuLi and quenched with trimethyltin chloride to give **26**.[13] The Stille reaction to **28** followed by reduction and mesylation afforded **29**.

Treatment of 4-aminotoluidine with 2,5-dimethoxytetrahydrofuran **30** in glacial acetic acid gave the pyrrole **31**. Formylation of **31** followed by treatment with hydroxylamine hydrochloride and acetic anhydride gave the nitrile **32**. The tetrazole derivative **33** was obtained by a 1,3-dipolar cycloaddition with trimethyltin azide followed by treatment with anhydrous hydrogen chloride to give the free tetrazole, [16] which was subsequently protected with triphenylmethyl chloride in the presence of triethylamine.

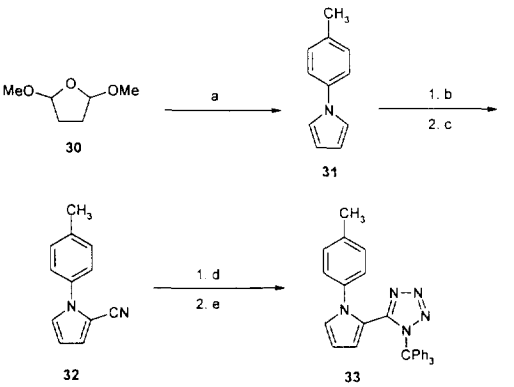
The obtained oxadiazoles **6, 7** and **23,** triazoles **8, 9** and **10**, thiadiazole **14,** tetrazoles **17, 22** and **33** were brominated with N-bromosuccinimide (NBS) in carbon tetrachloride in the presence of a catalytic amount of azoisobutyronitrile (AIBN) at reflux. The triazole **8** was protected with triphenylmethylchloride in acetone in the presence of an equivalent amount of triethylamine *prior* to bromination under similar conditions.

Scheme 4



Reagents: a) Me_3SnN_3 then HCl, b) CPh_3Cl, Et_3N, c) BuLi then Me_3SnCl, d) $Pd(PPh_3)_2Cl_2$, DMF, e) $LiAlH_4$, f) MsCl, Et_3N

Scheme 5

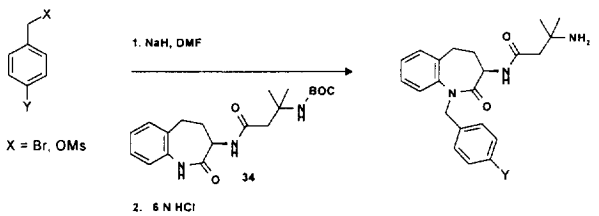


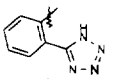
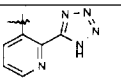
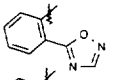
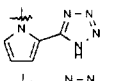
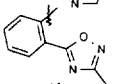
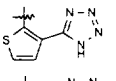
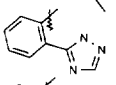
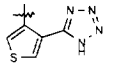
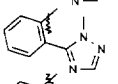
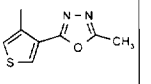
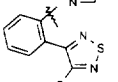
Reagents: a) 4-Aminotoluidine, AcOH, b) DMF, $POCl_3$, c) $NH_2OH{\cdot}HCl$, Ac_2O, d) Me_3SnN_3, HCl, e) CPh_3Cl, Et_3N

NMR showed variation in dibromination from 0% (for **6** and **9**) to 30% (for **8**), but the crude product was carried on to the next step without further purification. The obtained bromides and mesylate **29** were added to a suspension of **34**[16] and sodium hydride in dimethylformamide or potassium hydroxide in dimethylsulfoxide followed by deprotection with 6 N hydrogen chloride to give the desired compounds.[17]

Results and Discussion: Growth hormone release *in vitro* was determined in rat pituitary cell assays by a modified method of Heiman[18] and Sartor[19]. All results presented, are the mean of minimum two separate experiments. In each assay two positive controls (L-692,429 and GHRP-6) were used. The potency (EC_{50}) of the compounds was determined as the concentration inducing half of maximal stimulation of the GH release. The potency of L-692-429 and GHRP-6 was determined to 125 nM and to 2 nM, respectively.

Table 1



Compound	Y	EC_{50} (nM)[a]	Compound	Y	EC_{50} (nM)[a]
2		125	40		2350
35		30	41		275
36		1050	42		140
37		90	43		150
38		265	44		125
39		1800			

[a]Rat Pituitary cell assay

The investigation of the biraryl function of **1** focused on two parts: The replacements of the tetrazole/carboxylic acid group with various 5-membered ring systems, and replacement of the biphenyl with heteroaromatic biaryls as shown in Table 1. Since the tetrazole group is a well known carboxylic acid isostere it was our initial belief that the tetrazole acted as a hydrogen bonding donor. However, as seen in Table 1 the oxadiazole **35** and the triazole **37** showed slightly higher affinity (EC_{50} = 30 nM and EC_{50} = 90 nM) suggesting that the tetrazole acts as a hydrogen bonding acceptor. Considering the less acidic nature of tetrazole compared to carboxylic acid this observation may also explain the higher affinity of **2** compared to **1**. The more bulky analogs **36**, **38** and **39** showed lower affinity suggesting limited space in the receptor binding site corresponding to the tetrazole moiety. The pyrrole analog **41** and thiophene analogs **42** and **43** showed slightly lower potency indicating acceptance of heteroatoms in the biarylic moiety. On the other hand the pyridine analog **40** showed a significant decrease in potency. This may result from a limited flexibility (i.e. planar pyridine-tetrazole system) caused by the potentiel tautomerism due to the amino acid like nature of the pyridine-tetrazole system. Since the results indicated an acceptance of thiophene in the biarylic system and an acceptance of bioisosters as tetrazole analogs we were prompted to incorporate an oxadiazole containing the biarylic system. As shown the analog **44** showed similar activity as the original compound **2**.

In summary, the structure-activity relationships of the biaryl-tetrazole system of L-692-429 demonstrated here, suggest that a non-polar aromatic moiety and a hydrogen bonding acceptor of various kinds may indeed replace the original biaryltetrazole moiety.

Reference and Notes

[1] Howard, A.D.; Feighner, S.D.; Cully, D.F.; Arena, J.P.; Liberator, P.A.; Rosenblum, C.I.; Hamelin, M.; Hreuniuk, D.L.; Palyha, O.C.; Anderson, J.; Paress, P.S.; Diaz, C.; Chou, M.; Liu, K.K.; McKee, K.K.; Pong, S.-S.;Chaung, L.-Y.; Elbrecht, A.; Dashkevicz, M.; Heavens, R.; Rigby, M.; Sirinathsinghji, D.J.S.; Dean, D.C.; Melillo, D.G.; Patchett, A.A.; Nargund, R.; Griffin, P.R.; DeMartino, J.A.; Gupta, S.K.; Schaeffer, J.M.; Smith, R. G.; Van der Ploeg, L.H.T. *Science* **1996**, *273*, 974

[2] Jaffe, C.A.; Ho, P.J.; Demott-Friberg, R; Bowers, C.Y.; Barkan, A.L. *J. Clin. Endocrinol. Metab.* **1993**, *77*, 1641.

[3] Gertz, B.J.; Barrett, J.S.; Eisenhandler, R.; Krupa, D.A.; Wittreich, J.M.; Seibold, J.R.; Schneider, S.H. *J. Endocrinol. Metab.* **1993**, *77*, 1393.

[4] 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.

[5] DeVita, R. J.; Schoen, W. R.; Fischer, M. H.; Frontier, A. J.; Pisano, J. M.; Wyvratt, M. J.; Cheng, K.; Chan, W. W.-S.; Butler, B. S.; Hickey, G. J.; Jacks, T. M.; Smith, R. G. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2249.

[6] Chu, L.; Mrozik, H.; Fisher, M. H.; Brown, J. E.; Cheng, K.; Chan, W. W.-S.; Schoen, W. R.; Wyvatt, M. J.; Butler, B. S.; Smith, R. G. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2245.

[7] DeVita, R. J.; Wyvatt, M. J. *Drugs of the Future*, **1996**, *21*, 273.

[8] Lin, Y.-i.; Lang, S.A.; Lovell, M.F.; Perkinson, N.A. *J. Org. Chem.* **1979**, *44*, 4160.

[9] Weinstock, L. M.; Davis, P.; Handelsman, B.; Tull, R *J. Org. Chem.* **1967**, *32*, 2823.

[10] Weinstock, L. M.; Davis, P.; Handelsman, B.; Tull, R. *Tetrahedron Lett.* **1966**, 1263.

[11] Sakamoto, T.; Kaneda, S.; Nishimura, S.; Yamanaka, H. *Chem. Pharm. Bull.* **1985**, *33*, 565.

[12] Alo, B. I.; Kandil, A.; Patil, P. A.; Sharp, M. J.; Siddiqui, M. A.; Snieckus, V.; Josephy, P. D. *J. Org. Chem.* **1991**, *56*, 3763.

[13] Casalnuovo, A. L.; Calbrese, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 4324.

[14] Allen, E.E:; Rivero, R.A.; Kevin, N. US.Pat 5,198,438.

[15] Shawali, A.S. *Chem. Rev.* **1993**, *93*, 2731.

[16] Bovy, P.R..; Reitz, D.B.; Collins, J.T.; Chamberlain, T.S.; Olins, G.M.; Corpus, V.M.; McMahon, E.G.; Palomo, M.A.; Koepke, J.P.; Smits, G.J.; McGraw, D.E.; Gaw, J.F. *J.Med.Chem.* **1993**, *36*, 101-110.

[17] Schoen, W.R.; Pisano, J.M.; Prendergast, K.; Wyvratt, M.J.; Fischer, M.H.; Cheng, K.; Chan, W.W.-S.; Butler, B.; Smith, R.G.; Ball, R.G. *J. Med. Chem.* **1994**, *37*, 897.

[18] Heiman, M.L.; Nekola, M.V.; Murphy, W.A.; Lance, V.A.; Coy, D.H. *Endocrinology* **1985**, *116*, 410.

[19] Sartor, O.; Bowers, C.Y.; Chang, D. *Endocrinology* **1985**, *116*, 952.

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