Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 2067–2072
Bioorganic & Medicinal Chemistry Letters

Optimization of 1*H*-tetrazole-1-alkanenitriles as potent orally bioavailable growth hormone secretagogues

Andrés S. Hernández,[a,*] Stephen G. Swartz,[a] Dorothy Slusarchyk,[b] Mujing Yan,[b] R. Krishna Seethala,[b] Paul Sleph,[b] Gary Grover,[b] Kenneth Dickinson,[b] Leah Giupponi,[b] Timothy W. Harper,[c] W. Griffith Humphreys,[c] Daniel A. Longhi,[b] Neil Flynn,[b] Brian J. Murphy,[b] David A. Gordon,[b] Scott A. Biller,[a] Jeffrey A. Robl[a] and Joseph A. Tino[a,*]

[a]*Discovery Chemistry, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543, USA*
[b]*Metabolic Disease Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543, USA*
[c]*Pharmaceutical Candidate Optimization, Bristol-Myers Pharmaceutical Research Institute, Princeton, NJ 08543, USA*

Received 11 September 2007; revised 25 January 2008; accepted 25 January 2008
Available online 31 January 2008

Abstract—1*H*-Tetrazole-1-alkanenitrile ***SR*-9g** exhibits a >10-fold in vivo potency enhancement over the lead nitrile **1** and has acceptable oral bioavailability in rats and dogs. An enantiospecific synthesis of 1*H*-tetrazole-1-alkanenitrile nitriles **9** has been developed.

Both growth hormone-releasing hormone (GHRH)[1] and the more recently discovered growth hormone secretagogues (GHS)[2] induce growth hormone (GH)[3] release from the pituitary upon binding, respectively, to the GHRH receptor[4] and the GHS receptor (GHS-R1a). The earliest reported GHS were oligopeptides, called growth hormone-releasing peptides (GHRPs), such as GHRP-6.[5] Several orally bioavailable small peptidomimetic GHS[6] were synthesized before ghrelin was identified as the endogenous GHS receptor ligand.[7]

A series of small molecule orally active GHS described in the literature[8] contain a dipeptide formed from *O*-benzyl-D-serine or its carbon- analogue, *R*-2-amino-5-phenylpentanoic acid, and 2-methyl alanine as a common feature. Structure–function studies[9] of ghrelin suggest this dipeptide may correspond to the N-terminus of the GHRPs and ghrelin. Members of the above series shown in Figure 1 are MK-677,[6b] CP-424391,[10] LY 444711[11] and the two tetrazoles **1** and **2** previously reported by us.[12]

The differences in polarity and lipophilicity between a cyano and a phenyl group of tetrazoles **1** and **2** suggest that the propanonitrile and phenylpropyl moieties may project into different binding pockets. We sought to coalesce the two structures to generate a high-affinity agonist. Accordingly a set of 1*H*-tetrazole analogs **9a**–**9d** were prepared bearing a variety of branched alkyl chains capped with phenyl and cyano in the hope of identifying the spacers that would maintain the optimal orientation of each substituent. This paper describes the SAR elucidation for these analogs, culminating with the identification of GHS agonists exhibiting >10-fold greater in vivo potency compared to that of **1**.

Mitsunobu promoted alkylation of the chiral *S*-tetrazole **6** with racemic alcohols **3**–**5** generated 1*H*-tetrazoles **7a**–**7d** along with the corresponding isomeric 2*H*-tetrazoles **8a**–**8d**.[12] As outlined in Scheme 1, alcohol **3** was readily obtained from a commercially available ketone; alcohols **4** and **5** were prepared as shown from the indicated esters. Following separation of the tetrazole regioisomeric mixture by column flash chromatography,[13] TFA-mediated cleavage of the BOC-protecting group of

Keywords: Growth hormone secretagogues; Tetrazole; Enantiospecific synthesis.
* Corresponding authors. Tel.: +1 609 818 7127; fax: +1 609 818 6810 (A.S.H); e-mail: andres.hernandez@bms.com

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.01.100

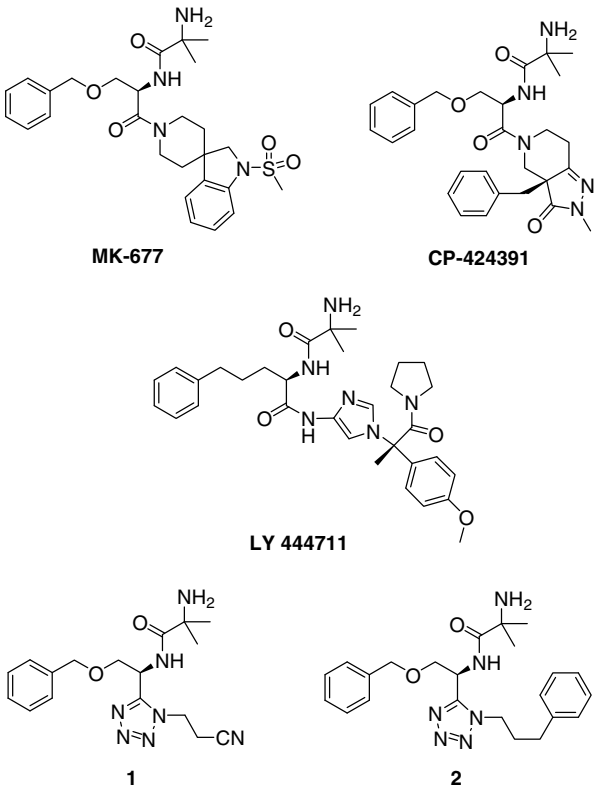
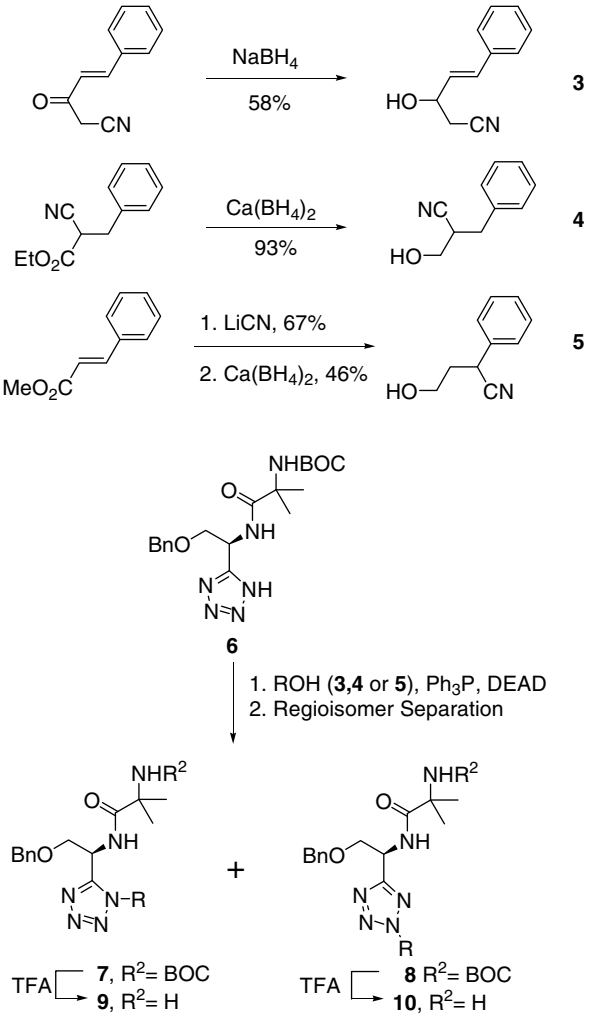


Figure 1. GHSs sharing a dipeptide structural motif.



Scheme 1. Preparation of alcohols **3**, **4** and **5**, and synthesis of tetrazole-alkanenitriles **9** and **10**.

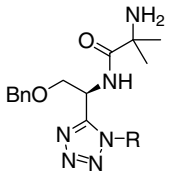
1*H*-tetrazoles **7a**, **7c** and **7d** yielded chiral mixtures of *SR* and *SS* diastereomeric tetrazoles **9a**, **9c** and **9d**. Hydrogenation (5% Pt/C) of the diastereomeric mixture **7a** followed by BOC cleavage generated saturated analogs **9b**. Although the mixture of *SR* and *SS* diastereomers **9a** and **9b** were separated into single epimers by reverse phase HPLC, the separation of the diastereomeric mixtures **9c** and **9d** was not attempted given their lack of in vitro activity relative to **9a** and **9b**. The BOC-protected mixture of 2*H*-tetrazole **8a** was separated into single chiral diastereomers by reverse phase HPLC and then converted to single epimers **10a**. The reduced 2*H* analogs **10b** were synthesized from single epimers **8a** by hydrogenation (5% Pt/C) followed by BOC cleavage. TFA treatment converts 2*H*-tetrazole **8c** and **8d** to the chiral diastereomeric mixture of *SR* and *SS* 2*H*-tetrazoles **10c** and **10d**.

Table 1 summarizes the results for nitrile analogs **9** and **10** in the in vitro binding and functional assays.[12] Since functional activity of these full agonists was 10- to 75-fold greater than binding affinity for the GHS receptor, the SAR discussion will focus on the functional data. Only diastereomer 1 of 1*H*-tetrazoles **9a** and **9b** offered any promise of meeting the criteria for enhanced activity, as they exhibited 60- and 40-fold greater agonist potency relative to **1**. This finding is consistent with the existence of two binding sites, since only the spacers of **9a** and **9b** are sufficiently long to allow the tethered phenyl and cyano moieties to simultaneously assume the preferred conformation of **1** and **2**. As would be expected given the three-dimensional nature of the binding site, the binding affinity and in vitro potency for one of the chiral diastereomers of **9a** and **9b** vastly exceeds that of the other. In other words, all the activity resides in just one diastereomer of the diastereomeric pair comprising **9a** or **9b.** The observation that **9c** and **9d** exhibit comparable in vitro agonist activity to **1** and **2** suggests that their spacers allows either the phenyl or the cyano but not both to assume the optimal orientation.

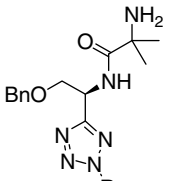
In agreement with previous SAR,[12] the 2*H*-tetrazole series is consistently less potent than the corresponding 1*H* regioisomer. The analogs in this series exhibit the following order in decreasing potency: **10a > 10b** ≈ **10d > 10c**. The difference in activity between epimers of a same 2*H* regioisomer **10** is far smaller than the differences found for the corresponding 1*H* regioisomer **9**. This observation infers that neither 2*H* diastereomer binds in an optimal fashion.

Evaluation of the more active single chiral diastereomers of **9a** and **9b** in the acute IV rat model[12] revealed that the respective 60- and 40-fold enhanced in vitro potency relative to the parent **1** collapsed to only a 3-fold improvement in ED_{250}[14] at best (Table 2). *C*log *P* increased from −0.3 for **1** to approximately 2 upon incor-

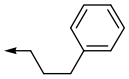
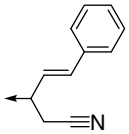
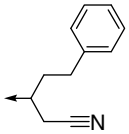
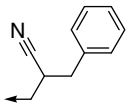
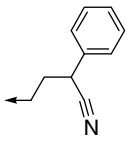
Table 1. In vitro activity of tetrazole nitriles **9** and **10**



9



10

Compound	R	Substitution	Diastereomer	K_i[a] (nM)	EC_{50}[a,b] (nM)
1		1*H*	NA	2940	30
2		1*H*	NA	200	17
9a		1*H*	1	12	0.5
		1*H*	2	737	62
10a		2*H*	1	453	19
		2*H*	2	2510	202
9b		1*H*	1	63	0.8
		1*H*	2	1380	139
10b		2*H*	1	2560	150
		2*H*	2	3940	244
9c		1*H*	1/1 mix	298	22
10c		2*H*	1/1 mix	>5000	2470
9d		1*H*	1/1 mix	611	27
10d		2*H*	1/1 mix	>5000	302

[a] See Ref. 12 for detailed description.
[b] All compounds are full agonists.

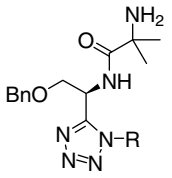
poration of a 2-phenylethene/2-phenylethyl substituent in **9a** and **9b**. This 200-fold predicted increase in lipophilicity may have adversely impacted ADME properties thereby accounting for the fact that the in vivo potency of **9a** and **9b** relative to **1** does not reflect the in vitro differential in functional activity (Table 2). In an attempt to modulate the increase in liphophilicity while enhancing in vitro potency, analogs **9e–9h** were prepared containing heteroatoms or polar functionality.

A previously developed regiospecific route[12] was employed to yield only the 1*H*-tetrazoles of analogs **9e–9h**. At this stage the SAR was not sufficiently developed to confidently state whether the *SR* or *SS* diastereomer was preferred. Consequently, (±)-methyl aziridine-2-carboxylate was utilized to prepare racemic 3-amino-butanonitrile **14** thereby insuring formation of both diasteremers (Scheme 2).

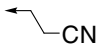
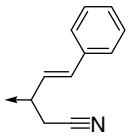
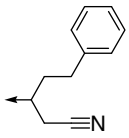
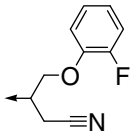
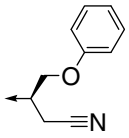
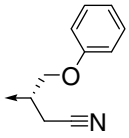
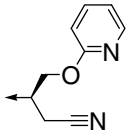
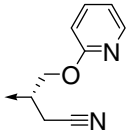
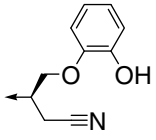
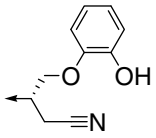
Methyl aziridine-2-carboxylate was BOC-protected and then reduced with calcium borohydride to afford alcohol **11** in 77% yield. Mitsunobu reaction of **11** with phenols **12e**, **12f**, **12h** or 2-hydroxypiridine (**12g**) provided the aryl ethers **13e**, **13f**, **13h** or the heteroaryl ether **13g** in 44–80%. The conversion to **13g** was particularly inefficient because of competitive N-alkylation. Cyanide addition to aziridines **13** followed by BOC cleavage furnished the 3-amino-butanonitriles **14** in an average 71% yield. Subsequent conversion of the racemic amines **14** to a mixture of *SR* and *SS* diastereomeric 1*H*-tetrazole nitriles **9e–9h** is summarized in Scheme 2. The benzyloxyphenol **15h** was quantitatively converted to its related phenol by hydrogenolysis before BOC cleavage. Following separation by reverse phase HPLC, evaluation of the individual diastereomers revealed that the faster eluting epimer was consistently more active.[15]

The absolute stereochemistry of the more active epimer was assigned following enantiospecific conversion of chiral amines **14** to individual epimers of **9** using the methodology of Scheme 2. The enantiospecific synthesis of amines ***R*-14** from readily available oxazolidine ***R*-16**

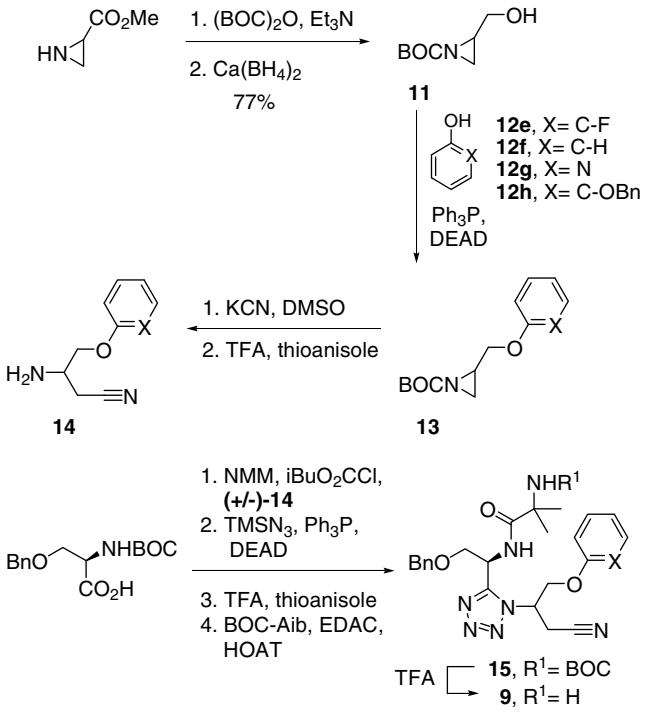
Table 2. Correlation between in vitro activity, in vivo activity and liphophilicity for 1*H*-tetrazole nitriles **9**



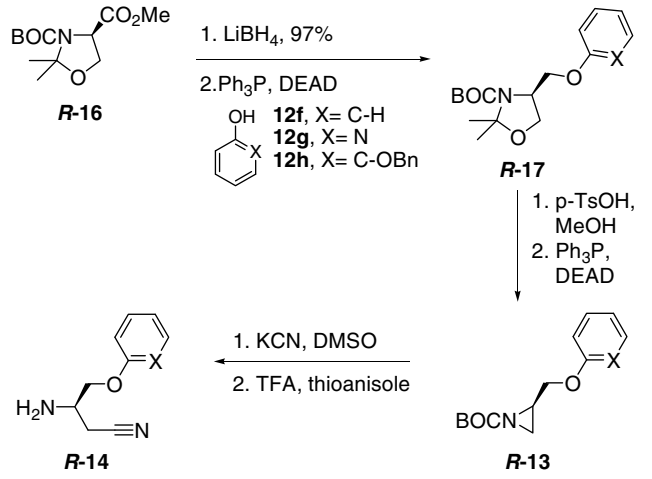
9

Compound	R	Diastereomer: (*SR*/*SS*)	K_i[a] (nM)	EC_{50}[a,b] (nM)	ED_{250}[c] (μmol/kg)	*C*log *P*
1		NA	2940	30	4.8	−0.3
9a		1 2	12 737	0.5 62	>2 ND	1.9
9b		1 2	63 1380	0.8 139	1.7 ND	2.0
9e		1 2	18 696	0.6 64	ND ND	1.4
9f		1:*SR*	7.5	0.4	0.87	1.3
9f		2:*SS*	197	19	ND	1.3
9g		1:*SR*	40	0.4	0.33	0.7
9g		2:*SS*	503	19	ND	0.7
9h		1:*SR*	5.7	0.3	<0.15	0.6
9h		2:*SS*	240	23	ND	0.6

[a] See Ref. 12 for detailed description.
[b] All compounds are full agonists.
[c] In vivo activity of 1*H*-tetrazoles in the acute anesthetized IV rat model. See Ref. 12 and 14 for detailed description.



Scheme 2. Preparation of 3-aminobutanenitriles **14** and synthesis of 1*H*-tetrazole nitriles **9e**–**9h**.



Scheme 3. Enantiospecific route to 3-aminobutanenitriles ***R*-14**.

and the appropriate phenol is depicted in Scheme 3. Reduction of the methyl ester ***R*-16** and condensation of the resulting alcohol with phenols **12f**, **12h** or 2-hydroxypiridine (**12g**), under Mitsunobu conditions, afforded the aryl ethers ***R*-17f**, ***R*-17h** and the hetero aryl ether ***R*-17g** in 76, 67 and 49% yields, respectively. Acid methanolysis of oxazolidines ***R*-17** and subsequent aziridine formation induced by Mitsunobu activation of the hydroxy function generated enantiomers ***R*-13** in an average 70% yield. Cyanide addition to ***R*-13** and BOC removal yielded enantiomerically pure amines ***R*-14**. Condensation of *N*-Boc-*O*-benzyl-D-serine with amines ***R*-14f**, ***R*-14g** or ***R*-14h** following the reaction sequence outlined in Scheme 2 yielded nitriles ***SR*-9f**, ***SR*-9g** or ***SR*-9h**, which spectroscopic analysis confirmed to be the more active epimer 1 of **9f**, **9g** and **9h**, respectively. To further support this conclusion, an analogous reaction sequence beginning with oxazolidine ***S*-16**, 2-hydroxypyridine (**12g**) and *N*-Boc-*O*-benzyl-D-serine yielded ***SS*-9g**, which was identical to epimer 2 of **9g**. Based on these structural correlations, we conclude that the *R* configuration at the epimeric center is preferred for activity.

Analysis of analogs **9e**, **9f**, **9g** and **9h** (Table 2) revealed that the impact of decreasing the calculated lipophilicity 20-fold had little impact on in vitro potency as exemplified by the near equivalence of **9h** and **9a**. However, an increase in polarity appears to have positively impacted ADME properties resulting in a respective 15- and 30-fold enhanced in vivo activity for ***SR*-9g** and ***SR*-9h** relative to nitrile **1**.

Further characterization of ***SR*-9g** and ***SR*-9h** revealed ***SR*-9h** to be a more potent inhibitor of CYPs 2D6 and 3A4 than ***SR*-9g** (Table 3). Consequently, despite being 2-fold less potent in the in vivo model, ***SR*-9g** was chosen as a lead compound for additional pharmacokinetic studies. The oral bioavailability of 1*H*-tetrazole-1-alkanenitrile ***SR*-9g** was 24% in rats and 55% in dogs, somewhat less than the corresponding values of 56% and 76% for the parent nitrile **1**.

In summary, an enantiospecific route for the synthesis of nitriles **9** has been developed. The potency of nitrile **1** has been optimized by introducing an 2-arylethyl moiety which provides an additional interaction with the GHS receptor. A precisely defined spatial relationship is required between the newly added aryl and the existing cyano group to achieve synergistic improvement in activity and otherwise potency is unaffected or even diminished. Heteroatoms were included in the 2-arylethyl moiety to maintain similar physicochemical properties, such as lipophilicity, to those exhibited by nitrile **1**. Only in vitro functional activity of polar analogs proportionally translated to in vivo activity. These modifications led to 1*H*-tetrazole-1-alkanenitrile ***SR*-9g** which exhibited >10-fold improved potency in vivo than the lead nitrile **1** with acceptable oral bioavailability in rats and dogs.

Table 3. Cytochrome P450 profile for ***SR*-9g** and ***SR*-9h**

Compound	1A2 IC_{50} (μM)	2C9 IC_{50} (μM)	2C19 IC_{50} (μM)	2D6 IC_{50} (μM)	3A4/BFC IC_{50} (μM)	3A4/Bz-RES IC_{50} (μM)
***SR*-9g**	>100	>100	33	>100	59	11
***SR*-9h**	>66	>66	42	5.1	9.8	5.5

Acknowledgments

We thank Dr. William Washburn, Dr. Peter T.W. Cheng and Dr. Robert Zahler for their suggestions on the manuscript.

References and notes

1. Rivier, J.; Spiess, J.; Thorner, M.; Vale, W. *Nature* **1982**, *300*, 276.
2. (a) Bowers, C. Y. In *Growth Hormone Secretagogues*; Bercu, B. B., Walker, R. F., Eds.; Springer-Verlag: New York, 1996; pp 9–28; (b) Bowers, C. Y.; Reynolds, G. A.; Durham, D.; Barrera, C. M.; Pezzoli, S. S.; Thorner, M. O. *J. Clin. Endocrinol. Metab.* **1990**, *70*, 975.
3. Reichlin, S.; Saperstain, R.; Jackson, I. M. D.; Boyd, A. E.; Patel, Y. *Ann. Rev. Physiol.* **1976**, *38*, 389.
4. Howard, A. D.; Feighner, S. D.; Cully, D. F.; Arena, J. P.; Liberator, P. A.; Rosenblum, C. I.; Hamelin, M.; Hreniuk, 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. P.; Elbrecht, A.; Dashkevicz, M.; Heavens, R.; Rigby, M.; Sirinathsinghji, D. J. S.; Dean, D. C.; Melillo, D. G.; Patchett, A. A.; Nargund, R. P.; 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.
5. Bowers, C. Y.; Momany, F. A.; Reynolds, G. A.; Hong, A. *Endocrinology* **1984**, *114*, 1537.
6. (a) DeVita, R. J.; Schoen, W. R.; Fischer, M. H.; Fontier, A. J.; Pisano, J. M.; Wyvratt, M. J.; Cheng, K.; Chan, W.-S.; Butler, B. S.; Hickey, G. J.; Jacks, T. M.; Smith, R. G. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2229; (b) Patchett, A. A.; Nargund, R. P.; Tata, J. R.; Chen, M.-H.; Barakat, K. J.; Johnston, D. B. R.; Cheng, K.; Chan, W. W.-S.; Butler, B.; Hickey, G.; Jacks, T.; Schleim, K.; Pong, S.-S.; Chaung, L.-Y. P.; Chen, H. Y.; Frazier, E.; Leung, K. H.; Chiu, S.-H. L.; Smith, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7001.
7. Kojima, M.; Hosoda, H.; Date, Y. *Nature* **1999**, *402*, 656.
8. Carpino, P. A. *Exp. Opin. Ther. Patents* **2002**, *12*, 1599.
9. Dodge, J. A.; Heiman, M. L. *Annu. Rep. Med. Chem.* **2003**, *38*, 81.
10. Pan, L. C.; Carpino, P. A.; Lefker, B. A.; Ragan, J. A.; Toler, S. M.; Pettersen, J. C.; Nettleton, D. O.; Ng, O.; Pirie, C. M.; Chidsey-Frink, K.; Lu, B.; Nickerson, D. F.; Tess, D. A.; Mullins, M. A.; MacLean, D. B.; Da Silva-Jardine, P. A.; Thompson, D. D. *Endocrine* **2001**, *14*, 437.
11. (a) Seyler, D. E.; Dodge, J. A.; Osborne, J. J.; Cox, K. L.; Viswanath, D.; Wilmot, A. F.; Keaton, M. J.; Heiman, M. L.; Bryant, H. U.; Cutler, G. B., Jr. *Drug Dev. Res.* **2000**, *49*, 260; (b) Lugar, C. W.; Clay, M. P.; Lindstrom, T. D.; Woodson, A. L.; Smiley, D.; Heiman, M. L.; Dodge, J. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5873.
12. Hernández, A. S.; Cheng, P. T. W.; Musial, C. M.; Swartz, S. G.; George, R. J.; Grover, G.; Slusarchyk, D.; Seethala, R. K.; Smith, M.; Dickinson, K.; Giupponi, L.; Longhi, D. A.; Flynn, N.; Murphy, B. J.; Gordon, D. A.; Biller, S. A.; Robl, J. A.; Tino, J. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5928.
13. ^{1}H NMR easily distinguished between the 1*H* and 2*H* isomers by comparison of the $\Delta\delta$ between the methyls of the 2-aminoisobutyric fragment. The $\Delta\delta$ is significantly larger in the 1*H* isomer than in the 2*H* isomer.
14. A dose response for MK-677 was performed in order to benchmark tetrazole analogs versus a well studied reference. At the highest dose utilized, 5.23 μmol/kg, there was no evidence of a plateau in the response. Higher doses could not be achieved as toxicity was observed and therefore an ED_{50} could not be determined. Since the maximal tolerated dose of MK-677 effects a 500% increase in GH, in vivo potencies for tetrazole based GHS are expressed as ED_{250} or the dose at which a 250% increase in plasma GH is achieved. The ED_{250} of MK-677 is 0.46 μmol/kg.
15. Analytical HPLC conditions: Column: Zorbax SB C18, 4.6 × 75 mm; Grad. T: 8 min; Flow R.: 2.5 mL/min.; Solvent Grad.: 0–100% B; Wave: 220 nm (A = 10% MeOH −90% H_2O −0.2% H_3PO_4; B = 90% MeOH −10% H_2O −0.2% H_3PO_4). ***SR*-9g** RT/***SS*-9g** RT = 5.30 min/5.65 min.