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Tetrahydroisoquinoline 1-carboxamides as growth hormone secretagogues

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Abstract—Several novel series of tetrahydroisoquinoline 1-carboxamides were prepared and shown to be potent growth hormone (GH) secretagogues. Among them, carbamate **12a**–**E2** displays excellent in vivo activity by increasing plasma GH 10-fold in an anesthetized IV rat model.

The availability of recombinant human growth hormone (GH) in mid 1980s has generated numerous potential uses for GH replacement therapy such as the treatment of aged related functional muscular decline in the elderly.[1] However, significant drawbacks, including cost, parental administration, and side effects, limit the clinical use of GH.[2] An alternative approach of promoting endogenous GH release with growth hormone releasing peptides (GHRPs) was later discovered by Bowers et al.[3] GHRPs and peptidomimetic analogs, collectively called GH secretagogues, can increase pulsatile GH release via activation of the novel G-protein coupled receptor GHS-R1a, mimicking physiological conditions in humans.[4] The discovery of new GHSs has received considerable attention since GHS therapy may offer potential advantages over existing GH replacement therapy.[5] Several GH secretagogues have advanced to clinic studies, including MK-677 (Merck),[6] CP-424391 (Pfizer),[7] and LY-444711 (Lilly).[8] One common structural feature among these compounds is the presence of a dipeptide scaffold containing an aminoisobutyric acid (Aib) and a phenylpropylglycine or *O*-benzylserine residue (Fig. 1). In an attempt to identify novel GH secretagogues, we initiated a high throughput screen of the BMS

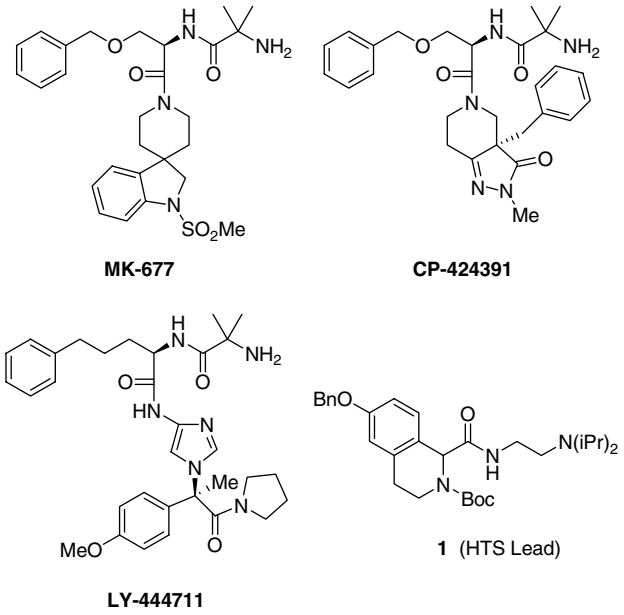

Figure 1. Representative GH secretagogues and HTS lead **1**.

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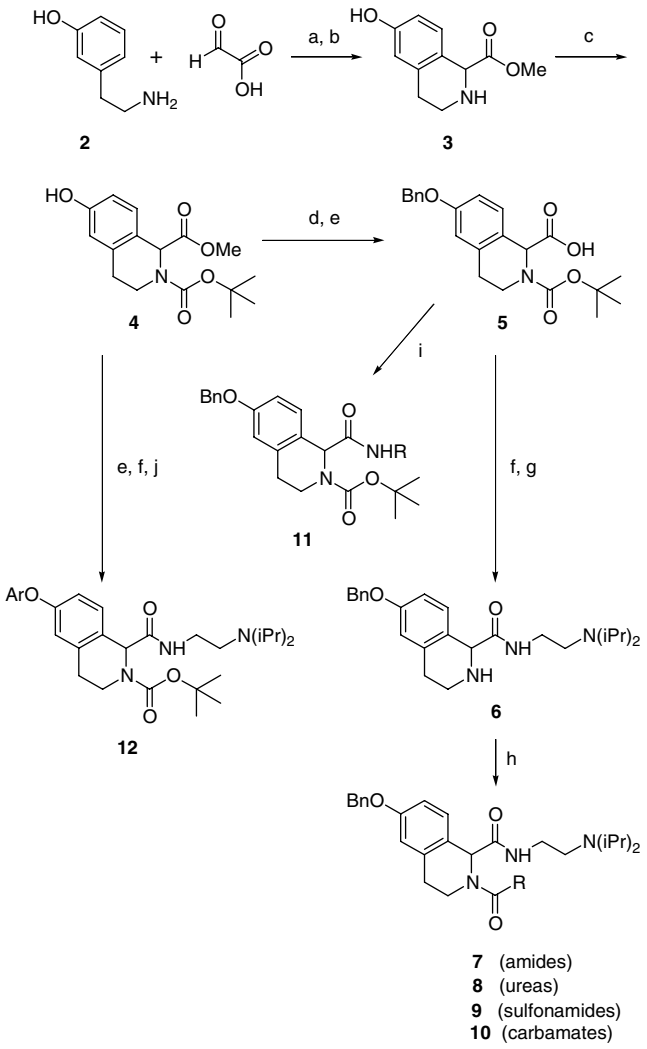

Scheme 1. Reagents: (a) 5% HCl; (b) MeOH, TMSiCl; (c) Boc_2–O, Et_3N; (d) NaH, BnBr; (e) NaOH; (f) 2-diisopropylaminoethylamine, EDAC, HOAT; (g) 4 N HCl in dioxane, MeOH; (h) see Ref. 14; (i) RNH_2, EDAC, HOAT; (j) $ArB(OH)_2$, $Cu(OAc)_2$, pyridine, Et_3N, 4 Å molecular sieves.

compound collection. Through this effort, tetrahydroisoquinoline **1** was identified as a full GHS agonist with an EC_{50} of 11.7 nM. Herein we describe the SAR surrounding this novel class of GH secretagogues.

One commonly used method for in vitro evaluation of GHS activity has been the measurement of GH release from isolated rat pituitary cells.[9] However, the results from this assay tend to be highly variable and the procedure is not amendable for high throughput screen.[10] Since the GHS receptor has been shown to signal through a calcium phospholipase C mediated pathway, it is expected that agonists can be identified by their ability to stimulate intracellular calcium mobilization using a calcium sensitive fluorescent probe in an automated instrument such as the fluorescence imaging plate reader (FLIPR).[11] A cell based functional FLIPR assay was subsequently developed from H4 glioma cells in which expression of the endogenous human GHS receptor was enhanced by RAGE-activation.[12,13]

The general synthetic sequence for analogs related to **1** is outlined in Scheme 1. The key step for tetrahydroisoquinoline formation was accomplished by condensation of glyoxylic acid with 3-hydroxy-phenethylamine (**2**) in the presence of dilute hydrochloric acid. The methyl ester **3** was generated from the intermediate acid to aid in isolation and purification. From methyl ester **3**, several standard synthetic manipulations provided the late-stage amine intermediate **6**. With **6** in hand, an automated parallel solution phase synthesis was used for the rapid preparation and isolation of analogs **7**–**10**.[14] Alternately, conventional amide coupling of acid **5** with various amines gave analogs **11**, allowing for the evaluation of the basic amino side chain. A copper promoted diaryl ether synthesis was adopted to provide analogs **12** in good yield.[15] During the initial round of optimization, all analogs were screened in vitro as racemic compounds.[16] Potent analogs were then evaluated in an anesthetized IV rat model to determine their effect on plasma GH levels.[17,18]

We hypothesized that there were three major pharmacophore components enabling lead **1** to function as a GHS agonist: a hydrophobic region, a basic amine, and a carbonyl hydrogen bond acceptor (Fig. 2).

Structure–activity relationships were first directed toward exploration of the Boc group in **1**. Several series of amides **7**, ureas **8**, sulfonamides **9**, and carbamates **10** were prepared and their in vitro data are summarized in Table 1. No clear SAR trend could be seen from amide analogs **7**, with most resulting in a decrease in potency except for compounds **7j**,**p**, and **q**. Several ureas **8a**–**c** and sulfonamides **9a**–**e** were also tested, but found to be less active in the functional activity. Carbamate analogs **10e**–**g** in which a methyl group of the Boc moiety is replaced with a methyl ester, methyl ketone or dimethylamide respectively, showed a 5- to 10-fold improvement of in vitro potency relative to **1**. As compared with the endogenous ligand Ghrelin, several analogs never reach maximum response even at 10 μM, as noted in Table 1.

A set of primary, secondary, and tertiary amines through analogs **11** (Table 2) were prepared to assess the importance of terminal diisopropylamino group in **1**. We also included compound **11e** which contained a terminal α,α-dimethyl amine in an attempt to model the aminoisobutyrate subunit found in the literature series (Fig. 2). In addition, different chain lengths between the amino group and tetrahydroisoquinoline core were

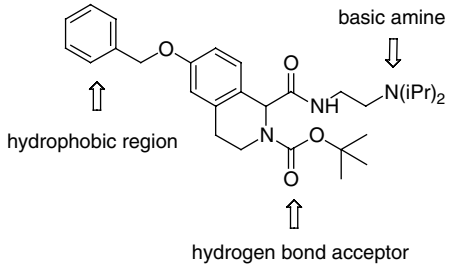

Figure 2. Proposed pharmacophore model for HTS lead **1**.

Table 1. In vitro potency of Boc replacement analogs **7**–**10**

Compd[a]	R	EC_{50} (nM)[b]
1	*t*-BuO	11.7
7a	*t*-BuCH_2	20.9
7b	*t*-Bu[c]	50% @ 10 μM
7c	Me	257
7d	Ph	7.6
7e	2-MeCOPh	7.3
7f	2-FPh	17.8
7g	2-EtOPh[c]	30% @ 10 μM
7h	PhCH_2	9.0
7i	2-FPhCH_2	43
7j	2-MeOPhCH_2	2.1
7k	3-MeOPhCH_2	6.4
7l	4-MeOPhCH_2	45
7m	PhCH_2CH_2	26.7
7n	Ph$CH_2CH_2CH_2$	13.1
7o	PhCH(OH)	39
7p	PhCH(CH_2OH)	5.6
7q	PhCH(Me)	3.4
7r	PhCH(COMe)	9.2
7s	PhCH(NHCOMe)	19.7
8a	*t*-BuNH	14.4
8b	PhNH[c]	43% @ 10 μM
8c	Me_2N	190
9a	$MeSO_2$[c]	47% @ 10 μM
9b	$BuSO_2$[c]	50% @ 10 μM
9c	$PhSO_2$	21
9d	$BnSO_2$	7.3
9e	Me_2NSO_2	21
10a	*n*-PrO	3.4
10b	*c*-HexO	4.9
10c	PhCH_2O	27
10d	PhO	<5% @ 10 μM
10e	MeO_2CCMe_2O	1.5
10f	$MeOCCMe_2O$	2.0
10g	$Me_2NCOCMe_2O$	0.85
10h	$NCCMe_2O$	9.8
Ghrelin		1.4

[a] Compounds **7o**–**s** are diastereoisomers, all others are racemates.
[b] See Ref. 12 for detailed description.
[c] These compounds have a maximum intrinsic activity less than 50% at 10 μM as compared to endogenous ligand Ghrelin.

Table 2. In vitro potency of analogs **11** with modification of 2-diisopropylaminoethyl amide side chain

Compd	R	EC_{50} (nM)[a]
1	$CH_2CH_2N(i\text{-Pr})_2$	11.7
11a	$CH_2CH_2NH(i\text{-Pr})$	219
11b	$CH_2CH_2NMe(i\text{-Pr})$	41
11c	$CH_2CH_2NEt_2$	19.6
11d	$CH_2CH_2NMe_2$	46% @ 10 μM
11e	$CH_2CMe_2NH_2$	47% @ 10 μM
11f	$CH_2CH_2CH_2NH(i\text{-Pr})$	<5% @ 10 μM
11g	$CH_2CMe_2CH_2NH_2$	257

[a] See Ref. 12 for detailed description.

probed. As can be seen in Table 2, the diisopropylamino moiety in **1** appears to be optimal for maintaining potent GHS functional activity since all modifications resulted in a substantial loss in potency for the series.

Simple replacement of the benzyl moiety in **1** with a phenyl group provided compound **12a** with a 20-fold (EC_{50} of 0.49 nM vs 11.7 nM) improvement in functional potency (Table 3). Further modifications with substituted phenyl ethers led to the identification of several subnanomolar GHS agonists such as **12f**,**h**. The two enantiomers of **12a** were separated to probe the stereochemical effect of tetrahydroisoquinoline 1-carboxamide side chain. The second eluting enantiomer (**12a**–**E2**) was 15-fold more potent than the first one (**12a**–**E1**) and both are full agonists.[19]

To assess the in vivo efficacy of this novel series of GH secretagogues, select analogs were evaluated in an anesthetized IV rat model (Table 4).[17,18] Two carbamates **10e** and **f** (EC_{50} of 1.5–2.0 nM) were found to be efficacious; however, a slightly more potent carbamate **12h** (EC_{50} = 0.49 nM) only showed a marginal effect in this model. Treatment of rats with the single enantiomer **12a**–**E2** (EC_{50} = 0.46 nM) generated over a 10-fold increase in plasma GH, resulting in the most potent analog identified in the IV rat model for the current series.

In summary, we have identified a novel series of tetrahydroisoquinolines as potent GH secretagogues. Systematic SAR studies have resulted in compounds **12a**,**g**, and **h** with excellent in vitro potency ($EC_{50} \sim 0.5$ nM) in the GHS functional assay. Carbamate **12a**–**E2** showed an 10-fold increase in plasma GH levels in an

Table 3. In vitro potency of phenyl analogs **12**

Compd[a]	Ar	EC_{50} (nM)[b]
1	PhCH_2	11.7
12a	Ph	0.49
12a–**E1**	Ph	7.6
12a–**E2**	Ph	0.46
12b	3-FPh	4.3
12c	4-ClPh	0.73
12d	3-CF_3Ph	1.5
12e	4-CF_3Ph	2.3
12f	3-NO_2Ph	0.66
12g	3-MeOPh	0.46
12h	4-MeOPh	0.49
12i	4-MeSPh	2.4
12j	4-CHOPh	1.4
12k	3-AcNHPh	12

[a] Compound **12a**–**E1** & **12a**–**E2** are single enantiomers separated from **12a**; all others are racemates.
[b] See Ref. 12 for detailed description.

Table 4. In vivo activity in the IV rat model

Compd	EC_{50} (nM)	GH ± SEM(%)[a]	Responder[c]
10e	1.5	746 ± 227	5
10f	2.0	666 ± 326	5
12h	0.49	172 ± 49	4
12a–**E2**	0.46	1283 ± 367	5
		367 ± 154[b]	5

[a] Percent (%) of plasma GH increase at an IV dose of 5.23 μmol/kg.
[b] Percent (%) of plasma GH increase at an IV dose of 1.74 μmol/kg.
[c] Number of rats with greater than 100% GH increase over the control group, there are five rats in both control and compound treated groups.

anesthetized IV rat model. Selected analogs are being further evaluated.

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References and notes

1. Rudman, D.; Feller, A. G.; Nagraj, H. S.; Gergans, G. A.; Lalitha, P. Y.; Goldberg, A. F.; Schlenker, R. A.; Cohn, L.; Rudman, I. W.; Mattson, D. E. *N. Engl. J. Med.* **1990**, *323*, 1.
2. Hintz, R. L. *Horm. Res.* **1992**, *38*(Suppl. 1), 44.
3. Bowers, C. Y.; Momany, F. A.; Reynolds, G. A.; Hong, A. *Endocrinology* **1984**, *114*, 1537.
4. Smith, R. G.; Van Der Ploeg, L. H.; Howard, A. D.; Feighner, S. D.; Cheng, K.; Hickey, G. J.; Wyvratt, M. J., Jr.; Fisher, M. H.; Nargund, R. P.; Patchett, A. A. *Endocr. Rev.* **1997**, *18*(5), 621.
5. For a review on GHS, see: Nargund, R. P.; Patchett, A. A.; Bach, M. A.; Murphy, M. G.; Smith, R. G. *J. Med. Chem.* **1998**, *41*, 3103.
6. (a) Chapman, I. M.; Bach, M. A.; Van Cauter, E.; Farmar, M.; Krupa, D.; Taylor, A. M.; Schilling, L. M.; Cole, K. Y.; Skiles, E. H.; Pezzoli, S. S.; Hartman, M. L.; Veldhuis, J. D.; Gormley, G. J.; Thorner, M. O. *J. Clin. Endocrinol. Metab.* **1996**, *81*, 4249; (b) Patchett, A. A.; Nargund, R. P.; Tata, J. R.; Chen, M. H.; Barakat, K. J.; Johnston, D. B. R.; Cheng, K.; Chan, W. S.; Butler, J. B.; Hickey, G. J.; Jacks, T.; Schleim, K.; Pong, S. S.; Chaung, L.-Y. P.; Chen, H. Y.; Frazier, E.; Leung, K. H.; Chiu, S. H.; Smith, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7001.
7. (a) Pan, L. C. *Endocrine* **2001**, *14*, 121; (b) Carpino, P. A. et al. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3279.
8. (a) 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*, 5873–5876; (b) 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.
9. Howard, A. D.; Feighner, S. D., et al. *Science* **1996**, *273*, 974.
10. Pong, S. S.; Chuang, L.-Y. P.; Dean, D. C.; Nargund, R. P.; Patchett, A. A.; Smith, R. G. *Mol. Endocrinol.* **1996**, *10*, 57.
11. (a) Sullivan, E.; Tucker, E. M.; Dale, I. L. *Methods Mol. Biol.* **1999**, *114*, 125–133; (b) Schroeder, K. S.; Neagle, B. D. *J. Biomol. Screen.* **1996**, *1*, 75.
12. The EC_{50} was measured by determining intracellular calcium concentration in a FLIPR assay with Ghrelin as having 100% intrinsic functional activity. All EC_{50} values are the averages of multiple measurements.
13. For details about random activation of gene expression (RAGE)^{TM} technology (Athersys, Inc.), see: Harrington, J. J.; Sherf, B.; Rundlett, S.; Jackson, P. D.; Perry, R.; Cain, S.; Leventhal, C.; Thornton, M.; Ramachandran, R.; Whittington, J.; Lerner, L.; Costanzo, D.; McElligott, K.; Boozer, S.; Mays, R.; Smith, E.; Veloso, N.; Klika, A.; Hess, J.; Cothren, K.; Lo, K.; Offenbacher, J.; Danzig, J.; Ducar, M. *Nat. Biotechnol.* **2001**, *19*, 440.
14. For details, see (a) Li, J. J.; Tino, J. A. US 6,469,024, **2002**; (b) Lawrence, R. M.; Biller, S. A.; Fryszman, O. M.; Poss, M. A. *Synthesis* **1997**, 553.
15. Evans, D. A.; Katz, J. L.; West, T. R. *Tetrahedron Lett.* **1998**, *39*, 2937.
16. The enantiomers were later separated for several analogs (unpublished results). It was typically observed that one enantiomer is about 5- to 15-fold more potent than the other, and both enantiomers are full GHS agonists.
17. Fasted male Wistar rats (200–250 g) were anesthetized via intraperitoneal injection with ketamine (30 mg) and xylazine (10 mg) per kilogram body weight. This treatment not only serves to anesthetize the animals, but also stabilizes the baseline plasma GH concentrations at approximately 60 ng/mL.[18] The drug or vehicle (10% ethanol, 0.09% saline V/V) was administered intravenously at a volume of 10 mL/kg to a group of five rats respectively. After 15 min, 1.5 mL of blood sample was drawn from the abdominal aorta. Plasma samples were then assayed for rat growth hormone by radioimmunoassay using a modification of the kit supplied by the National Pituitary Hormone Center (Dr. A. Parlow, Harbor-UCLA Medical Center, Los Angeles, CA).
18. Chihara, K.; Arimura, A.; Schally, A. V. *Endocrinology* **1979**, *104*, 1434.
19. Chiral separations were performed on a preparative ChiralPakAD Column (5×50 cm) with 20% isopropanol/hexanes. The first eluting isomer is assigned as **12a**–**E1**, and second one is assigned as **12a**–**E2**, but absolute configurations have not been assigned.