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Tetrazole based amides as growth hormone secretagogues

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Abstract—A novel series of *N*1 substituted tetrazole amides were prepared and showed to be potent growth hormone (GH) secretagogues. Among them, hydroxyl containing analog **31** displayed excellent in vivo activity by increasing plasma GH 10-fold in an anesthetized IV rat model.

Human growth hormone (GH) replacement therapy has shown promising results for the prevention of muscle functional decline in the elderly in a number of preliminary studies.[1] However, longer term use is associated with multiple side effects such as edema, arthralgia, carpal tunnel syndrome, and hyperglycemia due to the need to administer superphysiological doses of GH in order to maintain an efficacious level in the systemic circulation.[2] Orally active GH secretagogues (GHS), on the other hand, have been shown to enhance the amplitude of GH during endogenous pulsatile release and are believed to more closely mimic human physiological condition.[3] Therefore, treatment with an oral GHS agent should offer a more optimal approach for restoring pulsatile GH secretion in the elderly population.[4] Several classes of GHS agonists such as **1** (MK-677),[5] **2** (CP-42491),[6] and **3** (LY-444711)[7] have advanced to the clinics (Fig. 1). More recently, we have reported a novel tetrazole based GHS **4** (BMS-317180) as our first clinical candidate. Tetrazole **4** was shown to be orally active and efficacious in promoting GH release in a preclinical beagle dog model.[8] As part of our efforts to further delineate structural features critical for activity within this series, SAR exploration of various amide and/or reverse amide linkers, in place of the corresponding carbamate in **4**, was undertaken. Herein is a summary of these SAR studies which led us to the identification of a novel series of *N*1 substituted tetrazole amides as potent GHS agonists.

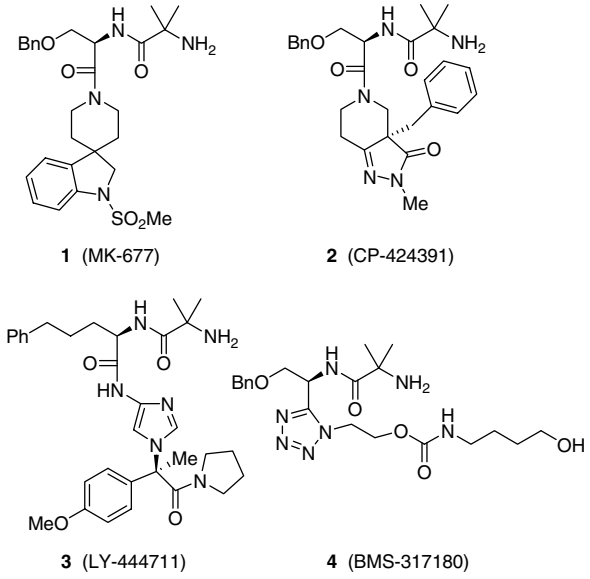

Figure 1. Representative examples of GH secretagogues.

The preparations of tetrazole amides were straightforward from readily available starting materials and are

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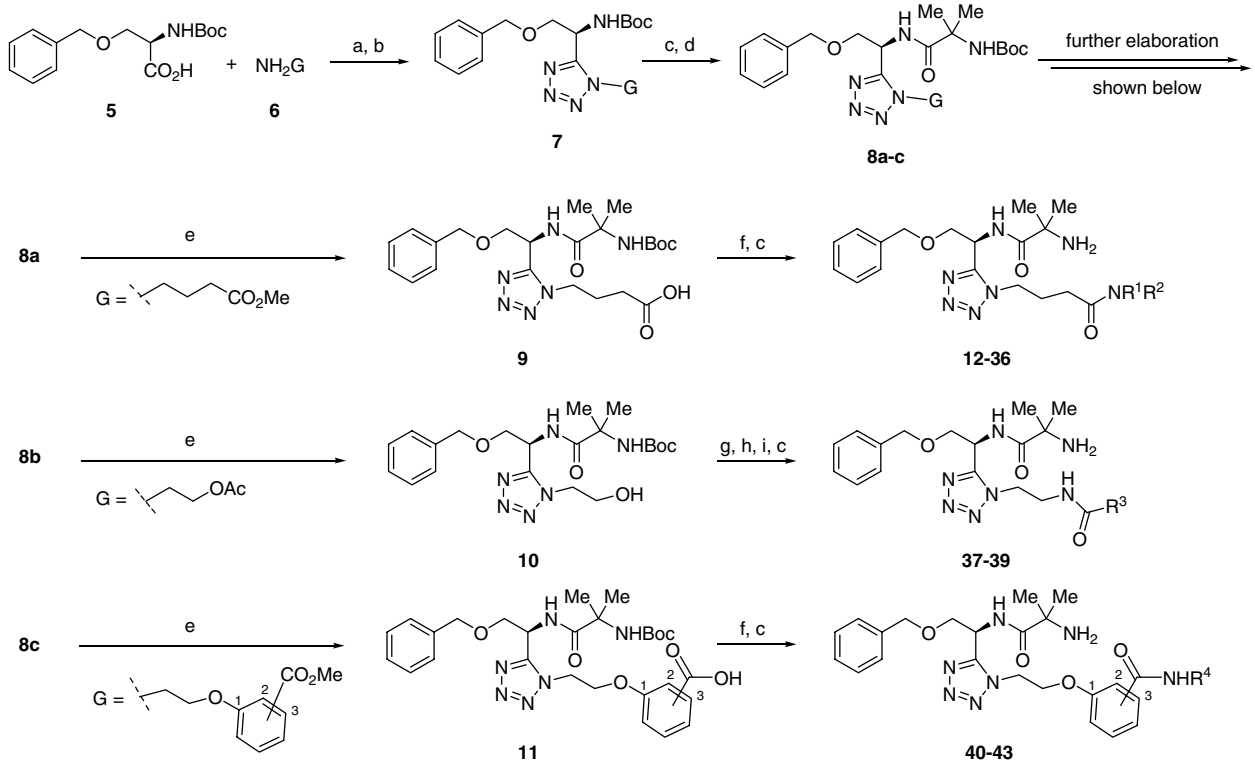
outlined in Scheme 1. The syntheses started with amide coupling of *N*-Boc-*O*-benzyl-*D*-Serine (**5**) and amines **6** via conventional mixed anhydride activation protocol. The formation of tetrazole intermediate **7** proceeded in the presence of triphenylphosphine, diethyl azodicarboxylate (DEAD), and azidotrimethylsilane ($TMSN_3$).[9] The removal of Boc protecting group in **7** with strong acids and subsequent reaction with Boc-Aib-OH gave **8a**–**c**. Hydrolysis under basic condition provided acid **9** or **11**; or the alcohol intermediate **10**. Amide formation from acid **9** or **11** via the activation of EDAC and HOAT followed by deprotection of Boc group gave the desired amides **12**–**36** or benzyl amides **40**–**43**. For reverse amide analogs, alcohol **10** was first converted to an azide intermediate using a modified Mitsunobu condition, followed by hydrogenation of the azide to provide the corresponding amine. Once again, reverse amides **37**–**39** were prepared in a similar manner as previously described using a variety of acids. The last two steps of these sequences, amide formation and de-Boc deprotection, were easily amended to a parallel synthesis procedure using automated solid phase extraction purification and generally afforded the final compounds in >95% purity.[10]

A H4 glioma cell based GHS functional FLIPR assay was developed for the in vitro evaluation of these GHS agents.[11,12] All the compounds reported herein showed an intrinsic activity greater than 85% as compared with endogenous Ghrelin (EC_{50} = 1.4 nM) and therefore are considered as full agonist.[13]

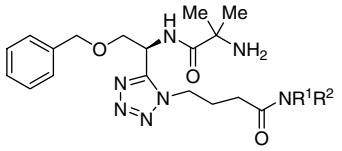
Simple amides with various terminal lipophilic groups such as **12**–**17** exhibited modest potency in the range of 26–70 nM (Table 1). The incorporation of more polar functional groups at the distal position, such as a methyl sulfone in **18**, a sulfonamide in **19**, a primary amide in **20**, or a urea in **23** improved GHS potency to give compounds with EC_{50} value less than 10 nM. The dramatic effect of the terminal moiety on the in vitro potency was evident by comparison to ester **21** or acid **22**. These modifications resulted in a significant loss of functional potency (EC_{50} > 140 nM).

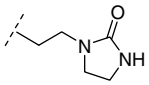
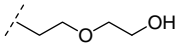
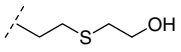
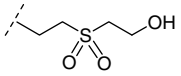
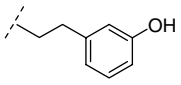
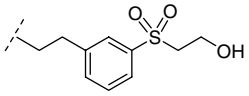
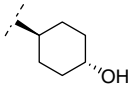
We have demonstrated earlier that the incorporation of a distal hydroxyl group in the tetrazole carbamate series (i.e., **4**) significantly improved oral activity in our preclinical beagle dog model.[8] Consequently, a series of tetrazole amides containing a terminal hydroxyl group were evaluated. A longer length in general appeared to be preferred as compounds **27**–**32** showed considerably greater potency than shorter length analogs **25**–**26** (Table 1). Interestingly, replacement with a phenolic hydroxyl moiety as in **33** provided a very potent subnanomolar GHS agonist. However, further extension of the terminal hydroxyl group as in **35**, or restriction as in cyclohexanol **36**, resulted in a substantial loss of activity. For two of the most potent amides **31** and **33** ($EC_{50} \sim 0.2$ nM), the corresponding N-methylated tertiary amides **32** and **34** were also generated, but an approximate 10-fold loss of the functional potency was observed in both cases.



Scheme 1. Reagents and conditions: (a) NMM, *i*-BuOCOCl, 70–95%, THF; (b) $TMSN_3$, DEAD, Ph_3P, 50–85%, THF; (c) 15–20% TFA/CH_2Cl_2 or 4 N HCl in dioxane/methanol; (d) Boc-Aib-OH, EDAC, HOAT, 65–83%, CH_2Cl_2; (e) 1 N aq NaOH, 80–90%; (f) EDAC, HOAT, appropriate amines, 30–90%, CH_2Cl_2; (g) PPh_3, DEAD, DPPA, 95%, THF; (h) H_2/5% Pd–C catalyst, 95%, MeOH; (i) EDAC, HOAT, appropriate acids, 30–90%, CH_2Cl_2.

Table 1. In vitro potency of amides

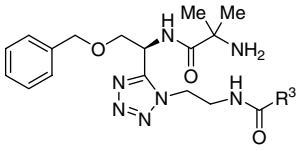


Compound[a]	R_1	EC_{50} (nM)
12	$-(CH_2)_3CH_3$	30
13	$-CH_2CF_3$	26
14	–Ph	70
15	$-CH_2Ph$	32
16	$-(CH_2)_2Ph$	23
17	$-(CH_2)_3Ph$	33
18	$-(CH_2)_2SO_2Me$	4.1
19	$-(CH_2)_2SO_2NH_2$	1.4
20	$-(CH_2)_2CONH_2$	7.6
21	$-(CH_2)_2CO_2Et$	140
22	$-(CH_2)_2CO_2H$	204
23		3.3
24	$-(CH_2)_2OH$	40
25	$-(CH_2)_3OH$	27
26	$-(CH_2)_4OH$	1.8
27	$-(CH_2)_5OH$	1.3
28	$-(CH_2)_6OH$	0.6
29		0.7
30		0.2
31		0.2
32[a]		3.1
33		0.2
34[a]		2.9
35		105
36		521
Ghrelin		1.4

[a] R_2 is hydrogen; R_2 is methyl for compounds **32** and **34**.

Three reverse amides, generated based on SAR observations in the initial amide series (e.g., **18**, **26**, and **33**), were also evaluated (Table 2). Analogs **37**–**39** showed approximately a two- to fourfold loss of potency as compared to their amide analogs, limiting our interest in further optimization in the reverse amide chemotype. Finally, selected benzyl amides were assessed as part of the linker evaluation. Similar to the results obtained in the simple amide series, longer chain lengths were also preferred (i.e., **42**, EC_{50} of 0.9 nM vs **40**, EC_{50} of 11 nM) but the difference in potency between 1,2 and 1,3 substitution scaffold was unremarkable in the two pairs evaluated (Table 3).

Table 2. In vitro potency of reverse amides



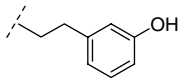
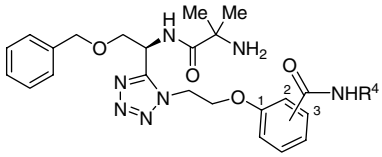
Compound	R_3	EC_{50} (nM)
37	$-(CH_2)_4OH$	4.4
38	$-(CH_2)_2SO_2Me$	14.7
39		0.64

Table 3. In vitro potency of benzyl amides



Compound	Position	R_4	EC_{50} (nM)
40	1,2	$-(CH_2)_2OH$	11
41	1,3		8.4
42	1,2	$-(CH_2)_4OH$	0.9
43	1,3		3.0

To assess the in vivo efficacy of these novel tetrazole amide based GHS compounds, analogs **19**, **28**, **31**, **33**, and **37** were selected for evaluation in an anesthetized IV rat model for their ability to stimulate GH release.[14] All the compounds showed good activity in generating 4- to 10-fold increases in plasma GH upon a single screening dose of 0.17 μmmole/kg, and were slightly more potent than carbamate **4** in this model (Table 4). Compound **31** afforded the greatest response and was subsequently tested at lower doses (0.017 or 0.052 μmmole/kg). The observed response indicates that analog **31** was the most potent in vivo analog of the series. Unfortunately, in a subsequent PK evaluation in rat, compound **31** was shown to have poor oral bioavailability (<3%), precluding it for further evaluation.

In summary, we have identified a novel series of tetrazole amides as exceptionally potent GHS agonists. The

Table 4. In vivo activity in the IV anesthetized rat model[a]

Compound	EC_{50} (nM)	GH ± SEM[b] (%)
4	1.9	264 ± 39
19	1.4	408 ± 69
28	0.6	522 ± 88
33	0.2	606 ± 104
37	4.4	722 ± 67
31	0.2	1290 ± 119
		956 ± 120 (0.052 μmole/kg)
		398 ± 65 (0.017 μmole/kg)

[a] Five rats per group are treated with each compound.

[b] The values represent the increase of GH ± SEM (%) relative to control after IV dosing of 0.17 μmole/kg of compound; lower doses were also evaluated for compound **31**.

incorporation of a terminal hydroxyl group in general was preferred for both potent in vitro and in vivo activity. The most potent analog **31** (EC_{50} = 0.2 nM) showed an increase in plasma GH levels of approximately 10-fold in an anesthetized IV rat model.

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12. Expression of the endogenous human GHS receptor was enhanced by Random Activation of Gene Expression (RAGE)™ 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.
13. 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 average of at least three measurements.
14. Fasted male Wistar rats (200–250 g) were anesthetized via intraperitoneal injection with ketamine (30 mg) and xylazine (10 mg) per kg body weight. 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, a 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).