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NOVEL SERIES OF HIGHLY POTENT NON-PEPTIDE GROWTH HORMONE SECRETAGOGUES WITH IMPROVED BIOAVAILABILITY

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Abstract – The discovery and the SAR of acylproline derivatives as highly potent growth hormone secretagogues (GHSs) with good oral bioavailability are described. One representative compound, *N*-[3-(2,2-dimethylpropylamino)-2-hydroxypropyl]-2(*R*)-[1-(2,2-dimethylpropionyl)pyrrolidine-2(*S*)-carbonylamino]-3-naphthalen-2-ylpropionamide **(4e)**, showed potent GHS activity (ED_{50}=1 nM) and good oral bioavailability (BA=33.2%). Moreover, the optically pure *N*-[3-(2,2-dimethylpropylamino)-2(*S*)-hydroxypropyl]-2(*R*)-[1-(2,2-dimethylpropionyl)pyrrolidine-2(*S*)-carbonylamino]-3-naphthalen-2-ylpropionamide **(**(2*S*)-**4e)** showed a good metabolic stability against *in vitro* clearance (human liver microsome) with potent GHS activity.

INTRODUCTION

Since the discovery of the peptidyl growth hormone secretagogue, named GHRP-6, by Bowers and Momany *et al*. in 1984,[1,2] GHSs have received considerable attention with the hope of using them as a replacement for the parenteral GH.[3,4] In addition to the stimulation of growth, GH has been known to play a number of important roles in the metabolic processes, e.g., the stimulation of protein synthesis and the mobilization of free fatty acids. Furthermore, the discoveries of the GHS receptors[5] and its endogenous ligand, Ghrelin[6] has spured the research activity on orally active small molecular GHSs for their potential use such as an appestat.

We have already created novel benzothiazepin derivatives represented as the compound **(1)** (Figure 1) with a nanomolar GHS activity using a 3D pharmacophore technique and the site-dependent fragment QSAR analysis,[7] but most of these derivatives turned out to have a low bioavailability (BA<0.4%). We have also

learned that the addition of non-polar groups to the R^2 position of benzothiazepin derivatives **(2)** could bestow a better gastrointestinal absorption on this series, but at the same time, resulted in weaker *in vitro* activities (EC_{50}>100 nM). In order to resolve this incompatibility, we resumed our research to create a new scaffold[8] that would be able to harmonize the GHS activity with the oral bioavailability. In this communication, we report new acylproline derivatives which have the acylprolinyl scaffold replacing the benzothiazepin group, and discuss their GHS activity and *in vivo* behavior.

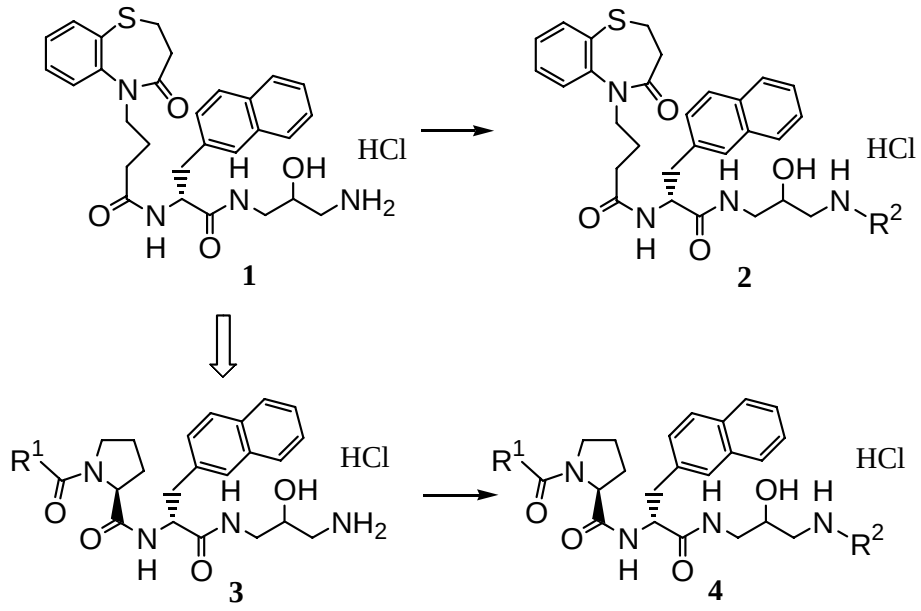
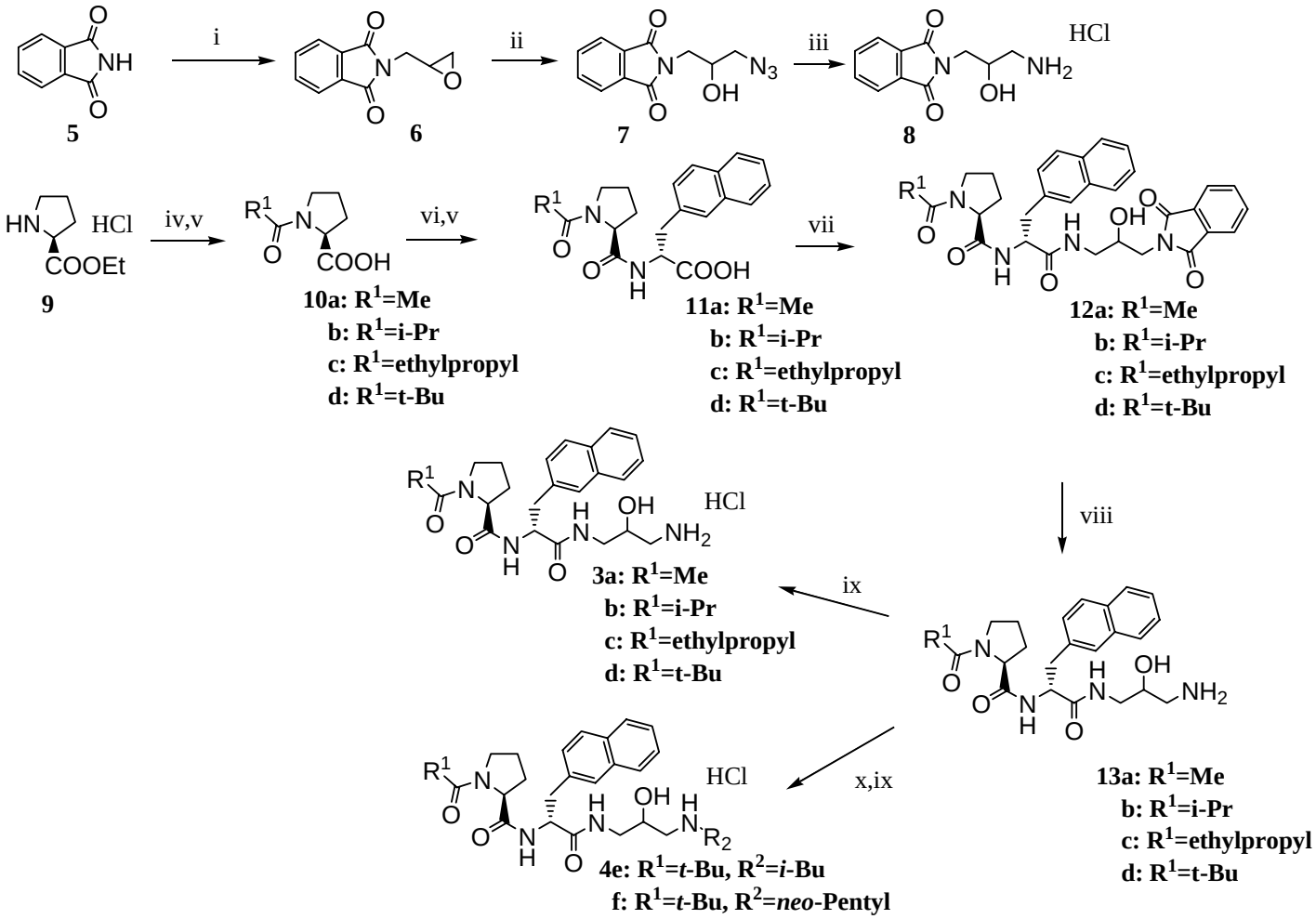


Figure 1.

RESULTS AND DISCUSSION

The general method for the synthesis of the acylproline derivatives as a mixture of two distereoisomers is outlined in Scheme 1. The epoxide **(6)** was obtained from the phtalimide **(5)** with glycidol by the Mitsunobu reaction[8] in 75%. The cleavage of the epoxide **(6)** with sodium azide followed by the Pd-catalyzed hydrogenation in the presence of conc. HCl produced the amino alcohol salt **(8)** in 42% from **6**. On the other hand, the L-proline ethyl ester hydrochloride **(9)** was converted to the corresponding amides with acetic acid, isobutyric acid, 2-ethylbutanoic acid or pivalic acid and then hydrolysis of the ethyl ester gave acylprolines **(10a-d)** in good to excellent yields. The coupling of **10a-d** with D-3-(2-naphtyl)alanine methyl ester and subsequent hydrolysis of the methyl ester produced the acid **(11a-d)** . The coupling of **11a-d** with **8** produced compound **(12a-d)**. The deprotection of **12a-d** with hydrazine hydrate gave the amine **(13a-d)**. The resulting amine **(13a-d)** was then converted to the HCl salt **(3a-d)** with 4M HCl/AcOEt in good to excellent yields from **10a-d**. The reductive amination of **13d** with

isobutyl aldehyde or pivaloyl aldehyde followed by treatment of the resulting secondary amine with 4M HCl/AcOEt provided the target compounds **(4d,e)** as the HCl salt in moderate yields from **10a-d**.

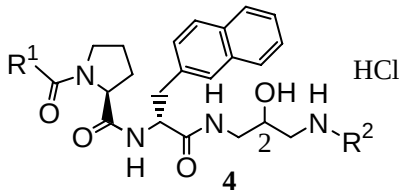


Scheme 1. Conditions: (i) glycidol, PPh_3, DEAD, THF; (ii) NaN_3, NH_4Cl, DMF, 80℃; (iii) 10% Pd-C, conc. HCl, EtOH, H_2/2MPa; (iv) R^1COOH, HOBt, EDC, *N*-Methylmorpholine, CH_2Cl_2; (v) 2M NaOH, MeOH; (vi) D-3-(2-Naphtyl)alanine methyl ester, HOBt, EDC, *N*-Methylmorpholine, DMF; (vii) **8**, HOBt, EDC, *N*-Methylmorpholine, DMF; (viii) NH_2NH_2-H_2O, EtOH, reflux; (ix) 4M HCl/AcOEt, (x) R^2CHO, $NaBH_3CN$, MeOH.

Table 1 shows the GHS activity and the oral bioavailability of the acylproline compounds. Several repeats of the appropriate structural modifications followed by SAR analyses of this scaffold yielded compounds with a strong GHS activity and eventually with a good oral bioavailability (Table 1). The SAR studies of the acylproline derivatives revealed that when R^1 was a small aliphatic group, the GHS activity was not improved at all (**4a**:1000 nM). However the activity significantly increased when the α-branched aliphatic alkyl groups were inserted in the R^1. The compounds (**4b**, R^1=isopropyl), (**4,** R^1=ethylpropyl) and (**4d**, R^1=pivalyl) depicted in the Table 1 showed high GHS activities (**4b**:10 nM, **4c**:1 nM, **4d**:10 nM), but their

bioavailavilities were not as good as we expected (BA=3.6% and 0.6%, respectively). Based on these results, we added a hydrophobic functional group at the amino moiety in order to increase the hydrophobisity of the entire molecule for the enhancement of the gastrointestinal absorption through the effect of the cell membrane permeability improvement. The amino moiety was the most influential pharmacophare on the GHS activity and the improvised change at that part in the series of benzodiazepine derivatives only caused a loss of the GHS activity. On the other hand, the acylproline derivatives tolerated the modification at this sensitive part toward the hydrophobisity. As shown in Table 1, the *t*-butyl substitution at R^1 in the presence of the isobutyl or neopentyl group at R^2 drastically improved the oral bioavailability while the GHS activity remained intact (**4e**:BA=33.2%, **4f**:BA=30.1%).

Table 1 GHS Activity and Oral Bioavailability of Acylproline Compounds



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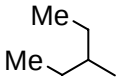
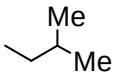
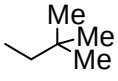
Compound	R^1	R^2	EC_{50}(nM)[a]	Bioavailability(%)[b]
4a		H	1000	——
4b		H	10	——
4c		H	1	3.6
4d		H	10	0.6
4e			1	33.2
4f			10	30.1

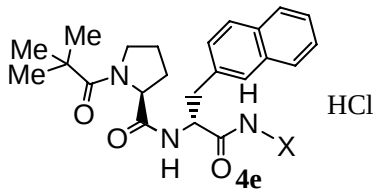
[a] EC_{50} values were measured with rat primary anterior pituitary cells.[7,10]

[b] Rats, 10mg/kg, po.

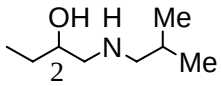
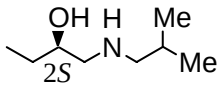
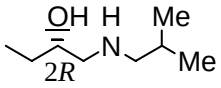
These results naturally prompted us to further examine compound (**4e)** as to whether it would have a suitable profile as a drug candidate. The *in vitro* clearance in the liver microsome of **4e** was tested and the results are shown in Table 2. The compound (**4e)** turned out to be quite stable against the rat liver

microsome, but relatively vulnerable to the human one. Furthermore, the optically pure compounds ((2*S*)-**4e**[11] and (2*R*)-**4e**[12]) were synthesized[13] and examined the GHS activity and the clearance in the liver microsome of humans (Table 2). The GHS activity of (2*S*)-**4e** was 100-times better than that of (2*R*)-**4e**. In addition, it should be noted that (2*S*)-**4e** was 2-fold more metabolically resistant to *in vitro* clearance (human liver microsome) than (2*R*)-**4e**. These results strongly suggested that the compound **(**(2*S*)-**4e)** would have an excellent *in vivo* behavior, i.e., a high Cmax and a long half-life time in the human body.

Table 2 *in vitro* Clearance in Liver Microsome of Compound (**4e)**



4e

Compound	X	EC_{50}(nM)[a]	CLint.(mL/min/mg) rat	human
4e		1	0.00	0.11
(2*S*)-**4e**		1	——	0.07
(2*R*)-**4e**		100	——	0.14

[a] EC_{50} values were measured with rat primary anterior pituitary cells.[7,10]

In conclusion, we discovered novel acylproline derivatives with a high a GHS activity and drastically improved oral bioavailability. Moreover, we demonstrated that (2*S*)-**4e** had a more potent GHS activity and a more favorable metabolic stability against *in vitro* clearance (human liver microsome) than (2*R*)-**4e**.

ACKNOWLEDGEMENTS

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11. Data for (2*S*)-**4e**: $[\alpha]_D^{25}$-40.9°(c=1.0, MeOH) ; ^{1}H-NMR (DMSO-d_6 ; 270 MHz) δ 0.93 (6H, d, *J*=6.6 Hz), 1.12 (9H, s), 1.20-1.40 (1H, m), 1.50-1.85 (3H, m), 1.90-2.10 (1H, m), 2.60-2.80 (3H, m), 2.85-3.05 (2H, m), 3.10-3.40 (3H, m), 3.45-3.70 (2H, m), 3.85-4.05 (1H, br s), 4.20-4.35 (1H, br s), 4.45-4.65 (1H, m), 5.68 (1H, d, *J*=4.6 Hz), 7.35-7.55 (3H, m), 7.71 (1H, s), 7.75-7.90 (3H, m), 8.19 (1H, t, *J*=5.6 Hz), 8.32 (1H, d, *J*=8.6 Hz), 8.40-8.70 (2H, br s) ; MS (FAB) m/z 525 $(M + H)^+$.
12. Data for (2*R*)-**4e**: $[\alpha]_D^{25}$-20.0°(c=0.1, MeOH) ; ^{1}H-NMR (DMSO-d_6 ; 270 MHz) δ 0.94 (6H, d, *J*=6.6 Hz), 1.12 (9H, s), 1.20-1.40 (1H, m), 1.60-1.90 (3H, m), 1.95-2.10 (1H, m), 2.65-2.85 (3H, m), 2.90-3.20 (3H, m), 3.20-3.70 (4H, m), 3.85-4.00 (1H, m), 4.20-4.35 (1H, br s), 4.45-4.65 (1H, m), 5.72 (1H, d, *J*=5.0 Hz), 7.35-7.55 (3H, m), 7.71 (1H, s), 7.75-7.90 (3H, m), 8.15-8.30 (1H, m), 8.40 (1H, d, *J*=8.3 Hz), 8.50-8.70 (2H, br s) ; MS (FAB) m/z 525 $(M + H)^+$.
13. (2*S*)-**4e** and (2*R*)-**4e** were synthesized from the (*S*)-glycidol and (*R*)-glycidol, respectively.