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Discovery and Biological Characterization of Capromorelin Analogues with Extended Half-Lives

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Abstract—New *tert*-butyl, picolyl and fluorinated analogues of capromorelin (**3**), a short-acting growth hormone secretagogue (GHS), were prepared as part of a program to identify long-acting GHSs that increase 24-h plasma IGF-1 levels. Compounds **4c** and **4d** (ACD LogD values ≥ 2.9) displayed extended plasma elimination half-lives in dogs, primarily due to high volumes of distribution, but showed weak GH secretagogue activities in rats ($ED_{50}s > 10$ mg/kg). A less lipophilic derivative **4** (ACD LogD = 1.6) exhibited a shorter canine half-life, but stimulated GH secretion in two animal species. Repeat oral dosing of **4** in dogs for 29 days (6 mg/kg) resulted in a significant down-regulation of the post dose GH response and a 60 and 40% increase in IGF-1 levels relative to pre-dose levels at the 8- and 24-h post dose time points. Compound **4** (CP-464709-18) has been selected as a development candidate for the treatment of frailty.

Over the past decade, two classes of growth hormone secretagogues (GHSs) have undergone extensive clinical evaluation. Short-duration GHSs stimulate growth hormone (GH) release after single or repeat administration and produce transient elevations in plasma insulin-like growth factor-1 (IGF-1) levels.[1] Compounds such as pralmorelin (**1**)[2] have been reported to increase growth velocity in GH-deficient children.[3] Long-duration GHSs increase the amplitudes and frequencies of endogenous GH peaks and produce sustained elevations of plasma IGF-1 levels.[4] The post-dose GH response of this class of drugs is attenuated due to negative feedback inhibition by IGF-1. One long-acting GHS, ibutamoren (**2**, MK-077, Fig. 1),[5] has been shown to: (1) increase markers of bone turnover in elderly adults and obese young males;[6] (2) increase energy expenditure and fat-free mass in obese subjects;[7] and (3) reverse diet-induced catabolism in healthy volunteers.[8] Both short- and long-acting GHSs exhibit high affinities for the human type 1a growth hormone secretagogue receptor (hGHS-R1a), also known as the receptor for the new gastrointestinal hormone ghrelin.

We have recently described the pharmacological characterization of a short-duration pyrazolinone-piperidine (PP) dipeptide GHS capromorelin (**3**, CP-424391-18).[9] Because of the growing clinical precedent established with long-acting GHSs such as ibutamoren, we sought an orally active derivative of **3** with an extended half-life ($t_{1/2}$) in a beagle dog model used to predict GH/IGF-1 activity in humans. In order to manipulate the pharmacokinetic (PK) parameters that determine $t_{1/2}$, potential metabolic sites in **3** were blocked and physicochemical properties of **3** were altered. Herein, the syntheses, biological activities, and PK properties of new *tert*-butyl, picolyl, and fluorinated PP dipeptidyl analogues are described. This work led to the discovery of **4**, a long-duration GHS that increases 24-h plasma IGF-1 levels in dogs.

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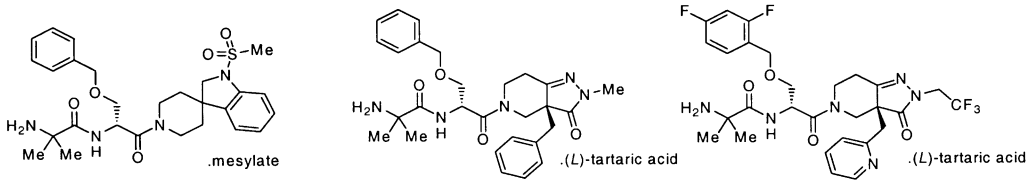
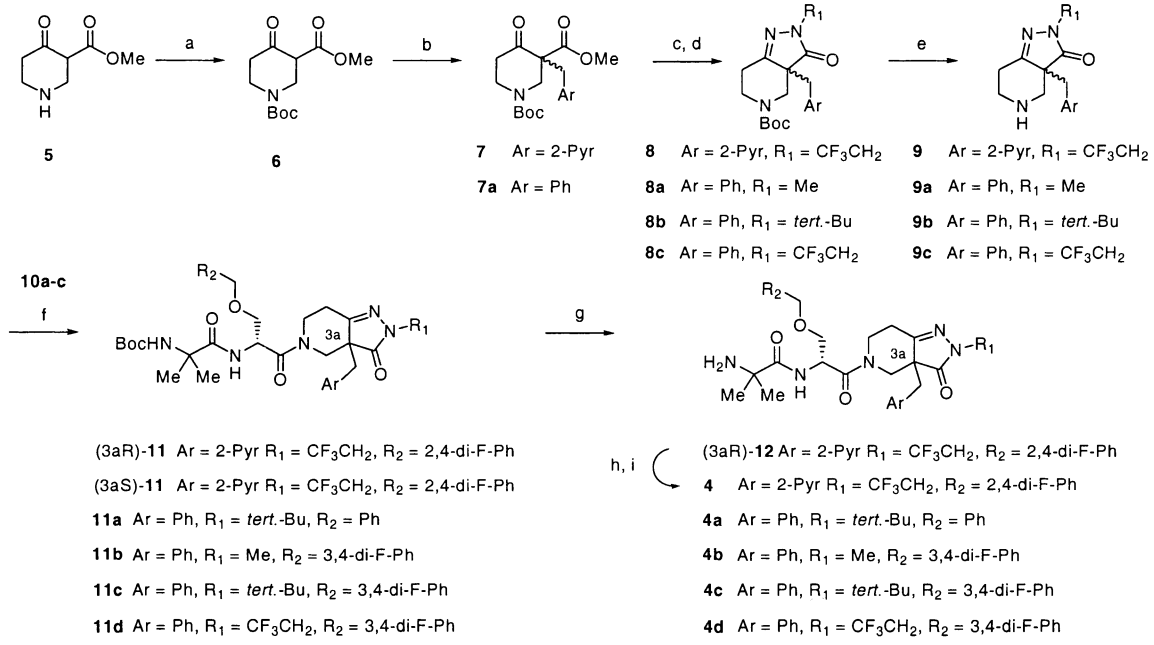


2 Ibutamoren (MK-0677) **3** Capromorelin (CP-424391-18) **4** CP-464709-18

Figure 1. Structures of selected GHSs.

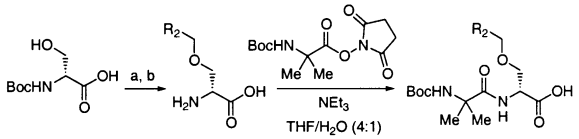


5 6 7 Ar = 2-Pyr 7a Ar = Ph 8 Ar = 2-Pyr, $R_1 = CF_3CH_2$ 8a Ar = Ph, R_1 = Me 8b Ar = Ph, R_1 = *tert.*-Bu 8c Ar = Ph, $R_1 = CF_3CH_2$ 9 Ar = 2-Pyr, $R_1 = CF_3CH_2$ 9a Ar = Ph, R_1 = Me 9b Ar = Ph, R_1 = *tert.*-Bu 9c Ar = Ph, $R_1 = CF_3CH_2$

Scheme 1. Syntheses of **4** and **4a–d**. Reagents and conditions: (a) Boc_2O, DMAP, CH_2Cl_2, 0 °C; (b) $PhCH_2Br$ or 2-$PyrCH_2Cl$, NaH, DMF; (c) R_1NHNH_2, EtOH, reflux; (d) toluene, cat. HOAc (if necessary), reflux; (e) TFA, 0 °C; (f) HOAt, EDC, NEt_3, CH_2Cl_2, 0 °C; (g) concd HCl, EtOH; (h) (L)-tartaric acid, MeOH; (i) crystallization from hot EtOAc.

The picolyl PP dipeptide GHS **4** was prepared from commercially available methyl 4-oxo-3-piperidinecarboxylate (**5**) as shown in Scheme 1. Treatment of **5** with di-*tert*-butyldicarbonate (Boc_2O) provided **6** which was alkylated with 2-picolyl chloride using sodium hydride as a base to give **7**. Condensation of the keto ester with 2,2,2-trifluoroethylhydrazine yielded the pyrazolinone-piperidine heterocycle **8**. Subsequent acid-catalyzed removal of the N-*tert*-butoxycarbonyl (*N*-Boc) protecting group gave **9**. Cross-coupling of the racemic PP amine with the dipeptide Boc-Aib-(D)-*O*-2,4-di-F-Bn-Ser-OH (**10a**, see Scheme 2 for preparation) using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxy-7-azabenzotriazole (HOAt)[10] provided a mixture of two diastereomers, (3a*R*)-**11** and (3a*S*)-**11**. Addition of HOAt to the reaction mixture minimized racemization of the dipeptide subunit. The desired, less polar (3a*R*)-**11** diastereomer was de-protected with aqueous hydrochloric acid and the product (3a*R*)-**12** was treated with (L)-tartaric acid to yield GHS **4**.[11] The stereochemistry of the 3a-carbon center in the PP ring of **4** was established by X-ray crystallographic analysis.[12] Fluorinated and *tert*-butyl analogues **4a–d** (HCl salts) were obtained by coupling the appropriately substituted PP intermediates **9a–c** with either **10b** (see Scheme 2) or Boc-Aib-(D)-*O*-Bn-Ser-OH (**10c**),[13] separating the diastereomers, and then removing the N-Boc protecting groups under acidic conditions. Compounds **9a–c** were prepared in an analogous manner to **9** using benzyl bromide in the initial alkylation step and an appropriately substituted alkyl hydrazine in the condensation step. The reaction of **7a** with *tert*-butylhydrazine in ethanol gave a hydrazone intermediate which underwent condensation in toluene only in the presence of acetic acid.

Pharmacokinetic evaluations of capromorelin analogues **4** and **4a–d** were carried out in male beagle dogs. Plasma elimination half-life, plasma clearance (CL) and volume of distribution (Vd) values were measured following intravenous (iv) administration of drug (1 mg/kg). Bind-



13a R_2 = 2,4-di-F-Ph 13b R_2 = 3,4-di-F-Ph 10a R_2 = 2,4-di-F-Ph 10b R_2 = 3,4-di-F-Ph

Scheme 2. Syntheses of **10a,b**. Reagents and conditions: (a) NaH, 3,4-di-F-$PhCH_2Br$ or 2,4-di-F-$PhCH_2Br$, DMF, 0 °C; (b) concd HCl, EtOH.

ing affinities (K_i values) were determined via competitive binding assays against [^{125}I]-ghrelin (Perkin–Elmer NEN-Life Sciences) in membranes prepared from HEK293 cells over-expressing the hGHS-R1a. Functional activities (in vitro GH release) were measured in rat pituitary cell cultures using rat specific double antibody radioimmunoassays (NIDDK reagents). The in vivo GHS activities of selected analogues were assessed in an anesthesized Wistar rat model following iv administration and in a beagle dog model following oral administration. The IGF-1 response was measured in a beagle dog model using previously described protocols.

The short half-life of capromorelin in the dog was attributed to moderate Vd and moderate-to-high plasma CL. Major routes of hepatic metabolism included *N*-de-methylation of the PP ring, oxidation and debenzylation of the (D)-*O*-Bn-Ser side chain, and oxidation of one of the methyl groups on the α-aminoisobutyramide (Aib) subunit (data not shown). Replacement of the methyl group on the PP ring in capromorelin with a *tert*-butyl group as in **4a** led to an increase in the canine $t_{1/2}$, primarily because of a decrease in plasma clearance (see Table 1). Introduction of two fluorine substituents on the phenyl ring in the (D)-*O*-Bn-Ser moiety of capromorelin (see **4b**) also resulted in an extension of the half-life, but now because of an increase in Vd. Incorporation of lipophilic substituents at metabolic sites on both the PP ring and the side chain in the dipeptide moiety provided compounds such as **4c** and **4d** with moderately long half-lives due to large volumes of distribution. Plasma clearances for many of the compounds were often unpredictable, probably because potential sites of metabolism in the Aib moiety were not blocked. Modification of the Aib group was not undertaken because of the restrictive structure–activity relationships in the N-terminal region of the dipeptide.

In an attempt to lower plasma clearance values in analogues with *tert*-butyl or trifluoroethyl groups on the PP ring and fluorine groups in the dipeptide moiety, the overall lipophilicity of the compounds was reduced by decreasing the calculated octanol–water distribution coefficient (ACD logD). Lipophilic compounds tend to exhibit high metabolic clearances because of the large hydrophobic binding sites in many of the cytochrome P450 enzymes that are involved in the biotransformation of xenobiotics.[14] The phenyl ring on the 3a-benzyl side chain in the PP moiety, the last remaining site for chemical manipulation, was replaced by polar, weakly basic heterocycles. Compound **4** is a close-in derivative of **4d** with a picolyl group in place of the benzyl group on the PP ring, a different fluorine substitution pattern on the (D)-*O*-Bn-Ser group in the dipeptide subunit, and a reduced calculated logD value (see Table 1). In dogs, the plasma clearance of **4** was low in comparison to more lipophilic analogues. Volume of distribution was also small even though multiple fluorine substituents were present throughout the molecule. Despite a decrease in Vd, the half-life of **4** was only slightly shorter than the half-lives of related analogues without picolyl groups. Intrinsic clearances of **4**, **4c**, and **4d** in dog microsome preparations were not measured, so the specific effects of reducing lipophilicity on metabolic clearance were not determined.

Selected capromorelin analogues with extended half-lives (**4** and **4b–d**) exhibited varying GHS activities in the primary screens (see Table 2). The picolyl derivative **4** and capromorelin were equipotent in the binding and pituicyte cell culture assays, but **4** was 2-fold less active in the anesthesized rat model. The fluorinated compound **4b** was approximately 3-fold less potent than capromorelin in the in vitro assays, but 6-fold less active in the anesthesized rat model. The most lipophilic analogues **4c** and **4d** exhibited subnanomolar affinities in the binding assay, with K_i values similar to ghrelin, the endogenous GHS-R1a ligand. These two analogues stimulated GH release in pituicyte cell cultures with EC_{50} values less than 5 nM, but surprisingly showed no in vivo activity in the anesthesized rat model.

Compounds **4**, **4c**, and **4d** possessed comparable half-lives in dogs ($t_{1/2}$s $\geq$ 3.7 h) and showed equivalent EC_{50} values in rat pituicyte cell cultures, but only **4** with a picolyl group and a low calculated logD value exhibited GHS activity in an in vivo rat model. The unbound (free) fraction of **4** in rat plasma was high (39%), providing a possible explanation for its robust activity. The unbound fractions of **4c** and **4d** were not measured, however, high protein binding could have reduced the amount of free drug capable of interacting with the GHS-R1a. Very large Vds in the rat as in the dog could also have limited the concentrations of these drugs in the plasma compartment.

Table 1. Canine $t_{1/2}$, Vd and CL values and calculated lipophilicities

Compd	$t_{1/2}$[a] (h)	Vd (L/kg)	CL (mL/min/kg)	ACD LogD[b,c]
Capromorelin (**3**)	1.3	2	19	1.7
4	3.7	1.5	5.3	1.6
4a	2.5	2.2	13	2.9
4b	2.7	3.5	17	1.7
4c	3.9	5.9	19	2.9
4d	4.7	5.1	13	3.0

[a]Plasma elimination half-life (0–8 h).
[b]Calculated using Advanced Chemical Development ACD/LogD Suite.
[c]pH = 7.0.

Table 2. Binding affinities and GHS activities

Compd	K_i (nM)[a,d]	EC_{50} (nM)[b,e]	ED_{50} (mg/kg)[c,e]
Ghrelin	0.42		
Capromorelin (**3**)	7	3	0.05
4	5	3	0.1
4b	18	10	0.3
4c	0.34	4	>10
4d	0.45	2	>10

[a]Competitive binding assay, [^{125}I]-ghrelin as radioligand.
[b]GH release in pituitary cell cultures.
[c]GH release in anesthesized rat model.
[d]Average of duplicate experiments.
[e]Average of triplicate experiments.

With a moderately long canine half-life, compound **4** behaved similarly to other long-duration GHSs in dog models. Compound **4** stimulated canine GH secretion after a single 1 mg/kg oral dose, with a mean peak height of 60 ± 17 ng/mL. Repeat oral administration of **4** (6 mg/kg) for 29 days resulted in a down-regulation of the post dose GH response, with peak GH levels declining from 46 ng/mL on day 1 to 12 ng/mL on day 29. The serum IGF-1 levels increased 60% above pre-dose levels at an 8-h post dose time point, though the levels declined to 40% above pre-dose levels at the 24-h post-dose time point.

Pharmacokinetic properties of **4** were measured in female Sprague–Dawley rats and in male and female beagle dogs. In rats, after an iv dose of 1 mg/kg of free base equivalents (1 mgA/kg), mean values for Vd, CL, and $t_{1/2}$ were 4.9 L/kg, 46 mL/min/kg and 1.9 h. Following a 1 mgA/kg oral dose, maximum plasma concentrations (C_{max}) of 88 ng/mL were achieved at 0.25 h (T_{max}). Bioavailability of **4** was 59%, with an apparent oral $t_{1/2}$ of 3.9 h due to slow absorption. In the dog, following a 1 mgA/kg oral dose, C_{max} was 260 ng/mL, with a T_{max} of 1.3 h. Bioavailability was 77%, with an apparent oral $t_{1/2}$ of 4.2 h.

In conclusion, introduction of *tert*-butyl and fluorinated alkyl groups on the PP ring in capromorelin (**3**) and fluorine substituents on the phenyl group in the Aib-(D)-*O*-Bn-Ser subunit provided new dipeptidyl analogues (**4c** and **4d**) with large Vds, moderate CLs, and long half-lives in dogs, but weak GH activities in rat models. Modulation of the lipophilicity of a close-in analogue of these compounds by replacing a benzyl group with a 2-picolyl group led to a decrease in plasma CL and Vd in the dog, but no significant change in $t_{1/2}$. Compound **4** possessed a suitably extended canine $t_{1/2}$, but unlike more lipophilic analogues, stimulated GH secretion in two animal species. Repeat oral administration of **4** in dogs increased 24-h plasma IGF-1 levels and resulted in a down-regulation of the post-dose GH reponse. Compound **4** is an orally active, long-duration GHS that has been selected as a development candidate for the treatment of frailty.

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