Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 581–590

BIOORGANIC & MEDICINAL CHEMISTRY

Pyrazolinone-piperidine Dipeptide Growth Hormone Secretagogues (GHSs): Discovery of Capromorelin

Philip A. Carpino,* Bruce A. Lefker, Steven M. Toler, Lydia C. Pan, John R. Hadcock, Ewell R. Cook, Joseph N. DiBrino, Anthony M. Campeta, Shari L. DeNinno, Kristin L. Chidsey-Frink, William A. Hada, John Inthavongsay, F. Michael Mangano, Michelle A. Mullins, David F. Nickerson, Oicheng Ng, Christine M. Pirie, John A. Ragan, Colin R. Rose, David A. Tess, Ann S. Wright, Li Yu, Michael P. Zawistoski, Paul A. DaSilva-Jardine, Theresa C. Wilson and David D. Thompson

Pfizer Global Research and Development, Groton Labs, MS8220-3004, Eastern Point Rd, Groton, CT 06340, USA

Received 24 May 2002; accepted 12 August 2002

Abstract—Novel pyrazolinone-piperidine dipeptide derivatives were synthesized and evaluated as growth hormone secretagogues (GHSs). Two analogues, capromorelin (**5**, CP-424391-18, hGHS-R1a $K_i = 7$ nM, rat pituicyte $EC_{50} = 3$ nM) and the des-methyl analogue **5c** (hGHS-R1a $K_i = 17$ nM, rat pituicyte $EC_{50} = 3$ nM), increased plasma GH levels in an anesthesized rat model, with ED_{50} values less than 0.05 mg/kg iv. Capromorelin showed enhanced intestinal absorption in rodent models and exhibited superior pharmacokinetic properties, including high bioavailabilities in two animal species [F(rat) = 65%, F(dog) = 44%]. This short-duration GHS was orally active in canine models and was selected as a development candidate for the treatment of musculoskeletal frailty in elderly adults.

Introduction

Growth hormone secretagogues (GHSs) are a new class of orally active drugs which increase plasma levels of growth hormone (GH) and insulin-like growth factor-1 (IGF-1), two hormones that play important roles in the regulation of body composition and metabolism.[1] These drugs stimulate GH secretion from the pituitary gland via a heptahelical G-protein coupled receptor called the type 1a growth hormone secretagogue receptor (GHS-R1a).[2] They also promote release of a GH stimulatory hormone called GH-releasing hormone (GHRH) from the hypothalamus, possibly by antagonizing the actions of a hormone that inhibits GH release called somatotrophin release inhibitory factor (SRIF, somatostatin).[3] Elevation of plasma GH levels leads to increased IGF-1 synthesis by the liver. Recently, the endogenous ligand for the GHS-R1a has been identified as a pleiotrophic hormone synthesized by the stomach called ghrelin.[4]

The first GHSs were discovered in the early 1980s by Bowers and co-workers and were called growth hormone-releasing peptides (GHRPs).[5,6] Many structurally distinct GHSs have since been reported in the literature, including tripeptidyl compounds such as NN703 (**1**),[7] dipeptidyl derivatives such as the spiroindane MK-0677 (**2**)[8] and L-163540 (**3**),[9] and several non-peptidyl small molecules (see Fig. 1).[10,11] Prominent features of dipeptidyl GHSs include groups such as α-amino-isobutyric (Aib) acid and various (D)-amino acids (i.e., Aib-(D)-*O*-Bn-Ser, Aib-(D)-Trp) that minimize protease degradation in the intestinal tract following oral administration. Only a few of the peptidyl and peptidomimetic compounds have exhibited sufficient bioavailability in animal models to advance into human clinical studies. The most extensively studied GHS, MK-0677, has been

*Corresponding author. Tel.: +1-860-441-5694; fax: +1-860-715-8067; e-mail: philip_a_carpino@groton.pfizer.com

0968-0896/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0968-0896(02)00433-9

shown to restore GH and IGF-1 levels in elderly subjects to 'youthful' levels,[12] increase fat-free mass and markers of bone formation in healthy obese men,[13,14] and stimulate growth velocity in growth hormone-deficient children.[15]

A new series of dipeptidyl GHSs with pyrazolinone-piperidine (PP) heterocycles (**4**) in the carboxy-terminal (C-terminal) region was derived from compounds **2** and **3** using a pharmacokinetic-oriented screening strategy that focused first on the identification of compounds with good absorption properties in rodent models and second on the optimization of the in vitro and in vivo GH secretagogue activities. Several PP analogues with an Aib-(D)-*O*-Bn-Ser dipeptide subunit as in **2**, rather than a Aib-(D)-Trp dipeptide moiety as in **3**, exhibited

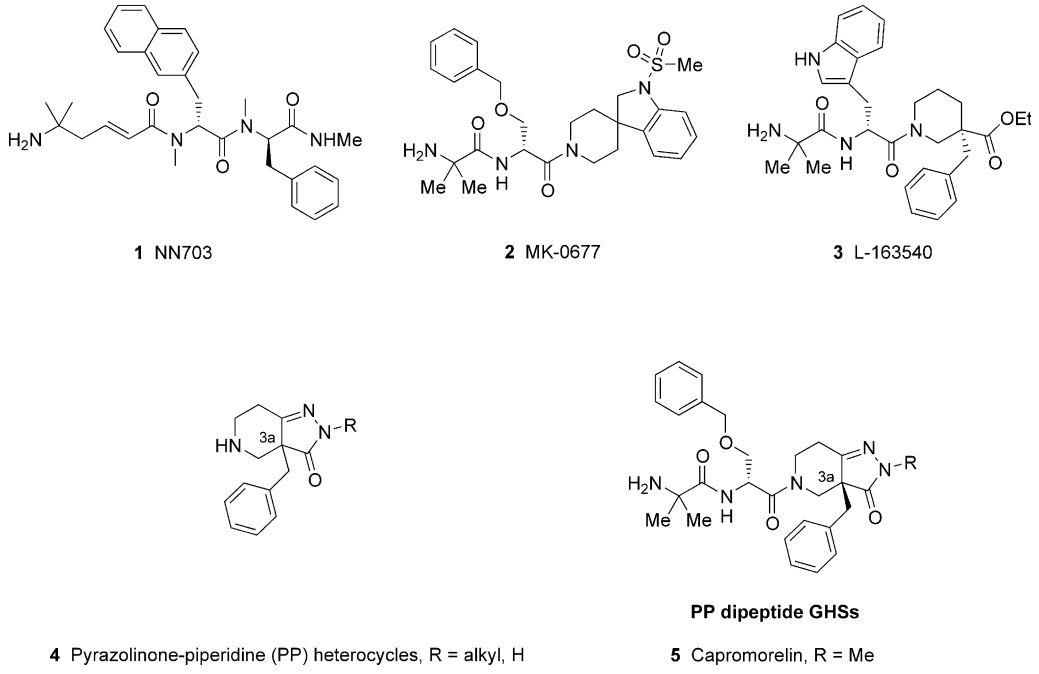
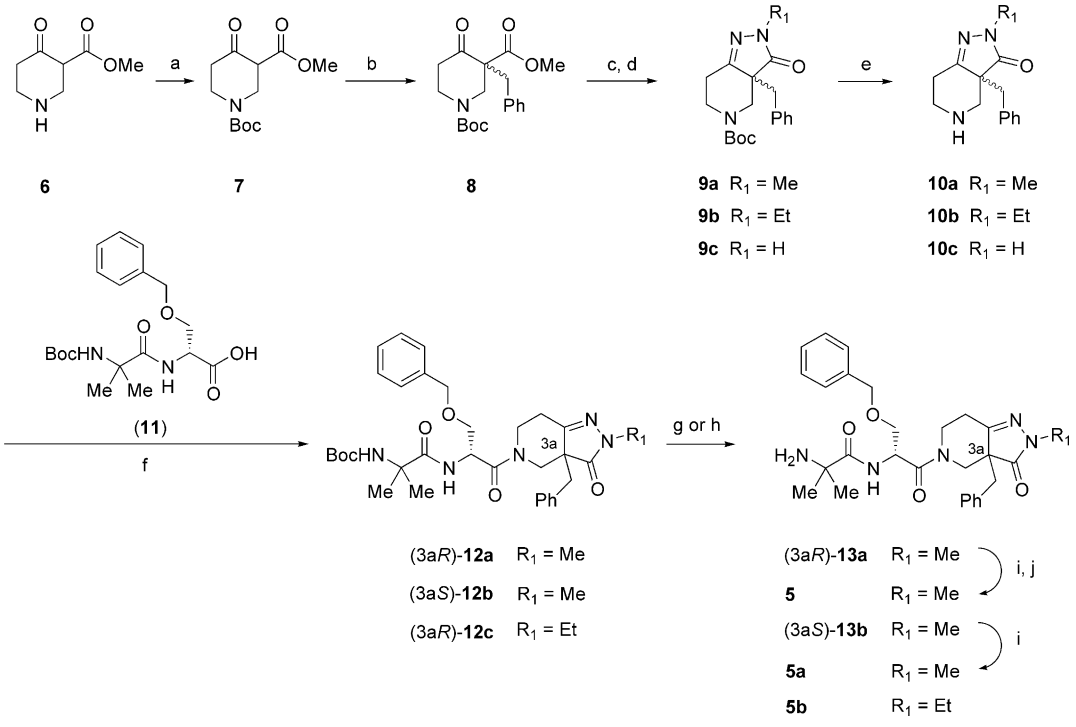


Figure 1. Peptidyl and peptidomimetic GHSs and pyrazolinone-piperidine (PP) heterocycles.



Scheme 1. Syntheses of GHSs **5**, **5a** and **5b**. Reagents and conditions: (a) Boc_2O, DMAP, CH_2Cl_2, 0 °C; (b) NaH, $PhCH_2Br$, DMF; (c) R_1NHNH_2, EtOH, reflux; (d) toluene, reflux; (e) TFA, CH_2Cl_2, 0 °C or concd HCl, EtOH; (f) HOAt, EDC, NEt_3, CH_2Cl_2, 0 °C; (g) TFA, CH_2Cl_2, 0 °C; (h) concd HCl, EtOH; (i) (L)-tartaric acid, MeOH; (j) EtOAc, reflux.

high intestinal absorption in rats and showed good biological activities in dog efficacy models. We have previously disclosed the preliminary pharmacological characterization of a short-duration GHS from this series, capromorelin (**5**, CP-424391–18).[16] Herein, we describe the discovery, biological activities, and syntheses of PP dipeptides and report the physicochemical and pharmacokinetic properties of one GHS, capromorelin, that led to its selection as a development candidate.

Chemistry

The synthesis of the pyrazolinone-piperidine dipeptide capromorelin **5** is shown in Scheme 1. The *N*-*tert*-butoxycarbonyl (*N*-Boc) piperidine derivative **7**, prepared from methyl 4-oxo-3-piperidinecarboxylate **6** and di-*tert*-butyldicarbonate (Boc_2O), was alkylated with benzyl bromide using sodium hydride as a base. The keto ester product **8** was then treated with methylhydrazine to provide the PP heterocycle **9a**. Acid-catalyzed cleavage of the *N*-Boc protecting group in **9a** yielded the amine **10a**, which was coupled with Boc-Aib-(D)-*O*-Bn-Ser-OH (**11**)[17] using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxy-7-azabenzotriazole (HOAt)[18] to give two diastereomers, (3a*R*)-**12a** and (3a*S*)-**12b**. Analysis of the reaction mixture by thin layer chromatography (TLC) revealed rapid formation of the more polar isomer (3a*S*)-**12b**, followed by slow appearance of (3a*R*)-**12a**. HOAt was added to the coupling reaction to minimize racemization of the dipeptide moiety. Removal of the *N*-Boc group in (3a*R*)-**12a** with aqueous hydrochloric acid gave (3a*R*)-**13a**. The (L)-tartrate salt of (3a*R*)-**13a** was prepared and crystallized from hot ethyl acetate to provide capromorelin. The absolute configuration of capromorelin was established by coupling the (3a*R*)-isomer of **10a** (confirmed by X-ray crystallographic analysis) with the (D)-isomer of dipeptide **11** under non-racemizing conditions, the results of which will be described separately in due course. Compound **5a** was obtained in two steps from (3a*S*)-**12b** by the procedure used to prepare **5** from (3a*R*)-**12a**. GHS **5b** was synthesized by first coupling dipeptide **11** with **10b** and then removing the *N*-Boc protecting group.

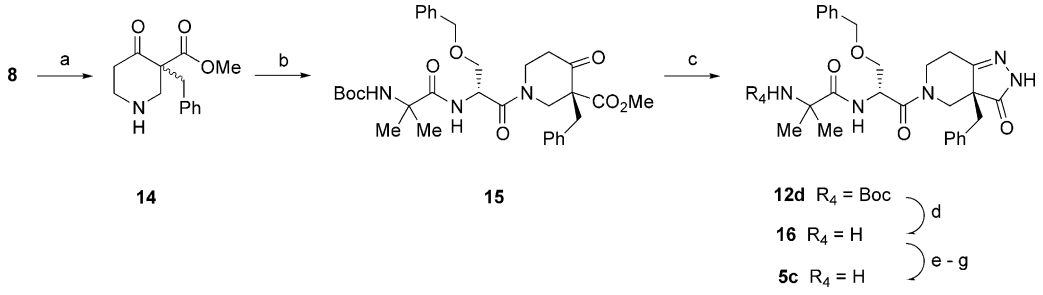
Because pyrazolinone-piperidine **10c** (HCl salt) was chemically unstable in CH_2Cl_2 or DMF solutions in the presence of bases such as triethylamine, the des-methyl GHS analogue **5c** was prepared by an alternate route that involved coupling dipeptide **11** with piperidine derivative **14**, obtained from the *N*-Boc derivative **8** by treatment with trifluoroacetic acid (see Scheme 2). Introduction of the PP heterocycle using standard methodology provided **12d**. Subsequent acid-catalyzed cleavage of the *N*-Boc protecting group yielded dipeptide **16**. The (L)-tartrate salt **5c** was prepared as described previously for **5**.

Results and Discussion

Several different dipeptidyl GHSs such as the clinical candidate MK-0677 (**2**) and the pre-clinical compound L-163540 (**3**) have been disclosed over the past decade. These compounds have generally exhibited good oral activities in canine models, but have required high pharmacological doses or shown unsatisfactory absorption properties in rat models used either for chronic oral efficacy studies or for prediction of absorption properties in humans.[1] The poor rat absorption was often ascribed to low aqueous solubility and low intestinal permeation, due in part to several unfavorable structural features in the molecules, including multiple amide bonds and high number of H-bond donor and acceptor groups in the polar dipeptide backbone. For some GHSs, high intestinal metabolism also contributed to the poor absorption.

The dipeptidyl backbones in GHSs **2** and **3** could not be easily modified without resulting in loss of biological activity. The amino group in the C-terminal region was therefore used to improve the physicochemical properties of the compounds and enhance oral absorption. New compounds were initially screened for intestinal absorption in rodent models and then evaluated as GHSs if exposure was found to be acceptable (a pharmacokinetic-oriented strategy). Intestinal absorption was assessed by measuring portal vein drug concentrations after oral dosing using protocols described in the literature for the identification of bioavailable peptidergic molecules.[19] Dipeptidyl analogues with an Aib-(D)-*O*-Bn-Ser moiety generally showed better intestinal absorption than analogues with an Aib-(D)-Trp group, possibly because they contained one less hydrogen bond and lacked a metabolically labile indole ring.

An unprecedented pyrazolinone-piperidine (PP, 3a-benzyl-2,3a,4,5,6,7-hexahydro-pyrazolo[4,3-c]pyridin-3-one)



Scheme 2. Synthesis of GHS **5c**. Reagents and conditions: (a) TFA, CH_2Cl_2, 0 °C; (b) **11**, EDC, HOAt, CH_2Cl_2, 0 °C; (c) $NH_2NH_2{\cdot}H_2SO_4$, NaOAc, EtOH, 78 °C; (d) concd. HCl, EtOH; (e) saturated aqueous $NaHCO_3$; (f) (L)-tartaric acid, MeOH; (g) EtOAc, 80 °C.

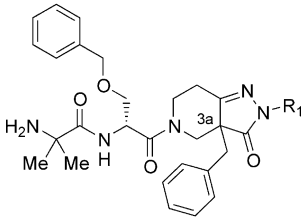
heterocycle **4** was identified as a suitable replacement for the C-terminal amino group in prototypical dipeptidyl GHSs. The PP heterocycle **4** was conceived as an enzymatically stable surrogate for the ethyl 3-benzylnipecotate moiety found in the short-duration GHS **3**, a compound that was designed to rapidly undergo esterase hydrolysis in the plasma to give a less active metabolite.[9] The PP group with its critical 3a-benzyl substituent proved sufficiently lipophilic to counterbalance the hydrophilic nature of the core dipeptidyl backbone. In the Aib-(D)-*O*-Bn-Ser series, analogues such as **5** and **5b** containing substituted PP heterocycles (**4**, R = Me, Et) possessed especially good physicochemical properties and exhibited high intestinal absorption in multiple animal models. An Aib-(D)-*O*-Bn-Ser analogue **5c** with an unsubstituted PP group (**4**, R = H) showed lower, but still satisfactory, absorption in the rat models, most likely due to the extra H-bond capacity. A representative substituted PP dipeptide derivative, capromorelin (**5**), was not only highly soluble in deionized water (>100 mgA/mL) and in a pH 7 buffer solution (4.9 mgA/mL) but also moderately lipophilic, with a calculated partition coefficient (clog *P*) in the optimal range for transcellular diffusion (clog *P* = 2.9). When administered orally to rats (1 mg/kg of free base equivalents or 1 mgA/kg), the calculated fraction of drug absorbed was approximately 1.0 (assuming most of the plasma clearance was due to hepatic elimination).[20] The unsubstituted PP dipeptide derivative **5c** (R = H, Fig. 1) exhibited similar physicochemical properties, but a lower calculated fraction absorbed (Fa = 0.6).

The biological activities and pharmacokinetic properties of several pyrazolinone-piperidine dipeptide derivatives with good absorption properties (capromorelin, **5a**–**c**) were characterized. Binding affinities were measured via competitive binding assays in HEK293 cells overexpressing the cloned human GHS-R1a (hGHS-R1a) using [^{125}I]-ghrelin as a radioligand. Capromorelin was found to be 17-fold less potent than ghrelin, the endogenous ligand for the GHS-R1a (see Table 1). The binding activity of capromorelin was shown to be stereospecific, with the 3a*R*-isomer of the pyrazolinone-piperidine heterocyclic ring preferred over the 3a*S*-isomer. Removal of the *N*-methyl group on the PP group led to a two-fold loss in activity. The *N*-ethyl PP derivative **5b** was equipotent to ghrelin in the assay.

Functional activities of the pyrazolinone-piperidine dipeptide GHSs were evaluated in a GH release assay in rat pituitary cell cultures. Despite exhibiting a 30-fold range of binding affinities, capromorelin and analogues **5b** and **5c** stimulated GH release with similar potencies (see Table 1). The 3a*S*-diastereomer of capromorelin (**5a**) showed significantly weaker activity, with an EC_{50} value greater than 1 μM. Interestingly, both the 3a*R*- and 3a*S*-diastereomers of compound **3** (structural precursors of PP dipeptides) potently stimulated GH secretion (EC_{50}s < 25 nM), though the 3a*S*-diastereomer was slightly preferred.[9] With the exception of **5a**, the binding affinities of the GHSs did not predict activity in the cellular assay, possibly because of the potentially confounding effects of protein binding in the whole cell assay.

The in vivo GH activities of the PP dipeptide analogues were measured in an anesthesized rat model following iv administration. Capromorelin and compounds **5b** and **5c** stimulated GH secretion after a single 1 mg/kg dose, though the mean GH peak heights for capromorelin and **5c** were significantly higher than the mean GH peak height for **5b** (data not shown). Capromorelin and **5c** showed similar dose–response relationships in the model, with ED_{50} values less than 0.05 mg/kg iv. The weaker in vivo activity of the *N*-ethyl PP derivative **5b** was attributed to its increased lipophilicity, which could have reduced the amount of unbound drug in the plasma compartment capable of interacting with the GHS-R1a.

Table 1. Binding affinities and GHS activities of PP dipeptide GHSs



Compound	C-3a stereochemistry	R_1	K_i (nM)[a]	EC_{50} (nM)[b]	ED_{50} (mg/kg iv)[c]
Ghrelin			0.42		
5 (Capromorelin)	*R*	Me	7	3	0.05
5a	*S*	Me	1430	>1000	ND[d]
5b	*R*	Et	0.66	4	ND
5c	*R*	H	17	3	0.04

[a]K_is for displacement of [^{125}I]-ghrelin in HEK293/hGHS-R1a cell membranes, calculated from average of duplicate experiments.
[b]GH release in rat pituitary cell cultures, mean EC_{50} values calculated from triplicate determinations.
[c]GH release in anesthesized rat model.
[d]Not determined.

Pharmacokinetic properties of two PP dipeptide GHSs, capromorelin and **5c**, were measured in female Sprague–Dawley rats. For capromorelin, plasma clearance (CL), volume of distribution (Vd) and plasma elimination half-life ($t_{1/2}$) following a 1 mgA/kg iv dose were 34 ± 5 mL/min/kg, 1.7 L/kg and 0.79 ± 0.21 h respectively. Capromorelin was rapidly absorbed after a 1 mgA/kg oral (po) dose, reaching maximum systemic concentrations (C_{max}) of 329 ± 158 ng/mL in 0.25 h. The unbound plasma free fraction was high (Fu = 27%), possibly explaining the robust GHS activity in the in vivo anesthesized rat model. Oral bioavailability was 65%, far greater than the reported bioavailabilities of other peptidyl or peptidomimetic GHSs in the literature. Compound **5c** was detected as a circulating metabolite, though exposure generally represented less than 10% of parent drug. Pharmacokinetic values for **5c** were similar to those for capromorelin. Following a 1 mgA/kg iv dose, systemic clearance was high and volume of distribution was moderate, resulting in a short half-life (CL = 56.7 mL/min/kg; Vd = 2.6 L/kg; $t_{1/2} = 0.83$ h). Oral bioavailability was low, most likely due to the combination of incomplete intestinal absorption and high CL ($F = 12\%$).

As the only PP GHS with superior bioavailability and good absorption properties in a rat model, capromorelin was selected for pharmacokinetic evaluation in the dog. After a 1 mgA/kg iv dose, the plasma clearance of the compound was 19 ± 5 mL/min/kg, the volume of distribution was 2.0 ± 0.4 L/kg and the elimination half-life was 1.3 h. Mean values for C_{max} and T_{max} following a 1 mgA/kg po dose were 180 ± 66 ng/mL and 1 h. The plasma free fraction of drug was 51% and the bioavailability was 44%. Compound **5c** was also identified as a circulating plasma metabolite.

Pharmacokinetic characterization of capromorelin in rats and dogs revealed short plasma elimination half-lives. In dogs, the short half-life was attributed to a moderate volume of distribution and a moderate-to-high clearance due in part to de-methylation of the PP ring and oxidation of both the benzyl group in the (D)-*O*-Bn-Ser moiety and a methyl group in the Aib moiety (data not shown). The compound did not undergo proteolytic degradation in plasma. The des-methyl metabolite **5c** exhibited a similarly short half-life, but because of its lower rat bioavailability, it did not offer any advantages over capromorelin. A short half-life was considered pharmacologically desirable for stimulating GH secretion (rather than increasing IGF-1 levels) and preventing attenuation of the post-dose GH response during repeat administration.[9]

The oral activity of capromorelin was examined in a dog model that was found to be predictive of GHS activity in humans.[8] Following a single 1 mg/kg po dose, capromorelin rapidly increased plasma GH levels, with a maximum peak height of 73 ng/mL. Significant GHS activity was observed at doses as low as 0.05 mg/kg. When administered at 1 mg/kg po for 5 days, capromorelin stimulated GH secretion on the first and last days of the study; however, the post-dose GH response on day 5 was somewhat attenuated.[16]

Conclusions

A novel series of pyrazolinone-piperidine (PP) dipeptide GHSs has been discovered using a pharmacokinetic-oriented screening strategy that focused on identifying compounds with good absorption properties. Several PP analogues with Aib-(D)-*O*-Bn-Ser subunits exhibited high intestinal absorption in rat models and possessed favorable physicochemical properties, including high aqueous solubility and moderate lipophilicity. One short-acting compound, capromorelin (**5**, CP-424391-18), stimulated GH secretion in several animal species following intravenous and oral administration and was selected as a clinical candidate for the treatment of musculoskeletal frailty in elderly subjects, the results of which will be reported elsewhere.

Experimental

General chemistry methods

Proton magnetic spectra were recorded on Bruker AC-250, Bruker AC-300, Varian XL-300 or Varian Unity 400 spectrometers. Chemical shifts are expressed in parts per million (δ) downfield from trimethylsilane; the deuterium lock signal of the deuterated solvent was used as an internal standard. Low resolution mass spectra (LRMS) were obtained on a Hewlett-Packard 5989A spectrometer using ammonia as the source of chemical ionization. All reagents, solvents and drying agents were obtained from commercial sources and were used without further purification unless otherwise specified. All moisture- or air-sensitive reactions were performed in flame-dried glassware equipped with septum inlets under a positive pressure of nitrogen. Analytical thin-layer chromatography was carried out using silica plates (E. Merck Kieselgel 60 F254). Preparative chromatographic separations were performed using flash chromatography with Amicon silica gel (30 μM, 60 Å pore size). Melting points were recorded on a Büchi 510 apparatus and are uncorrected. Combustion analyses were obtained from Micromass.

4-Oxo-piperidine-1,3-dicarboxylic acid 1-*tert*-butyl ester 3-methyl ester (7). To a mixture of 4-oxo-piperidine-3-carboxylic acid methyl ester (**6**, 7.0 g, 36.2 mmol) and 4,4-dimethylaminopyridine (8.82 g, 72.3 mmol) in CH_2Cl_2 (200 mL) at 0 °C was added a solution of di-*tert*-butyldicarbonate (7.88 g, 36.2 mmol) in CH_2Cl_2 (150 mL). The reaction mixture was allowed to warm to room temperature and stir for 19 h. The solvents were removed in vacuo and the residue was diluted with $CHCl_3$. The organic solution was washed with 10% aqueous HCl and saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuo to yield the desired product as an amorphous solid (9.18 g, 99%). ^1H NMR ($CDCl_3$) δ 4.0 (s, 2H), 3.75 (s, 3H), 3.74 (s, 1H), 3.55 (t, 2H), 2.32 (t, 2H), 1.45 (s, 9H). LRMS *m*/*z* 258 ($M^+ + 1$).

3-Benzyl-4-oxo-piperidine-1,3-dicarboxylic acid 1-*tert*-butyl ester 3-methyl ester (8). Sodium hydride (745 mg, 7.4 mmol, 60% oil dispersion) was added to a solution

of **7** (5.0 g, 19.4 mmol) in DMF (10 mL). The reaction mixture was stirred at room temperature for 0.25 h, then benzyl bromide (3.32 g, 19.4 mmol) in DMF (15 mL) was added. The reaction mixture was stirred for 42 h at room temperature and diluted with ethyl acetate. The organic solution was washed with H_2O and saturated aqueous NaCl, dried over $MgSO_4$, and concentrated in vacuo to give the desired product (6.0 g, 89%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.26–7.12 (m, 5H), 4.57 (bs, 1H), 4.17 (bs, 1H), 3.6 (s, 3H), 3.23 (d, 1H, J = 14.1 Hz), 3.14 (t, 1H), 3.01 (d, 2H, J = 13.29 Hz), 2.7 (m, 1H), 2.42 (d, 1H, J = 14 Hz), 1.43 (s, 9H). LRMS m/z 348 ($M^+ + 1$).

3a-Benzyl-2-methyl-3-oxo-2,3,3a,4,6,7-hexahydro-pyrazolo[4,3-c]pyridine-5-carboxylic acid *tert*-butyl ester (9a). A mixture of **8** (4.0 g, 11.5 mmol) and methylhydrazine (530 mg, 11.5 mmol) in ethanol (100 mL) was heated under reflux for 8 h. The reaction was cooled to room temperature and the ethanol was removed in vacuo. The residue was dissolved in toluene (100 mL) and heated under reflux for 17 h. After cooling to room temperature, the reaction mixture was concentrated under vacuum and the residue was purified on silica gel using a solvent gradient (15:85 v/v ethyl acetate/hexanes to 75:25 v/v ethyl acetate/hexanes) to give the desired product (2.6 g, 66%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.25–7.02 (m, 5H), 4.65 (bs, 1H), 4.55 (bs, 1H), 3.2 (d, 1H, J = 12.87 Hz), 3.05 (s, 3H), 3.0 (d, 2H, J = 13.27 Hz), 2.7 (bs, 3H), 2.52 (d, 1H, J = 9.97), 1.51 (s, 9H). LRMS m/z 344 ($M^+ + 1$).

3a-Benzyl-2-ethyl-3-oxo-2,3,3a,4,6,7-hexahydro-pyrazolo[4,3-c]pyridine-5-carboxylic acid *tert*-butyl ester (9b). The title compound **9b** was prepared from **8** by the procedure described for **9a** except ethylhydrazine was used. ^{1}H NMR ($CDCl_3$) δ 7.24–7.02 (m, 5H), 4.6 (bs, 1H), 4.4 (bs, 1H), 3.55 (m, 1H), 3.35 (m, 1H), 3.16 (d, 1H, J = 13.29 Hz), 3.0 (d, 1H, J = 13.29 Hz), 2.7 (bs, 3H), 2.53 (d, 1H, J = 9.55 Hz), 1.5 (s, 9H), 0.8 (t, 3H). LRMS m/z 358 ($M^+ + 1$).

3a-Benzyl-3-oxo-2,3,3a,4,6,7-hexahydro-pyrazolo[4,3-c]-pyridine-5-carboxylic acid *tert*-butyl ester (9c). The title compound **9c** was prepared from **8** by the procedure described for **9a** except hydrazine hydrate was used. ^{1}H NMR ($CDCl_3$) δ 8.08 (s, 1H), 7.2–7.07 (m, 5H), 4.66 (s, 1H), 4.46 (s, 1H), 3.2 (d, 1H, J = 12.46 Hz), 3.03 (d, 1H, J = 13.75 Hz), 2.7 (bs, 3H), 2.56 (d, 1H, J = 9.14 Hz), 1.5 (s, 9H). LRMS m/z 330 ($M^+ + 1$).

3a-Benzyl-2-methyl-2,3a,4,5,6,7-hexahydro-pyrazolo[4,3-c]pyridin-3-one (10a). To a solution of **9a** (13.8, 40 mmol) in CH_2Cl_2 (140 mL) at 0 °C was added cold trifluoroacetic acid (70 mL). The reaction mixture was stirred for 2.5 h and then concentrated under vacuum. Ethyl acetate was added and the solution was concentrated to dryness. The residue was then diluted with $CHCl_3$ and the organic solution was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated. The crude residue was chromatographed on silica gel using a gradient of 1:99 v/v MeOH/CH_2Cl_2 to 1:10 v/v MeOH/CH_2Cl_2 to give the desired product **10a** (7.8 g, 80%). ^{1}H NMR ($CDCl_3$) δ 7.24–7.13 (m, 5H), 3.50 (m, 1H), 3.40 (d, 1H, J = 12.45 Hz), 3.33 (d, 1H, J = 13.29 Hz), 3.16 (d, 1H, J = 13.29), 3.08 (s, 3H), 3.05 (m, 1H), 2.60 (m, 3H). LRMS m/z 244 ($M^+ + 1$).

3a-Benzyl-2-ethyl-2,3a,4,5,6,7-hexahydro-pyrazolo[4,3-c]pyridin-3-one (10b). The title compound **10b** was prepared from **9b** by the procedure described for **10a**. ^{1}H NMR ($CDCl_3$) δ 7.22–7.07 (m, 10H), 3.58 (m, 1H), 3.46 (m, 1H), 3.35 (m, 1H), 3.28 (m, 2H), 3.10 (d, 1H, J = 13.7 Hz), 2.55 (m, 4H), 1.73 (bs, 1H), 0.82 (t, 3H). LRMS m/z 258 ($M^+ + 1$).

3a-Benzyl-2,3a,4,5,6,7-hexahydro-pyrazolo[4,3-c]pyridin-3-one hydrochloride (10c). A solution of **9c** (1.0 g, 3 mmol) in a 2:1 ethanol/concentrated HCl solution (15 mL) was stirred at room temperature for 2 h. The reaction mixture was diluted with ethanol (30 mL) and concentrated under vacuum to give the desired product as a colorless solid (0.6 g, 100%). ^{1}H NMR (MeOH-d_4) δ 7.3–7.0 (m, 5H), 4.7 (d, 1H), 4.5 (d, 1H), 3.8–3.6 (m), 3.1–2.6 (m). LRMS m/z 230 ($M^+ + 1$).

{1-[2-(3a-(*R*)-Benzyl-2-methyl-3-oxo-2,3,3a,4,6,7-hexahydro-pyrazolo[4,3-c]pyridin-5-yl)-1-(*R*)-benzyloxymethyl-2-oxo-ethylcarbamoyl]-1-methyl-ethyl}-carbamic acid *tert*-butyl ester [(3a*R*)-12a] and {1-[2-(3a-(*S*)-benzyl-2-methyl-3-oxo-2,3,3a,4,6,7-hexahydro-pyrazolo[4,3-c]pyridin-5-yl)-1-(*R*)-benzyloxymethyl-2-oxo-ethylcarbamoyl]-1-methyl-ethyl}-carbamic acid *tert*-butyl ester [(3a*S*)-12b]. To a solution of the PP derivative **10a** (7.8 g, 32 mmol) in CH_2Cl_2 (120 mL) at 0 °C was sequentially added Boc-Aib-(D)-*O*-Bn-Ser-OH (**11**, 18.2 g, 48 mmol), 1-hydroxy-7-azabenzotriazole (6.4 g, 48 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (9.2 g, 48 mmol), and triethylamine (6.7 mL, 48 mmol). The reaction mixture was allowed to warm to room temperature and stir for 17 h. Ethyl acetate (100 mL) was added and the organic solution was washed with 10% HCl, saturated aqueous $NaHCO_3$, saturated aqueous NaCl, dried over Na_2SO_4, and concentrated under vacuum. The crude residue was purified by silica gel chromatography using a solvent gradient (1:1 v/v ethyl acetate/hexanes to 100% ethyl acetate) to give a less polar diastereomer, (3a*R*)-**12a** (9 g, 46%), and a more polar diastereomer, (3a*S*)-**12b** (9.5 g, 49%). Compound (3a*R*)-**12a**: ^{1}H NMR ($CDCl_3$) δ 7.30–7.14 (m, 10H), 7.05 (d, 1H, J = 7.47 Hz), 5.22 (q, 1H), 5.05 (d, 1H, J = 12.04 Hz), 4.85 (d, 2H, J = 3.74 Hz), 3.68 (m, 1H), 3.62 (t, 1H), 3.13 (d, 1H, J = 13.28 Hz), 3.05 (s, 3H), 2.90 (m, 2H), 2.53 (d, 1H, J = 10.80 Hz), 2.47 (d, 1H, J = 13.29 Hz), 1.47 (s, 3H), 1.44 (s, 3H), 1.35 (s, 9H). LRMS m/z 606 ($M^+ + 1$). Compound (3a*S*)-**12b**: ^{1}H NMR ($CDCl_3$) δ 7.30–7.06 (m, 10H), 6.75 (d, 1H, J = 6.65 Hz), 5.3 (m, 1H), 5.05 (d, 1H, J = 12.46 Hz), 4.61 (dd, 2H, J = 11.21, 29 Hz), 3.8 (m, 1H), 3.65 (t, 1H), 3.1 (d, 1H, J = 13.7 Hz), 2.99 (s, 3H), 2.90 (d, 1H, J = 13.29 Hz), 1.54 (s, 3H), 1.45 (s, 3H), 1.4 (s, 9H). LRMS m/z 606 ($M^+ + 1$).

{1-[2-(3a-(*R*)-Benzyl-2-ethyl-3-oxo-2,3,3a,4,6,7-hexahydro-pyrazolo[4,3-c]pyridin-5-yl)-1-(*R*)-benzyloxymethyl-2-oxo-ethylcarbamoyl]-1-methyl-ethyl}-carbamic acid

***tert*-butyl ester [(3a*R*)-12c].** The title compound was prepared by coupling **10b** with **11** using the procedure described for (3a*R*)-**12a**. The desired compound was obtained as the less polar compound of the diastereomeric mixture after silica gel chromatography using a solvent gradient of 1:1 v/v ethyl acetate/hexanes to 100% ethyl acetate. ^{1}H NMR ($CDCl_3$) δ 7.5–7.1 (m, 10H), 5.25 (t, 1H), 5.05 (d, 1H, $J=13.7$ Hz), 4.48 (d, 2H), 3.7 (m, 1H), 3.6 (m, 1H), 3.35 (m, 1H), 3.13 (d, 1H, $J=13.7$ Hz), 2.9 (m, 2H), 2.58 (m, 1H), 2.47 (d, 1H, $J=13$ Hz), 1.54 (s), 1.47 (s, 3H), 1.43 (s, 3H), 1.35 (s, 9H), 0.8 (t, 3H). LRMS m/z 621 ($M^+ + 1$).

2-Amino-*N*-[2-(3a-(*R*)-benzyl-2-methyl-3-oxo-2,3,3a,4,6,7-hexahydro-pyrazolo[4,3-c]pyridin-5-yl)-1-(*R*)-benzyloxymethyl-2-oxo-ethyl]-isobutyramide [(3a*R*)-13a]. To a solution of (3a*R*)-**12a** (10 g, 17 mmol) in CH_2Cl_2 (69 mL) at 0 °C was slowly added trifluoroacetic acid (35 mL). The reaction mixture was allowed to warm to room temperature. After 3 h, the organic solution was concentrated under vacuum. The residue was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated in vacuo to afford (3a*R*)-**13a** (7.4 g, 86%). ^{1}H NMR (MeOH-d_4) δ 7.35–7.1 (m, 10H), 5.2 (t, 1H), 4.55 (s, 2H), 3.75 (d, 2H), 3.05 (m), 2.61 (d, 1H), 2.55 (d, 1H), 1.45 (s, 3H), 1.42 (s, 3H). LRMS m/z 506 ($M^+ + 1$).

2-Amino-*N*-[2-(3a-(*R*)-benzyl-2-methyl-3-oxo-2,3,3a,4,6,7-hexahydro-pyrazolo[4,3-c]pyridin-5-yl)-1-(*R*)-benzyloxymethyl-2-oxo-ethyl]-isobutyramide (L)-tartrate (5). To a solution of (3a*R*)-**13a** (385 g, 0.761 mol) in methanol (4 L) at room temperature was added (L)-(+)-tartaric acid (114 g, 0.761 mol). The reaction mixture was stirred for 17 h, filtered to remove any insoluble residues, and concentrated under vacuum. Ethyl acetate (12 L) was added and the solution was concentrated in vacuo. The residue was re-dissolved in ethyl acetate (12 L) and heated under reflux for 16 h. After slowly cooling to room temperature, a crystalline white solid slowly precipitated out of solution and was collected by filtration to give the desired product (482 g, 96.8%), mp 174–176 °C. ^{1}H NMR (MeOH-d_4) δ 7.3–7.1 (m, 10H), 5.2 (t, 1H), 4.55 (s, 2H), 4.4 (s), 3.78 (m, 2H), 3.1 (d, 1H, $J=13.3$ Hz), 3.01 (s, 3H), 2.97 (d, 1H), 2.65 (d, 1H, $J=12.4$ Hz), 2.55 (d, 1H, $J=11$ Hz), 1.59 (s, 3H), 1.58 (s, 3H). LRMS m/z 506 ($M^+ + 1$). Anal. calcd for $C_{32}H_{41}N_5O_{10}$: C, 58.62; H, 6.30; N, 10.68. Found: C, 58.65; H, 6.14; N, 10.78.

2-Amino-*N*-[2-(3a-(*S*)-benzyl-2-methyl-3-oxo-2,3,3a,4,6,7-hexahydro-pyrazolo[4,3-c]pyridin-5-yl)-1-(*R*)-benzyloxymethyl-2-oxo-ethyl]-isobutyramide [(3a*S*)-13b]. A solution of dipeptide (3a*S*)-**12b** (0.5 g, 0.83 mmol) in a 3:1 ethanol/concentrated HCl solution (26 mL) was stirred at room temperature for 5 h. The reaction mixture was diluted with ethanol (50 mL) and concentrated under vacuum. $CHCl_3$ (10 mL) was added and the organic solution was washed with saturated aqueous $NaHCO_3$, saturated aqueous NaCl, dried over $MgSO_4$, and concentrated in vacuo to give the desired product as a colorless solid (0.41 g, 98%). ^{1}H NMR ($CDCl_3$) δ 7.36–7.1 (m, 9H), 6.75 (d, 1H, $J=6.65$ Hz), 5.3 (bs, 1H), 5.06 (1H, dd, $J=12.88$, 23.89 Hz), 4.7–4.45 (m, 3H), 3.85–3.7 (m, 2H), 3.05 (q, 1H), 2.99 (s, 3H), 2.89 (t, 1H), 2.6 (m, 1H), 2.47 (d, 1H, $J=13.28$ Hz), 2.4 (d, 1H, $J=11.2$ Hz), 1.55 (s, 6H). LRMS m/z 506 ($M^+ + 1$).

2-Amino-*N*-[2-(3a-(*S*)-benzyl-2-methyl-3-oxo-2,3,3a,4,6,7-hexahydro-pyrazolo[4,3-c]pyridin-5-yl)-1-(*R*)-benzyloxymethyl-2-oxo-ethyl]-isobutyramide (L)-tartrate (5a). (L)-(+)-Tartaric acid (122 mg, 0.81 mmol) in MeOH (5 mL) was added to a solution of (3a*S*)-**13b** (410 mg, 0.81 mmol) in MeOH (5 mL). The reaction mixture was stirred at room temperature for 17 h and concentrated under vacuum to give the desired product (530 mg, 100%). ^{1}H NMR (MeOH-d_4) δ 7.37–7.0 (m, 9H), 6.8 (d, 1H, $J=6.65$ Hz), 5.30 (t, 1H), 4.60 (m, 2H), 4.37 (s, 2H), 3.88 (t, 1H), 3.80 (t, 1H), 3.72 (m, 1H), 3.42 (d, 1H, $J=13.7$ Hz), 3.2–3.07 (m, 2H), 2.96 (s, 3H), 2.75 (m, 1H), 2.6 (d, 1H, $J=12.87$ Hz), 2.5 (d, 1H, $J=13.29$ Hz), 1.55 (s, 6H). LRMS m/z 506 ($M^+ + 1$).

2-Amino-*N*-[2-(3a-(*R*)-benzyl-2-ethyl-3-oxo-2,3,3a,4,6,7-hexahydro-pyrazolo[4,3-c]pyridin-5-yl)-1-(*R*)-benzyloxymethyl-2-oxo-ethyl]-isobutyramide hydrochloride (5b). A solution of dipeptide (3a*R*)-**12c** (300 mg, 0.48 mmol) in a 2:1 ethanol/concentrated HCl solution (30 mL) was stirred at room temperature for 5 h. The reaction mixture was diluted with ethanol (30 mL) and concentrated under vacuum. Additional ethanol was added to the residue and the solution was concentrated in vacuo. This process was repeated until the product was obtained as a solid residue (250 mg, 100%). ^{1}H NMR (MeOH-d_4) δ 7.3–7.1 (m, 10H), 5.18 (t, 1H), 4.58 (s, 2H), 3.77 (m, 2H), 3.56 (m, 2H), 3.36 (m, 2H), 3.13 (d, 1H, $J=13$ Hz), 3.0 (m, 2H), 2.6 (t, 2H), 1.6 (s, 3H), 1.59 (s, 3H), 0.8 (t, 3H). LRMS m/z 521 ($M^+ + 1$).

3-Benzyl-4-oxo-piperidine-3-carboxylic acid methyl ester (14). To a solution of **8** (20.0 g, 5.8 mmol) in CH_2Cl_2 (200 mL) at 0 °C was added cold trifluoroacetic acid (100 mL). The reaction mixture was stirred for 2 h at 0 °C and for 3 h at room temperature and was then concentrated under vacuum. Ethyl acetate was added and the solution was concentrated again in vacuo. The residue was then diluted with $CHCl_3$ and the organic solution was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated under vacuum to give the desired product as a white solid (13.8 g, 97%). ^{1}H NMR ($CDCl_3$) δ 7.3–7.25 (m, 4H), 7.05 (m, 1H), 3.69 (s, 3H), 3.45 (m, 1H), 3.36 (d, 1H, $J=14.12$ Hz), 3.2 (m, 1H), 2.95 (m, 1H), 2.85 (d, 1H, $J=14.12$ Hz), 2.68 (d, 1H, $J=13.71$ Hz), 2.5 (m, 2H). LRMS m/z 248 ($M^+ + 1$).

3-(*R*)-Benzyl-1-[3-benzyloxy-2-(*R*)-(2-*tert*-butoxycarbonylamino-2-methyl-propionylamino)-propionyl]-4-oxo-piperidine-3-carboxylic acid methyl ester (15). To a solution of **14** (13.8 g, 56 mmol) in CH_2Cl_2 (200 mL) at 0 °C was added Boc-Aib-(D)-*O*-Bn-Ser-OH (**11**, 21 g, 56 mmol), 1-hydroxy-7-azabenzotriazole (11.4 g, 84 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (11 g, 56 mmol). The reaction mixture was allowed to warm to room temperature and

stir for 72 h. The organic solution was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over $MgSO_4$, and concentrated under vacuum. The crude residue was chromatographed on silica gel using a solvent gradient (2:1 v/v ethyl acetate/hexanes to 100% EtOAc) to give the desired product as the less polar isomer of the diastereomeric mixture (2.6 g, 28%). ^{1}H NMR (MeOH-d_4) δ 7.35–7.15 (m, 10H), 5.40–4.70 (m, 3H), 4.42 (s, 2H), 3.70–3.40 (m, 5H), 3.2 (d, 1H, $J=14$ Hz), 2.88 (m, 1H), 2.55 (m, 2H), 1.45 (s, 3H), 1.42 (s, 1H), 1.38 (s, 9H). LRMS m/z 611 ($M^+ + 1$).

{1-[2-(3a-(*R*)-Benzyl-3-oxo-2,3,3a,4,6,7-hexahydro-pyrazolo[4,3-c]pyridin-5-yl)-1-(*R*)-benzyloxymethyl-2-oxo-ethylcarbamoyl]-1-methyl-ethyl}-carbamic acid *tert*-butyl ester (12d). A solution of **15** (820 mg, 1.32 mmol), hydrazine sulfate (343 mg, 2.6 mmol) and sodium acetate (431 mg, 5.26 mmol) in EtOH (25 mL) was heated at 78 °C for 17 h. The reaction mixture was cooled to room temperature and concentrated under vacuum. The crude residue was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over $MgSO_4$, and concentrated under vacuum. The crude residue was chromatographed on silica gel using a solvent gradient (3:1 v/v EtOAc/hexane to 100% EtOAc) to give the desired product as a white solid (550 mg, 14%). ^{1}H NMR ($CDCl_3$) δ 9.0 (bs, 1H), 7.30–7.10 (m, 10H), 5.3 (q, 1H), 4.96 (d, 1H, $J=12$ Hz), 4.50 (s, 2H), 3.75–2.80 (m, 2H), 3.12 (d, 1H, $J=13$ Hz), 2.92 (m, 2H), 2.5 (m, 2H), 1.46 (s, 3H), 1.42 (s, 3H), 1.37 (s, 9H). LRMS m/z 592 ($M^+ + 1$).

2-Amino-*N*-[2-(3a-(*R*)-benzyl-3-oxo-2,3,3a,4,6,7-hexahydro-pyrazolo[4,3-c]pyridin-5-yl)-1-(*R*)-benzyloxymethyl-2-oxo-ethyl]-isobutyramide hydrochloride (16). The title compound was obtained from **12d** using the method described for the preparation of **5b**. ^{1}H NMR (MeOH-d_4) δ 7.36–7.17 (m, 10H), 5.26 (t, 1H), 4.56 (s, 2H), 3.80 (d, 2H, $J=4.27$ Hz), 3.1 (d, 1H, $J=13.67$ Hz), 3.0 (m, 2H), 2.63 (d, 1H, $J=12.6$ Hz), 2.56 (d, 1H, $J=10.26$ Hz), 1.61 (s, 3H), 1.60 (s, 3H). LRMS m/z 492 ($M^+ + 1$).

2-Amino-*N*-[2-(3a-(*R*)-benzyl-3-oxo-2,3,3a,4,6,7-hexahydro-pyrazolo[4,3-c]pyridin-5-yl)-1-(*R*)-benzyloxymethyl-2-oxo-ethyl]-isobutyramide (L)-tartrate (5c). Compound **16** (343 mg, 0.65 mmol) was added to $CHCl_3$ (10 mL) in a separatory funnel and the organic solution was washed with saturated aqueous $NaHCO_3$, saturated aqueous NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in MeOH (5 mL) and a solution of (L)-tartaric acid (99 mg, 0.65 mmol) in MeOH (3 mL) was added. The reaction mixture was stirred at room temperature for 17 h and concentrated under vacuum. The residue was dissolved in EtOAc (5 mL) and heated at 80 °C for 1 h and 62 °C for 17 h, after which time a white, finely crystalline solid precipitated out of solution. After cooling to room temperature, the solid was collected by filtration to give the desired product (320 mg, 77%), mp 211–213 °C. ^{1}H NMR (MeOH-d_4) δ 7.35–7.16 (m, 10H), 5.19 (t, 1H), 4.55 (s, 2H), 4.37 (s, 2H), 3.77 (d, 2H, $J=5.39$ Hz), 3.10 (d, 1H, $J=13.7$ Hz), 3.0 (m, 2H), 2.63 (d, 1H, $J=12.87$ Hz), 2.56 (d, 1H, $J=10.8$ Hz), 1.59 (s, 3H), 1.58 (s, 3H). LRMS m/z 492 ($M^+ + 1$).

Biological methods

Binding assay. Membranes were prepared from HEK293 cells (ATCC) stably transfected with the human GHS-R1a receptor cDNA in the plasmid pcDNA3.1neo (Invitrogen).[21] Competition radioligand binding assays were performed in 96-well format with GF/C filters pre-soaked in 0.3% polyethyleneimine. Assays were performed at room temperature for 1 h in duplicate using 50 pM [^{125}I]-ghrelin (Perkin–Elmer NEN-Life Sciences) and 1 μg membrane per well in 50 mM HEPES, pH 7.4, 10 mM $MgCl_2$, 0.2% bovine serum albumin and the following protease inhibitors: 100 μg/mL bacitracin, 100 μg/mL benzamidine, 5 μg/mL aprotinin, 5 μg/mL leupeptin. The membranes were harvested and washed three times with ice-cold wash buffer containing 50 mM HEPES, pH 7.4 and 10 mM $MgCl_2$. IC_{50} and K_i values were determined using Prism by Graphpad™. The K_d of [^{125}I]-ghrelin at membranes expressing human GHS receptors was calculated to be 0.2 nM. No detectable binding was observed in untransfected HEK293 cell membranes (data not shown).

Functional activity in vitro (GH release in rat pituitary cell cultures). Primary pituitary cell cultures were established by enzymatic dissociation of anterior pituitary glands from 6-week-old male Wistar rats.[16] Cells were suspended in Dulbecco's modified Eagle's medium (DMEM, 4.5 g/L glucose supplemented with 1 mM sodium pyruvate, 1% MEM non-essential amino acids, 10% heat-inactivated horse serum, 2.5% fetal bovine serum plus antibiotics), plated at 1×10^5 cells per well in 24-well tissue culture plates (Costar, Corning, NY) and incubated in a humidified 5% CO_2/95% air incubator at 37 °C. Hormone release was performed 3–4 days after plating. Cell cultures were rinsed twice and then equilibrated at 37 °C in release medium (DMEM with 25 mM HEPES buffer, pH 7.4 and 5 mg/mL bovine serum albumin) for 30 min. This medium was aspirated and replaced with pre-warmed release medium containing test agents. After a 15 min incubation period at 37 °C, the medium was removed and assayed for GH. Results are expressed as mean ± SEM of quadruplicate wells. Groups were compared by unpaired Student's 2-tailed t-test unless otherwise indicated. The data are reported as the concentration necessary to produce a half-maximum response (EC_{50}). MK-0677 was used as a reference standard in control plates.

Functional activity in vivo (GH release in anesthesized rat and canine models). Wistar rats (Charles River Laboratories, Wilmington, MA) were anesthesized with pentobarbital. Compounds were administered intravenously and the serum GH levels were measured 10 min post-dose. The ratio of the serum GH concentration at 10 min to the serum GH level prior to injection was calculated and used to determine ED_{50} values. Beagle dogs, male and female, 2–5 years old, were purchased from Marshall Farms (North Rose, NY). Dogs were

fasted overnight, dosed in the early morning by oral gavage, then fed approximately 3 h after dosing. Access to water was provided at all times. Canine blood samples were withdrawn by direct venipuncture of the jugular vein into heparinized vacutainers. Serum and plasma samples were stored frozen pending hormone assays. Rat and canine GHs were measured by species-specific double antibody radioimmunoassays using reagents and protocols obtained from the NIDDK National Hormone and Pituitary Program (Dr. A. F. Parlow, Harbor-UCLA Medical Center Torrance, CA). All animal studies were conducted under approved protocols in accordance with NIH Guidelines for the Care and Use of Laboratory.

Pharmacokinetics of capromorelin in rats. Eight adult female Sprague–Dawley rats were prepared for use by surgical implantation of a cannula in the femoral vein while under methoxyflurane anesthesia the day before study initiation. Study rats were fasted overnight prior to dosing, allowed free access to water and housed in standard polycarbonate rodent cages. The rodents were allowed access to food 4 h post-dose. The dose (intravenous and oral) was prepared as a 0.5 mg/mL base equivalent (0.5 mgA/kg) solution of capromorelin in deionized water. Four Sprague–Dawley rats (weighing 0.280–0.305 kg) received an iv dose of 1 mgA/kg of capromorelin. The dose was administered via the femoral vein catheter followed by a 1 mL infusion of normal saline to rinse the catheter. Blood samples (0.5 mL) were taken from the femoral vein catheter pre-dose, 0.083, 0.17, 0.25, 0.5, 0.75, 1, 2, 4, 6 and 8 h post-dose and transferred into heparinized microtainers. The blood volume withdrawn was replaced with normal saline. Plasma was harvested from the centrifuged microtainers and stored frozen at −70 °C in 500 μL polypropylene microcentrifuge tubes. Four Sprague–Dawley rats (weighing 0.275–0.290 kg) received an oral dose of 1 mgA/kg capromorelin. The dose was administered via an 18-gauge, 3 in., curved gavage needle. Blood samples (0.5 mL) were taken from the femoral vein catheter pre-dose, 0.083, 0.17, 0.25, 0.5, 0.75, 1, 2, 4, 6 and 8 h post-dose and transferred into heparinized microtainers. The blood volume withdrawn was replaced with normal saline. Plasma was harvested from the centrifuged microtainers and stored frozen at −70 °C in 500 μL polypropylene microcentrifuge tubes.

Sample extraction of capromorelin and analysis. A 100 μL aliquot of plasma (obtained as described above) was added to a 5 mL glass conical tube. The sample was vortexed with 100 μL of deionized water (containing 250 ng/mL MK-0677 as the internal standard), 25 μL of 1.0 M sodium hydroxide and 1.5 mL of ethyl acetate followed by centrifugation. The organic layer was transferred to a 15 mL conical tube and dried under a stream of nitrogen at 60 °C. The samples were reconstituted in 100 μL of water, vortexed, centrifuged and transferred into injection vials. The samples (40 μL) were injected onto a Keystone Inertsil ODS-2 2.0×50 mm 5 μm C_{18} analytical HPLC column. The compounds of interest were eluted from the column using a gradient elution program. The mobile phases consisted of $A = 10{:}90{:}0.01$ (v/v/v) acetonitrile/water/formic acid and $B = 90{:}10{:}0.01$ (v/v/v) acetonitrile/water/formic acid. A switching valve was used to divert the HPLC flow after the column to waste from 0 to 4 min and 8 to 13 min. Samples were detected via a Hewlett-Packard 5989B Mass Spectrometer (equipped with a hexapole ion guide driver). Ions were generated with a Hewlett-Packard 59987A Electrospray source. Capromorelin and internal standard (MK-0677) were monitored at m/z ratios of 506.05 and 529.00, respectively. Capromorelin and internal standard had retention times of approximately 5.5 min. The dynamic range was 5–1000 ng/mL with a run time of 13 min. Pharmacokinetic parameters were determined using PK_PARAM version 1.040.

Pharmacokinetics of compound 5c in rat. The protocol described for capromorelin was followed in preparing and dosing animals.

Sample extraction of compound 5c and analysis. A 100 μL aliquot of plasma was added to a 5 mL glass conical tube. The sample was vortexed with 100 μL of deionized water (containing 250 ng/mL MK-0677 as the internal standard), 25 μL of 1.0 M sodium hydroxide and 1.5 mL of ethyl acetate followed by centrifugation. The organic layer was transferred to a 15 mL conical tube and dried under a stream of nitrogen at 60 °C. The samples were reconstituted in 100 L of water, vortexed, centrifuged and transferred into injection vials. The samples (40 μL) were injected onto a Keystone Inertsil ODS-2 2.0×50 mm 5 μm C_{18} analytical HPLC column. The compounds of interest were eluted from the column using a gradient elution program. The mobile phases consisted of $A = 10{:}90{:}0.01$ (v/v/v) acetonitrile/water/formic acid and $B = 90{:}10{:}0.01$ (v/v/v) acetonitrile/water/formic acid. Samples were detected via a Hewlett-Packard 5989B Mass Spectrometer. Ions were generated with a Hewlett-Packard 59987A Electrospray source. Compound **5c** and internal standard (MK-0677) were monitored at m/z ratios of 492.05 and 529.00, respectively. Compound **5c** and internal standard had retention times of approximately 5.5 min. The dynamic range was 5–1000 ng/mL with a run time of 13 min.

Pharmacokinetics of capromorelin in dogs. Four adult beagle dogs (2 males and 2 females weighing 8.9–12.9 kg) were fasted overnight prior to dosing but were allowed free access to water. The dogs were allowed access to food 4 h post-dose. The iv dose was prepared as a 10 mgA/mL solution of capromorelin in deionized water and filter sterilized. The oral dose was prepared as a 2 mgA/mL solution of capromorelin in deionized water. Four beagle dogs (weighing 8.9–12.9 kg) received an oral dose of 1 mgA/kg of capromorelin. The dose was administered via an oral gavage tube. Blood samples (2 mL) were taken from the jugular vein pre-dose, 0.17, 0.33, 0.5, 0.75, 1, 2, 4, 6 and 8 h post-dose. Blood samples were collected directly into heparinized vacutainers. Plasma was harvested from the centrifuged vacutainers and stored frozen at −70 °C in 500 μL polypropylene microcentrifuge tubes. Following a 2 day

wash-out period, the same four beagle dogs received an iv dose of 1 mgA/kg of capromorelin. The dose was administered via the cephalic vein. Blood samples (2 mL) were taken from the jugular vein pre-dose, 0.083, 0.17, 0.33, 0.5, 0.75, 1, 2, 4, 6 and 8 h post-dose. Blood samples were collected directly into heparinized vacutainers. Plasma was harvested from the centrifuged vacutainers and stored frozen at −70 °C in 500 μL polypropylene microcentrifuge tubes.

Solubility measurements. Capromorelin (an amount in excess of its solubility) was added to 2 mL of deionized water. The sample was gently agitated for 24 h at room temperature (25 °C). After this time the samples were filtered, and the filtrate concentration of capromorelin was quantitated by HPLC. The same protocol was followed to measure the solubility of capromorelin in 2 mL of a 0.02 M sodium phosphate/0.02 sodium acetate combination buffer at pH 7.

References and Notes

1. Nargund, R. P.; Patchett, A. A.; Bach, M. A.; Murphy, M.G; Smith, R. G. *J. Med. Chem.* **1998**, *41*, 3103.
2. 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.; Elbrecht, A.; Dashkevicz, M.; Heavens, R.; Rigby, M.; Sirinathsinghji, D. J. S.; Dean, D. D.; Melillo, D. G.; Patchett, A. A.; Nargund, R.; 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.
3. Smith, R. G.; Van der Ploeg, L. H. T.; Howard, A. D.; Feighner, S. D.; Cheng, K.; Hickey, G. J.; Wyvratt, M. J.; Fisher, M. H.; Nargund, R. P.; Patchett, A. A. *Endocr. Rev.* **1997**, *18*, 621.
4. Kojima, M.; Hosoda, H.; Date, Y.; Nakazato, M.; Matsuo, H.; Kangawa, K. *Nature* **1999**, *402*, 656.
5. Bowers, C. Y.; Momany, F. A.; Reynolds, G. A.; Hong, A. *Endocrinology* **1984**, *114*, 1537.
6. Momany, F. A.; Bowers, C. Y.; Reynolds, G. A.; Hong, A.; Newlander, K. *Endocrinology* **1984**, *114*, 1531.
7. Hansen, B. S.; Raun, K.; Nielsen, K. K.; Johansen, P. B.; Hansen, T. K.; Peschke, B.; Lau, J.; Andersen, P. H.; Ankersen, M. *Eur. J. Endocrinol.* **1999**, *141*, 180.
8. 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. 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.
9. Yang, L.; Morriello, G.; Patchett, A. A.; Leung, K.; Jacks, T.; Cheng, K.; Schleim, K. D.; Feeney, W.; Chan, Wanda W.-S.; Chiu, S.-H. L.; Smith, R. G. *J. Med. Chem.* **1998**, *41*, 2439.
10. Schoen, W. R.; Pisano, J. M.; Prendergast, K.; Wyvratt, M. J.; Fisher, M. H.; Cheng, K.; Chan, W. W.-S.; Butler, B.; Smith, R. G.; Ball, R. G. *J. Med. Chem.* **1994**, *37*, 897.
11. Nagamine, J.; Nagata, R.; Seki, H.; Nomura-Akimaru, N.; Ueki, Y.; Kumagai, K.; Taiji, M.; Noguchi, H. *J. Endocrinol.* **2001**, *171*, 481.
12. Chapman, I. M.; Bach, M. A.; Van Cauter, E.; Farmer, 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.
13. Svensson, J.; Lonn, L.; Jansson, J. O.; Murphy, G.; Wyss, D.; Krupa, D.; Cerchio, K.; Polvino, W.; Gertz, B.; Boseaus, I.; Sjostrom, L.; Bengtsson, B. A. *J. Clin. Endocrinol. Metab.* **1998**, *83*, 362.
14. Svensson, J.; Ohlsson, C.; Jansson, J. O.; Murphy, G.; Wyss, D.; Krupa, D.; Cerchio, K.; Polvino, W.; Gertz, B.; Baylink, D.; Mohan, S.; Bengtsson, B. A. *J. Bone Miner. Res.* **1998**, *13*, 1158.
15. Codner, E.; Cassorla, F.; Tiulpakov, A. N.; Mericq, M. V.; Avila, A.; Pescovitz, O. H.; Svensson, J.; Cerchio, K.; Krupa, D.; Gertz, B. J.; Murphy, G. *Clin. Pharmacol. Ther.* **2001**, *70*, 91.
16. 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.; DaSilva-Jardine, P. A.; Thompson, D. D. *Endocrine* **2001**, *14*, 121.
17. Dorziotis, I.; Houpis, I.; Molina, A.; Volante, R. US Patent 6 046 333, 2000.
18. Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
19. Samanen, J.; Wilson, G.; Smith, P. L.; Lee, C.-P.; Bondinell, W.; Ku, T.; Rhodes, G.; Nichols, A. *J. Pharm. Pharmacol.* **1996**, *48*, 119.
20. The fraction absorbed (*fa*) was calculated according to the formula: $fa = F/(1-ER)$ where F is oral bioavailability and ER is hepatic extraction ratio ($ER = CL_h/Q$).
21. Bass, R.; Strnad, J.; Price, L. A.; Pausch, M. H.; Hadcock, J. R. *Mol. Pharmacol.* **1996**, *50*, 709.