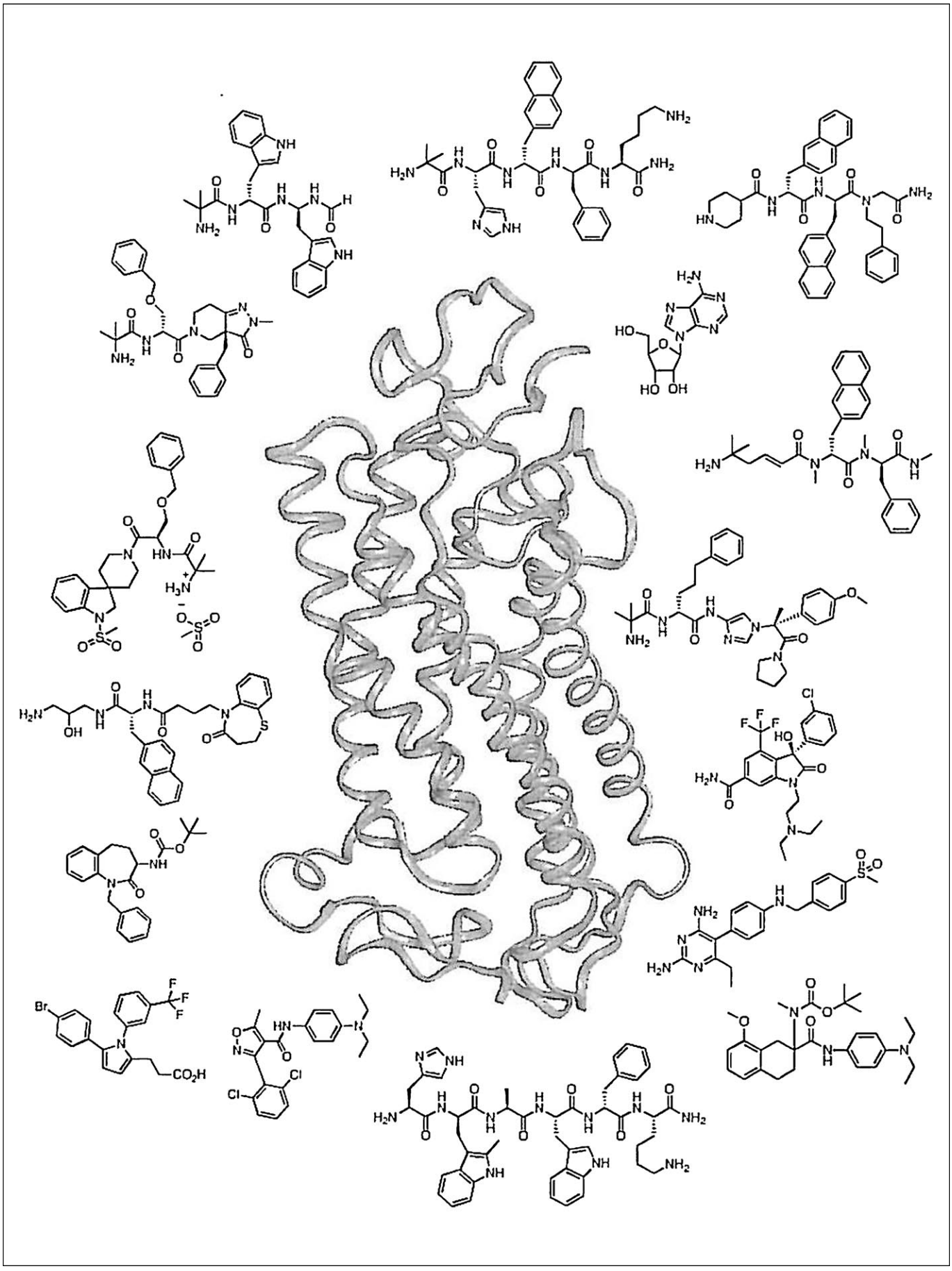


 DOI: 10.1002/cmdc.200700015

DOI: 10.1002/cmdc.200700015

Recent Developments in Ghrelin Receptor Ligands

Aline Moulin, Joanne Ryan, Jean Martinez, and Jean-Alain Fehrentz*[a]

The 28-amino acid peptide ghrelin is a neuroendocrine hormone synthesized primarily in the stomach. It stimulates growth hormone secretion and appetite, thus promoting food intake and body-weight gain. The pharmacological properties of this peptide are mediated by the growth hormone secretagogue receptor type 1a (GHS-R1a). Given its wide spectrum of biological activities, it is evident that the discovery of ghrelin and its receptor has opened up many perspectives in the fields of neuroendocrine and metabolic research and has had an influence on such fields of internal medicine as gastroenterology, oncology, and cardiology. It is therefore increasingly likely that synthetic, peptidyl, and nonpeptidyl GHS-R1a ligands, acting as agonists, partial agonists, antagonists, or inverse agonists, could have both clinical and therapeutic potential. This review summarizes the various types of GHS-R1a ligands that have been described in the literature and discusses the recent progress made in this research area.

Introduction

Ghrelin is a 28-residue peptide acylated with an *n*-octanoyl group on the Ser 3 residue (Figure 1) and is predominantly produced by the stomach.[1] Ghrelin displays strong growth hormone (GH)-releasing activity, which is mediated by the activation of the so-called GH secretagogue receptor type 1a (GHS-R1a).[2] GHS-R1a is concentrated in the hypothalamus–pituitary unit, but is also distributed in other central and peripheral tissues.[2–6] Indeed, apart from stimulating GH secretion, ghrelin exhibits hypothalamic activities that result in the stimulation of prolactin (PRL) and adrenocorticotropic hormone (ACTH) secretion.[7,8] It effects a negative feedback on the pituitary–gonadal axis,[9,10] stimulates appetite and a positive energy balance,[11–16] influences sleep and behavior,[17–21] controls gastric motility and acid secretion,[22–26] modulates pancreatic exocrine and endocrine function, and affects glucose levels.[27–33] Ghrelin has an influence on the cardiovascular system[34–36] and has been shown to modulate the proliferation of tumor cells.[37–40]

Figure 1. Human ghrelin.

Given this wide spectrum of biological activities, it is evident that the discovery of ghrelin and its receptor has opened up many avenues for neuroendocrine and metabolic research and has had an influence on fields of internal medicine such as gastroenterology, oncology, and cardiology. It is therefore increasingly likely that synthetic, peptidyl, and nonpeptidyl GHS-R1a ligands, acting as agonists, partial agonists, antagonists, or inverse agonists, could have both clinical and therapeutic potential. This review summarizes the different types of GHS-R1a ligands that have been described in the literature, and details the recent progress made in this research area.

1. hGHS-R1a Agonists

Pharmacological agents that mimic the actions of ghrelin, that is, GHS-R1a agonists, have several potential therapeutic applications. These compounds may be orally active alternatives to the recombinant human growth hormone (rhGH). Indeed, because GH is a large protein that must be administered by injection or inhalation, ghrelin agonists offer the promise of a more convenient and acceptable oral delivery of a substance stimulating endogenous GH secretion. In most cases, GH deficiency is due to a dysfunction in the GH secretion stimulation pathway and not in the GH production process. These new therapeutic tools could be effective in reversing metabolic dysfunctions in GH-deficient adults or in stimulating growth in GH-deficient children,[41–43] and also in improving some measures of physical performance in elderly men.[44–46] As recombinant GH has also shown promising results in the treatment of patients with burns, wounds, and bone fractures, and have helped combat the catabolism accompanying the treatment of critical illness such as cancer and AIDS, ghrelin agonists are interesting potential alternatives for reversing these syndromes.[47–51] Furthermore, agents that mimic ghrelin and thus stimulate food intake and induce a positive energy balance may be useful in restoring body weight in patients with eating disorders such as anorexia nervosa.[52] Clinical investigators have also exploited the ability of ghrelin agonists to release GH, particularly in combination with GH-releasing hormone (GHRH)[53,54] as a diag-

[a] *A. Moulin, J. Ryan, Prof. J. Martinez, Dr. J.-A. Fehrentz*
Institut des Biomolécules Max Mousseron, Faculté de Pharmacie
15 avenue Charles Flahault, BP 1441, 34093 Montpellier Cedex 5 (France)
Fax: (+33) 4-67-54-86-54
E-mail: jean-alain.fehrentz@univ-montp1.fr

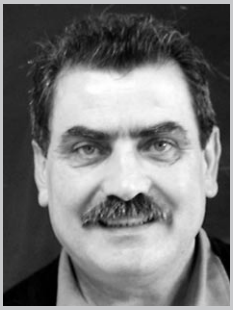
nostic tool.[42,55–59] This stimulation test is one of the most potent and reliable for evaluating the capacity of the pituitary to release GH for the diagnosis of GH deficiency. Due to the fact that ghrelin stimulates gastric motility,[22,60] ghrelin analogues could also be candidates for the treatment of postoperative gastric ileus. Furthermore, administration of ghrelin improves cardiac structure and function, and attenuates the development of cardiac cachexia in rats with heart failure.[34,61–63] These results suggest that ghrelin has cardiovascular protective effects, and thus ghrelin agonists may be used to treat heart failure and hypertension.[64–67] Finally, ghrelin receptors were recently identified in human cancer,[68,69] and it has been suggested that ghrelin agonists may have antiproliferative actions in some carcinoma cell lines.[70]

1.1. Peptide and pseudopeptide agonists

1.1.1. Bowers and co-workers

In 1977, while researching enkephalin analogues (compounds **1** and **2**, Figure 2), Bowers et al. reported the in vitro GH-releasing properties of various peptide compounds.[71] These compounds were able to stimulate GH secretion in the primary cultures of rat pituitary cells. Seven years later, they described the first growth-hormone-releasing peptide (GHRP) displaying in vivo activities.[72] This synthetic hexapeptide **3**, named GHRP-6 (Figure 2), was able to elicit a dose-dependent release of GH in vitro and in vivo in a variety of animal species accompanied by an increase in body weight. There was no concomitant release of luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), or prolactin (PRL).

Aline Moulin was born in Guilherand-Granges-lès-Valence (Ardèche), France, in 1981. She was awarded by the National Graduate Chemistry School of Montpellier in organic chemistry and received her MSc in biomolecular chemistry from the University Montpellier II in 2004. She is currently in the final stage of her PhD studies under the supervision of Dr. Jean-Alain Fehrentz. Her research interests are focused on the design and synthesis of bioactive compounds.



Jean Martinez studied chemistry at the Ecole Nationale Supérieure de Chimie de Montpellier (France). After receiving his PhD in 1972 he was awarded a permanent position at the CNRS. He completed his Thèse d'Etat in 1976 under the direction of Professor F. Winternitz, and performed postdoctoral studies with Professor E. Bricas in Orsay (France) and at Case Western University (Ohio, USA) with Professor M. Bodansky. Upon his return to France, he pursued research activities in the field of peptides and successively became head of various research laboratories in Montpellier, including the "Chemistry and Pharmacology of Biologically Interesting Molecules" Laboratory. In 1998, he was appointed Professor of the Faculty of Sciences and in 2001, Professor of the Faculty of Pharmacy. He is currently head of the Institut des Biomolécules Max Mousseron and president of the European Peptide Society. His research interests are peptide chemistry and pharmacology, stereoselective synthesis of amino acids, chemistry on polymeric supports, mass spectrometry, artificial protein synthesis, computer-assisted peptide searching, and green chemistry.

Joanne Ryan obtained a BSc degree with honors from the University of Melbourne, Australia in 1998 and an MSc in biostatistics from Monash University, Australia in 2006. She worked for four years as a research assistant at the University of Melbourne under the supervision of Professor Gething in the Department of Biochemistry and Molecular Biology (1999–2000) and in the Department of Surgery under the supervision of Dr. Baldwin (2001–2002). She also worked for three years (2004–2006) as a research technician for Professor Jean Martinez at the Institut des Biomolécules Max Mousseron, in the Faculty of Pharmacy, University Montpellier 1, France. In 2007, she started a PhD project in biostatistics and epidemiology at the Institute of Nervous System Pathologies, University Montpellier 1.

Jean-Alain Fehrentz was born in Nancy, France in 1955. He received his PhD in chemistry from the University of Nancy in 1983 and joined the Centre CNRS-INSERM de Pharmacologie Endocrinologie of Montpellier, in the research group of Professor Bertrand Castro. From 1989 to 1992, he was appointed as researcher at Sanofi Research. He then moved to the School of Pharmacy of Montpellier, working under the direction of Professor Jean Martinez. His research interests focus on peptide aldehydes, enzyme inhibitors, and receptor ligands.

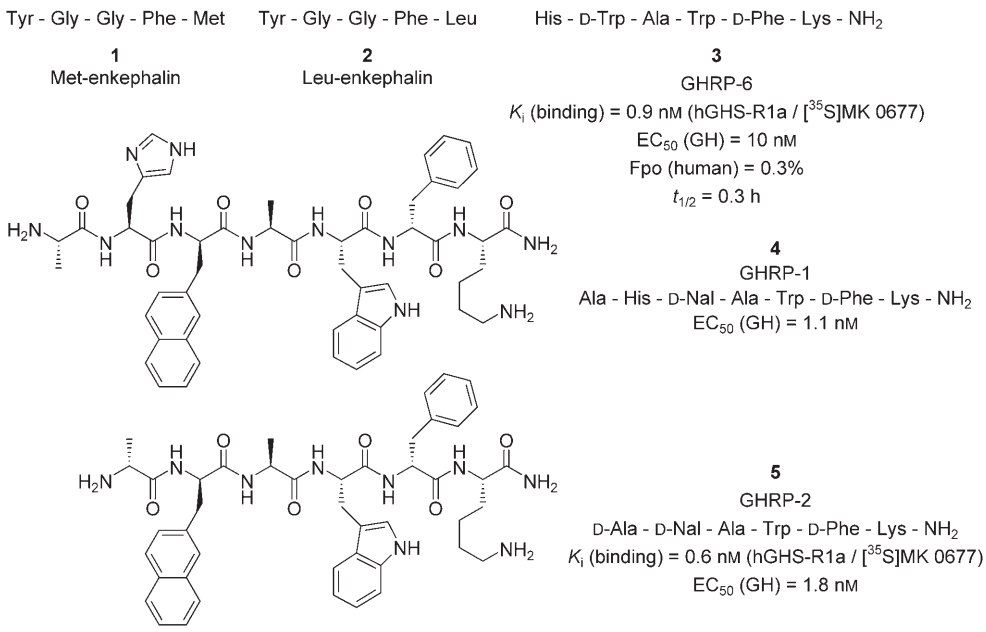


Figure 2. Peptide and pseudopeptide agonists developed by Bowers et al. (See Section 1.2.2 for information on compound MK 0677.)

In 1989, a study examining the activity and specificity of GHRPs in human subjects[73] suggested that such compounds could replace GH treatment, which is expensive even as a recombinant product, and must be administered by daily injection. The discovery of short peptides or pseudopeptides that stimulate the release of endogenous GH rapidly increased research in this field.

In 1993, two analogues of GHRP-6 were discovered,[74,75] which were slightly more potent than GHRP-6 in vitro and two- to threefold more efficient by subcutaneous administration in rats. These compounds, GHRP-1 (**4**) and GHRP-2 (**5**) (Figure 2), conserve the four C-terminal amino acids of GHRP-6, with the other residues replaced by a fragment containing a D-β-naphthylalanine (Nal) residue.

1.1.2. Mediolanum Farmaceutici

With GHRP-6 as the starting compound, examorelin or hexarelin (compound **6**, Figure 3), was synthesized by researchers at Mediolanum Farmaceutici by substituting D-tryptophan with 2-methyl-D-tryptophan. The resulting compound had enhanced potency and chemical stability, and a toxicity lower than that of GHRP-6.[76] Further research demonstrated the GH-releasing activity of examorelin in humans.[77]

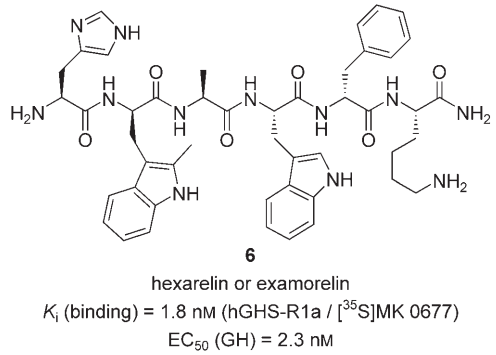


Figure 3. Peptide agonist **6** developed by Mediolanum Farmaceutici.

1.1.3. Truncated ghrelin analogues from Merck

Following the discovery of the original structure of the peptide ghrelin in 1999,[1] researchers at Merck synthesized ghrelin analogues with the aim of establishing the structural elements necessary to conserve its biological activity.[78] Several key findings resulted from this structure–activity relationship: 1) Acylation at the Ser3 residue with a bulky lipophilic residue is necessary to maintain good affinity toward human GHS-R1a. The *n*-octanoyl group can be replaced by flexible lipophilic groups such as undecanoyl or palmitoyl groups, or by more rigid groups such as benzoyl or adamantyl groups. 2) The ester function on Ser3 can be replaced by an amide function without any loss in biological activity. 3) The entire sequence of ghrelin is not necessary for binding and activation of GHS-R1a. The four N-terminal residues constitute the minimal active sequence of the peptide. Indeed, truncated peptides such as compounds **7** and **8** (Figure 4) activate GHS-R1a with the same potency as ghrelin, despite their lower affinity toward this receptor. However, these shortened ghrelin analogues seemed unable to elicit GH secretion in vivo.[79]

These results were confirmed by similar structure–activity studies undertaken by Matsumoto et al.[80] These types of ghrelin analogues were claimed in two patent applications.[81,82]

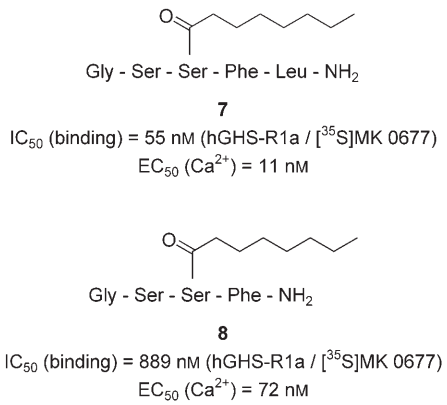


Figure 4. Peptide agonists developed by Merck.

1.1.4. LAPP/Europeptides

Research carried out by Europeptides (originally a subsidiary of Zentaris, but acquired by Ardana Bioscience in 2002) identified the tripeptide **9** (EP 51 389,[83] Figure 5), derived by truncating

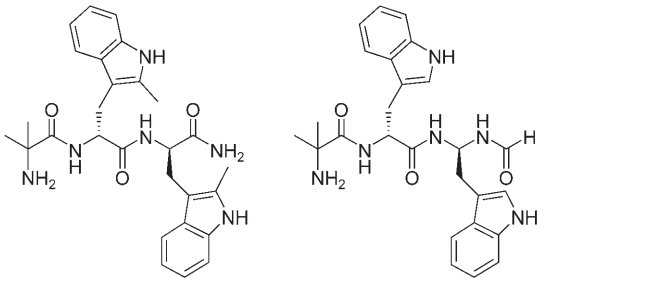


Figure 5. Peptide and pseudopeptide agonists developed by LAPP/Europeptides.

hexarelin. EP 51 389 was further modified in our laboratory at its C-terminal region to incorporate a *gem*-diaminotryptophan residue, which was formylated to give compound **10**, initially named JMV 1843, then EP 01572.[84,85] JMV 1843 is a potent in vitro agonist of hGHS-R1a,[86] despite its moderate receptor affinity; it is more active than hexarelin in subcutaneous administration in a rat model. It is also orally bioavailable in dogs and exhibits high oral activity and specificity in human volunteers.[87] This compound has been selected for further clinical investigation and successfully passed phase I clinical trials in 2004.

1.1.5. Genentech

Inspired by the discovery of GHRP-6, researchers at Genentech attempted to generate short peptidomimetics in order to obtain good oral bioavailability.[88] Compound **11** (G 7134, Figure 6) was claimed as a GHS-R1a agonist.

1.1.6. Novo Nordisk

The strategy taken at Novo Nordisk was to shorten GHRP-1 (**4**) to obtain compounds with lower molecular weight and good oral

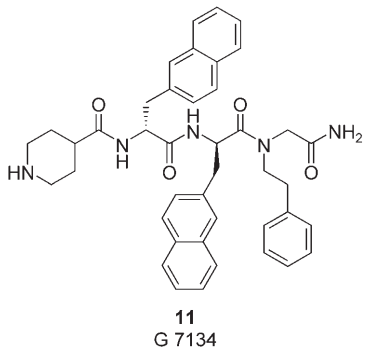


Figure 6. Pseudopeptide agonist **11** developed by Genentech.

bioavailability, while retaining the activity and specificity of the original peptide.[89] Molecular modeling studies were carried out, in which compound L 692 429, a peptidomimetic agonist discovered by Merck (see Figure 9 below), was superimposed with GHRP-1. This study suggested the possibility of removing the central sequence Ala–Trp. The resulting compound **12** (NNC 26 0039, Figure 7) displayed in vitro biological activity, but was inactive in vivo. By replacing the N-terminal L-Ala residue with D-Ala (compound **13**, NNC 26 0133), the in vivo activity was recovered. The replacement of this Ala residue with an α-aminoisobutyric (Aib) group gave compound **14** (NNC 26 0161), also known as ipamorelin.

Two other GHSs derived from ipamorelin were then generated: compounds **15** and **16**. Compound **15** (NNC 26 0235, Figure 7) was obtained by replacing the Aib–His fragment with

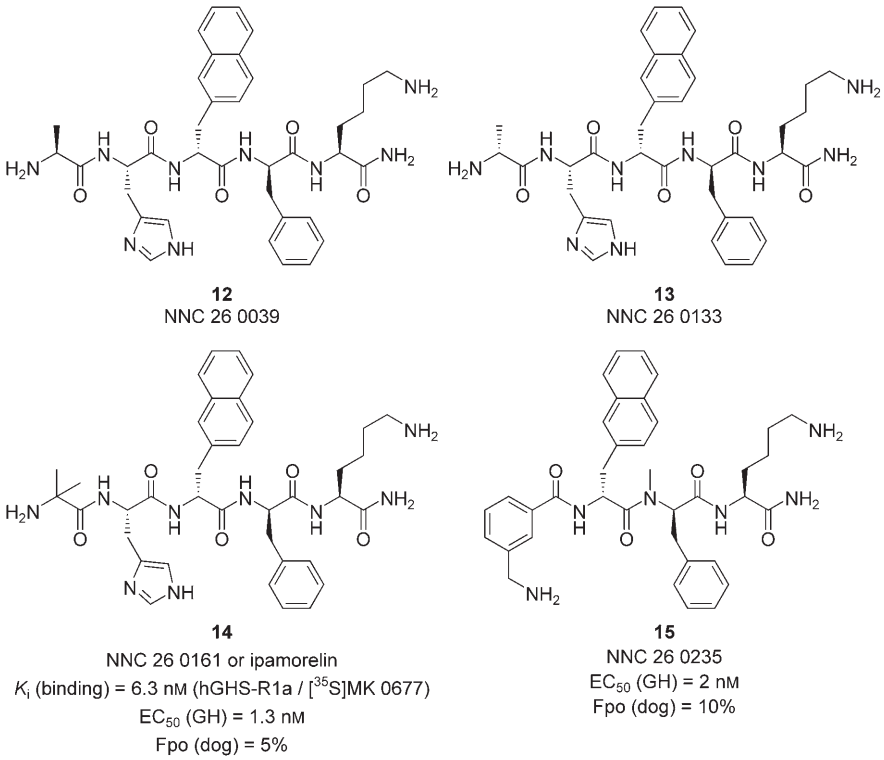


Figure 7. The first series of peptide and pseudopeptide agonists developed by Novo Nordisk.

a 3-aminomethyl benzoic acid moiety, which is less hydrophilic.[90] In both rat and porcine models, this compound displayed an in vivo activity similar to that of GHRP-6 (**3**) and ipamorelin. However, this compound displayed only a slight improvement in oral bioavailability despite the decreased potential for hydrogen bonding relative to ipamorelin. Compound **16** (NNC 26 0323, Figure 8) was obtained by removing the C-terminal Lys residue. It is the first compound of this series displaying an interesting oral bioavailability (20% in rat), but it is less active than compound **15**.

A new class of compounds was then synthesized.[91] The lead of this series is compound **17** (NN 703 or tabimorelin, Figure 8), which displays 35% oral bioavailability in dogs.[92] The compounds of both these series were claimed in two patent applications.[93,94]

To obtain GHSs displaying different pharmacokinetic properties, Novo Nordisk synthesized a wide range of NN 703 analogues. The effect of numerous modifications were examined, especially those concerning the C-terminal carboxamide function. Modification of this part of the molecule seems to have a substantial influence on the pharmacological properties of these compounds.[95–99] An example is compound **18** (NNC 26 1167, Figure 8), which displays a shorter half-life.

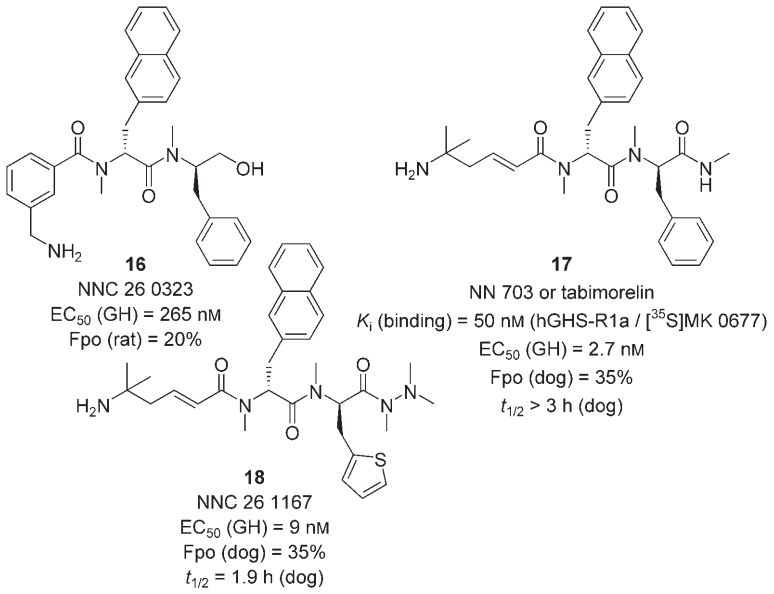


Figure 8. The second series of pseudopeptide agonists developed by Novo Nordisk.

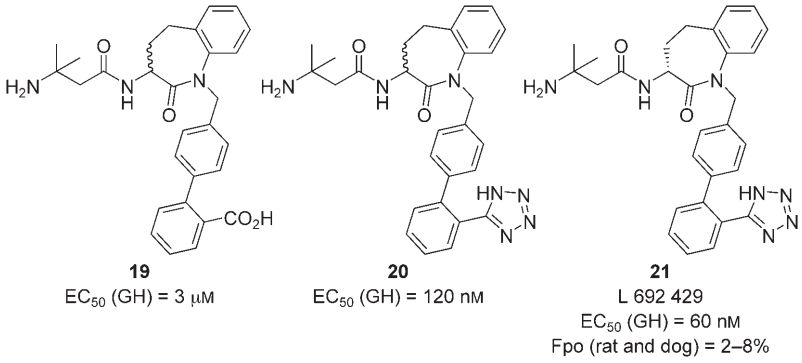


Figure 9. The first series of benzolactams developed by Merck.

1.2. Peptidomimetic agonists

Owing to the fact that peptides have low oral bioavailability (0.3% for GHRP-6 in comparison with parenteral absorption), extensive research has been carried out with the aim of identifying nonpeptide analogues with better oral availability.

1.2.1. Benzolactams from Merck

Merck was the first to report nonpeptide compounds capable of stimulating GH release.[100–102] To select the compounds with potential activity as growth hormone secretagogues, researchers at Merck used the work of Bowers et al.[71] as a starting point. Bowers had shown the significance of certain structural features, such as the presence of an amine function at the N terminus and of two aromatic moieties to retain GH-secreting activity. Initial in vitro screening of selected compounds from Merck's compound library identified benzolactam **19**, with an EC_{50} value of 3 μM (Figure 9).[103] A structure–activity study led to replacement of the carboxylic acid function by a tetrazole moiety to obtain compound **20**. Only the *R* enantiomer **21** (L 692429) is biologically active, with an EC_{50} value of 60 nM. Compound **21** stimulates GH secretion in vivo in several species and has no noticeable effect on the secretion of other hormones.[104] However, it has low oral bioavailability (2–8%) in both rat and dog models.[105]

Subsequent work was centered around obtaining analogues of compound **21** that are more potent and that have better oral bioavailability. Several modifications were performed on the dimethyl-β-alanine group,[106] heterocyclic analogues of the benzolactam ring were synthesized,[107] and replacements of the tetrazole[108] and biphenyl groups[109] were examined. Of the multiple analogues generated, the most studied were compounds **22** (L 692585) and **23** (L 700653) (Figure 10). Compound **22** is the reference compound in this series, as it was the first analogue more potent than GHRP-6 (**3**) in stimulating pituitary GH secretion in vitro (EC_{50} = 3 nM versus 10 nM for GHRP-6). It is also twofold more potent than GHRP-6 administered intravenously in dog.[110] However, its oral bioavailability is low, from 1 to 8% depending on the model. Modifications on the dimethyl-β-alanine group enhanced activity, but did not improve oral bioavailability. More potent analogues were also obtained by replacing the tetrazole moiety, such as with compound **23** (Figure 10), however oral bioavailability remained low. Acyclic analogues of compound **21** were also syn-

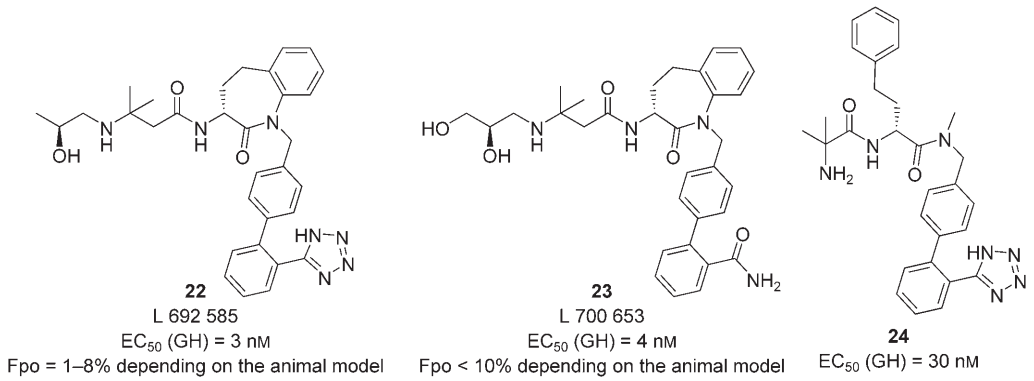


Figure 10. The second series of benzolactams developed by Merck.

thesized[111] and some of these molecules, such as compound **24**, have similar activity to that of compound **21**.

1.2.2. Spiropiperidines from Merck

The screening of Merck's library that resulted in the discovery of the benzolactam series also revealed compound **25** (Figure 11), which exhibits modest activity. Attempts to optimize the activity of this compound were unsuccessful.[112] However, other syntheses that involved the spiroindanylpiperidine moiety as the privileged structure were performed. It was thought that a complementary derivatization of this core structure could allow the generation of potent ligands for several receptors, particularly for those involved in GH secretion. One of the first derivatives resulting from this work was compound **26** (Figure 11). This compound stimulates pituitary GH secretion in vitro with an EC_{50} value of 50 nM,[113] but it does not stimulate in vivo GH secretion in dogs. Good oral activity was obtained with the D-tryptophan analogue, in which the ureidoquinuclidine group was replaced by an α-aminoisobutyric acid group to give compound **27** (L 162 752).[114] An even more potent analogue was obtained by introducing a sulfonamide group and by replacing the D-tryptophan residue with an *O*-benzyl-D-serine residue. This compound, L 163 191 (**28**), also named MK 0677 or ibutamoren, allowed the characterization and cloning of the GHS receptor GHS-R1a by introducing a radioisotopic label (^{35}S) in the structure.[2,115]

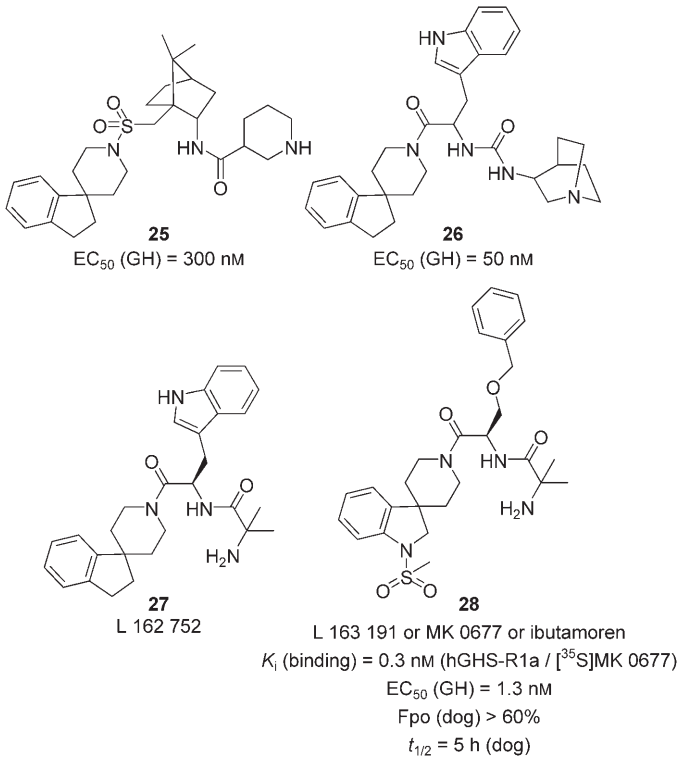
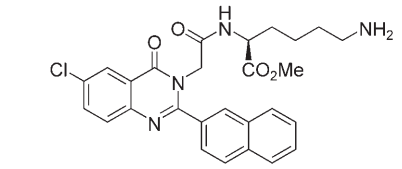


Figure 11. Spiropiperidines developed by Merck.

MK 0677 stimulates pituitary GH secretion in vitro, with an EC_{50} value of 1.3 nM and possesses high affinity toward GHS-R1a (0.3 nM). This compound is very stable against peptidases, thanks to the D configuration of the *O*-benzylserine residue and to the steric bulk of the α-aminoisobutyric group. These features, along with lipophilicity ($\log P = 3.0$), water solubility ($> 250\ mg\ mL^{-1}$ due to the presence of the mesylate salt), and moderate basicity ($pK_a = 7.8$) all contribute to its excellent oral bioavailability in dog (>60%). The half-life of MK 0677 is 5 h,[105] classifying this compound as a long-term GHS.[116] This is the first time a low-molecular-weight nonpeptide molecule has been claimed[102] to stimulate GH secretion. MK 0677 was chosen for clinical trials, and reached phase II development. However, by December 1998, further development had been discontinued.

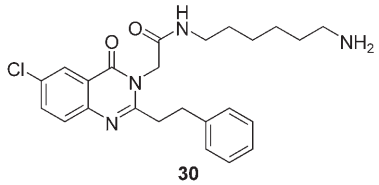
1.2.3. Quinazolinones from Merck

The cloning and characterization of GHS-R1a allowed Merck to discover another hit. Compound **29** (Figure 12), also a nonpeptide molecule, is characterized by modest affinity and contains a unique quinazolinone moiety. The activity of this family of GHSs was optimized by molecular modeling, based on the supposed binding site of MK 0677. Compound **30** displays good in vitro activity in stimulating GH secretion ($EC_{50} = 0.63$ nM) and high affinity toward the receptor ($IC_{50} = 16$ nM).[117]



29

IC_{50} (binding) = 3.5 μM (hGHS-R1a / [^{35}S]MK 0677)

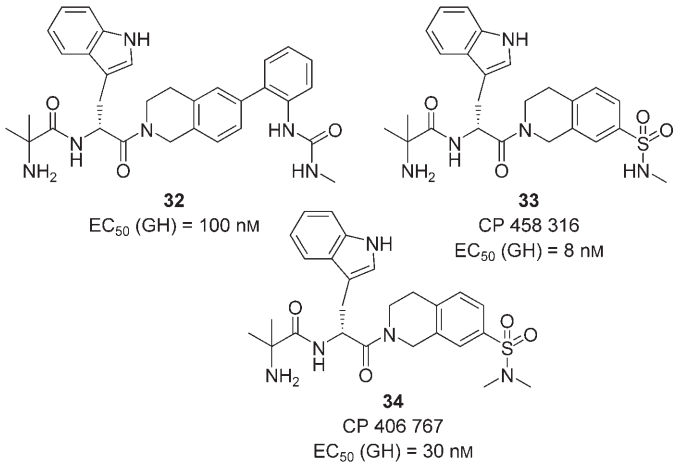


30

IC_{50} (binding) = 16 nM (hGHS-R1a / [^{35}S]MK 0677)

EC_{50} (GH) = 0.63 nM

Figure 12. Quinazolinones developed by Merck.



32

EC_{50} (GH) = 100 nM

33

CP 458 316

EC_{50} (GH) = 8 nM

34

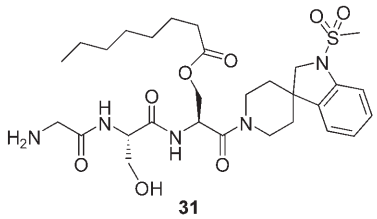
CP 406 767

EC_{50} (GH) = 30 nM

Figure 14. The first series of peptidomimetics developed by Pfizer.

1.2.4. Truncated peptides/MK 0677 hybrids

To obtain more potent GHSs that display different pharmacokinetic properties, Merck synthesized hybrids between truncated peptide **8** derived from ghrelin and MK 0677.[118] These analogues contain the spiropiperidine moiety of MK 0677 and the N-terminal portion of ghrelin. Compound **31** (Figure 13) is an



31

IC_{50} (binding) = 128 nM (hGHS-R1a / [^{35}S]MK 0677)

EC_{50} (Ca^{2+}) = 68 nM

Figure 13. Hybrid molecule **31** developed by Merck.

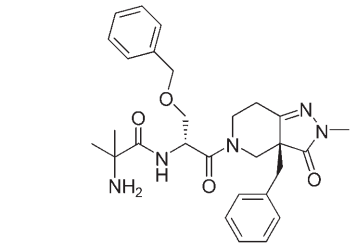
example of this type of hybrid. It has an affinity of 128 nM toward human GHS-R1a and an EC_{50} value of 68 nM. The ability of this type of compound in stimulating GH secretion has not yet been reported.

1.2.5. Pfizer

In 1998 researchers at Pfizer discovered a new series of GHSs composed of tetrahydroisoquinoline- and isoindoline-derived molecules.[119] These compounds were designed from molecular modeling studies of compounds **21** (Figure 9), **27** (Figure 11), and **30** (Figure 12). Compound **32** (Figure 14) was designed with this approach. A structure–activity study then allowed the discovery of potent GHSs such as compounds **33** (CP 458316) and **34** (CP 406767).

More recently, by optimizing the pharmacokinetics and pharmacodynamics of these compounds, Pfizer discovered a new series of original GHSs derived from MK 0677 which share a pyrazolinone–piperidine moiety as a common structural feature.[120] One of these compounds, CP 424391-18 or capromorelin (**35**), displayed particularly interesting properties (Figure 15).[121] It is able to stimulate GH secretion in vitro with an EC_{50} value of 3 nM, and its affinity toward GHS-R1a is approximately 7 nM. In vivo, capromorelin is a short-term GHS with high oral bioavailability (65% in rat).

Other GHSs with longer-term action were identified, such as compound **36** (CP 464709-18, Figure 15).[122] This compound was selected for clinical trials in the treatment of bone frailty.[123,124] Pfizer also claimed two series of dipeptide analogues containing heterocyclic scaffolds, such as compounds **37** and **38** (Figure 16), which are distinct from the pyrazolinone–piperidine-containing species.[123,125] Compound **37** contains a 3-aminomethylbenzoic acid group, which was used in another series of molecules derived from ipamorelin (compound **15** for example), and displays good oral bioavailability.[90]



35

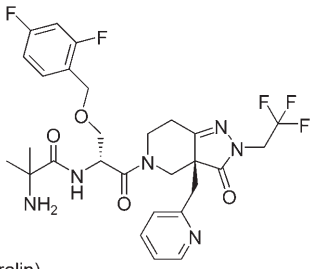
CP 424 391-18 or capromorelin

IC_{50} (binding) = 7 nM (hGHS-R1a / [His^{9}-^{125}I]ghrelin)

EC_{50} (GH) = 3 nM

Fpo (rat) = 65%

$t_{1/2}$ = 0.8 h (rat)



36

CP 464 709-18

IC_{50} (binding) = 5 nM (hGHS-R1a / [His^{9}-^{125}I]ghrelin)

EC_{50} (GH) = 3 nM

$t_{1/2}$ = 3.7 h (rat)

Figure 15. The second series of peptidomimetic agonists developed by Pfizer.

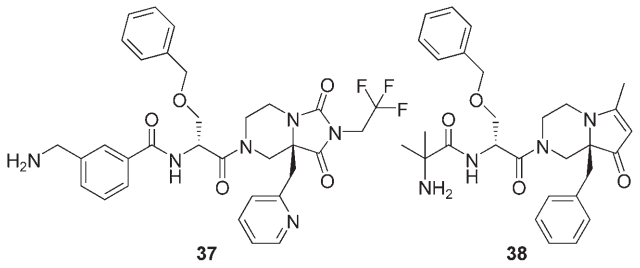


Figure 16. The third series of peptidomimetic agonists developed by Pfizer.

1.2.6. Kaken Pharmaceutical

Researchers at Kaken Pharmaceutical identified a new series of GHSs by using a virtual screening method.[126] For this purpose they developed a three-dimensional model of the GHS pharmacophore by molecular modeling based on structurally different GHSs (peptidyl and nonpeptidyl). This study led to the identification of several hits, such as compound **39** (Figure 17),

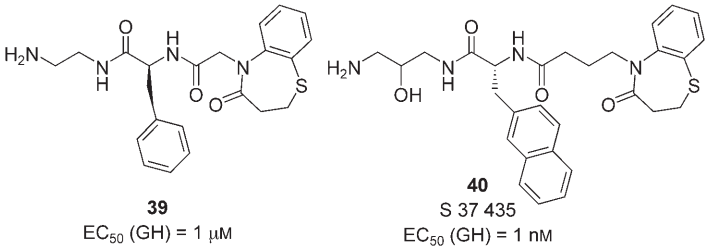


Figure 17. Peptidomimetic agonists developed by Kaken Pharmaceutical.

which presents a modest in vitro stimulating activity. Optimization of this compound by molecular modeling led to the discovery of compound **40** (S 37 435),[127,128] which has better in vitro GH secreting activity (EC_{50} = 1 nM compared with 1 μM for compound **39**) and was described as orally bioavailable in rat.

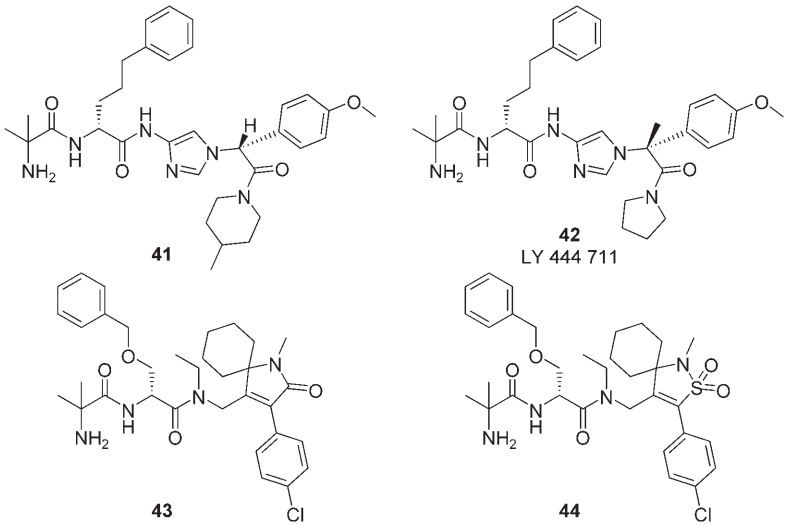


Figure 18. Peptidomimetics developed by Eli Lilly.

1.2.7. Eli Lilly

Eli Lilly discovered and claimed a series of compounds containing a 2-aminoimidazolyl group at the C terminus.[129] In another patent application, compounds such as **41** or **42** (LY 444 711) are specifically claimed (Figure 18). Pyrazolyl, pyrrolyl, and other heterocyclic moieties were introduced to replace the imidazolyl group in the C-terminal region of compound **41**.[129] Compound **42**, which presents good oral bioavailability in dog, increased GH secretion by 50-fold and plasma IGF-1 levels by 60% following a single oral dose of 1 mg kg^{-1}.[130] Two other series of GHSs are described in another patent application.[131] They contain new heterocyclic groups such as those exemplified by compounds **43** and **44** (Figure 18). 2-Acylaminopropanamides were also claimed as GHSs[132] in addition to their use in the treatment of cardiac disease.[133]

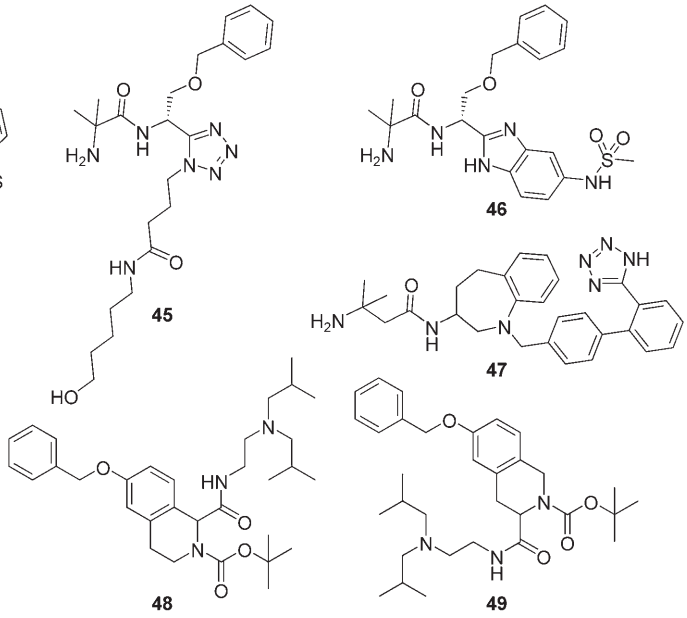


Figure 19. Peptidomimetic agonists developed by Bristol–Myers Squibb.

1.2.8. Bristol–Myers Squibb

Bristol–Myers Squibb identified ibutamoren (Figure 11) analogues in which the C-terminal amide function is replaced by a tetrazole or benzimidazole ring.[134] Compounds such as **45** and **46** (Figure 19) could have better bioavailability than their dipeptide analogues because of the heterocyclic group, which is less likely to form hydrogen bonds than the amide group.

A series of benzoazepine analogues such as compound **47** (Figure 19), derived from L 692 429 (**21**, Figure 9) by reduction of the amide function of the heterocyclic ring, was also identified.[135] A series of tetrahydroisoquinolines, exemplified by compounds **48** and **49**, was also claimed.[136]

2. hGHS-R1a Partial Agonists

2.1. Adenosine and derivatives

Adenosine (**50**, Figure 20) is an endogenous ligand of human GHS-R1a with varied pharmacological properties depending on the transfected cell line. According to Smith et al.,[137] adenosine exhibits partial agonist activity in HEK 293/hGHS-R1a cells with an EC_{50} value of 2 μM. Through site-directed mutagenesis studies, Smith and co-workers also showed that adenosine binds to a unique site on hGHS-R1a, distinct from the binding pockets of either ghrelin or ibutamoren. Tullin et al.[138] found that adenosine exhibited full agonist activity in baby hamster kidney (BHK) cells overexpressing hGHS-R1a, with an EC_{50} value of 50 nM. Several adenosine analogues were also reported to activate hGHS-R1a, including *N*-ethylcarboxamide adenosine (NECA, **51**) and compound **52**. An analogue of adenosine, N^6-(*R*)-phenylisopropyladenosine ((*R*)-PIA, **53**) was also found to be a potent hGHS-R1a agonist, with an EC_{50} value of 0.5 nM. Neither adenosine nor (*R*)-PIA stimulated GH secretion in rat pituitary cells.[138]

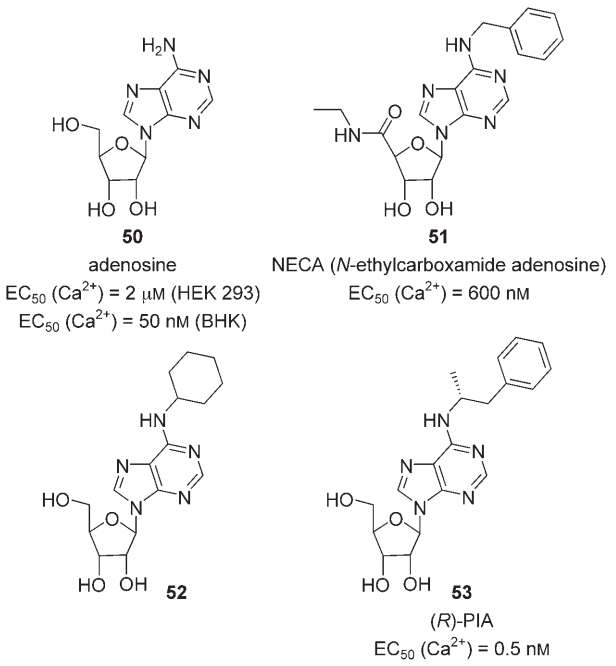


Figure 20. Partial agonists derived from adenosine.

2.2. Peptidomimetic partial agonists: Sumitomo Pharmaceuticals

A new generation of GHSs with low molecular weight and good pharmacokinetic properties was discovered by Sumitomo Pharmaceuticals.[139–141] These compounds are distinct from other GHSs because of their oxoindole-based structure and their pharmacokinetic properties. Indeed, they are the first synthetic partial agonists of hGHS-R1a to be discovered. Following the optimization of compound **54** (Figure 21), identified by virtual screening, compound **55**, (SM 130686) was synthesized. Compound **55** stimulates GH secretion in vitro with an EC_{50} value of 6.3 nM and with 50% of the maximal stimulatory response of ghrelin. It also displays an affinity toward GHS-R1a similar to that of MK 0677.

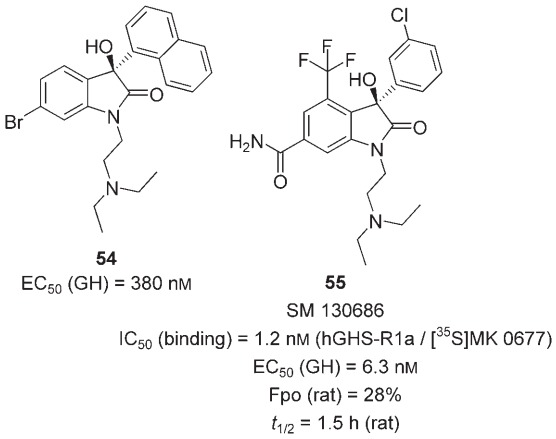


Figure 21. Partial agonists developed by Sumitomo Pharmaceuticals.

3. hGHS-R1a Antagonists

As ghrelin appears to be an important peripheral orexigenic signal to the brain,[142,143] blocking or neutralizing ghrelin's action may be a reasonable approach to reversing chronic obesity. The possibility of using a ghrelin antagonist for the treatment of obesity,[144] which is closely associated with a number of chronic diseases such as diabetes, cardiovascular disorders, and some types of cancer, has triggered a rush by many groups in the hunt to find such a valuable agent. Developing anti-obesity therapies, however, is complicated because the biological system that controls the energy balance in the body is complex and poorly understood.[145] On the other hand, it is conceivable that such ghrelin antagonists may help to treat patients with obese Prader–Willi syndrome.[146,147] Furthermore, in some cancers, ghrelin antagonists may act as antiproliferative agents.[37]

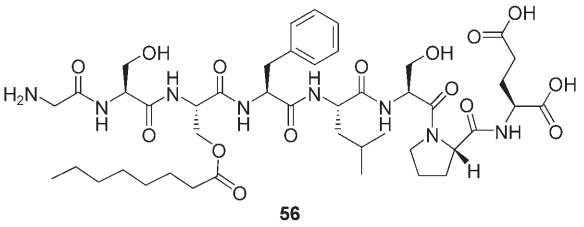
3.1. Peptide and pseudopeptide antagonists

3.1.1. Zentaris

Zentaris researchers have described truncated ghrelin analogues having antagonist activities.[148] Compound **56** (Figure 22) blocks GH secretion stimulated by ghrelin after subcutaneous injection at a dose of 300 $\mu g\,kg^{-1}$ in rats.

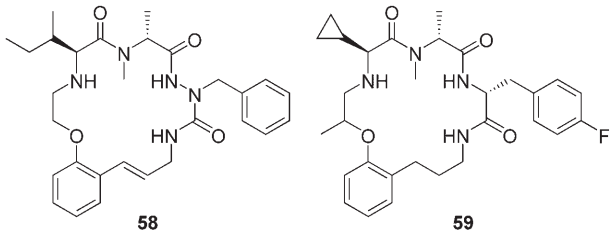
3.1.2. Ipsen

By producing ghrelin analogues with better metabolic stability and greater affinity toward GHS-R1a, researchers at Ipsen iden-



56

Figure 22. Peptide antagonist **56** developed by Zentaris.



58
K_i (binding) = 10–100 nM

59
K_i (binding) = 1–10 nM

Figure 23. Peptide antagonists developed by Tranzyme Pharma.

tified a competitive GHS-R1a antagonist.[149,150] This compound, BIM 28163 (**57**), the structure of which is not described, displays good affinity (K_i = 8 nM) in transfected cells and the ability to completely inhibit the GHS-R1a activation induced by ghrelin in a dose-dependent manner. Administered intravenously in rat, BIM 28163 completely blocked ghrelin-induced GH secretion. Administered as a unique product, it is able to decrease the rate of GH circulation and the amplitude of GH-secretion peaks without modifying their frequency. Surprisingly, it modulates the feeding behavior in rats, as a complete agonist, by reproducing the effects of ghrelin in body-weight gain and food intake.[149]

3.1.3. Tranzyme Pharma

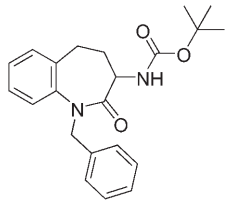
Tranzyme Pharma has claimed extensively on conformationally defined macrocyclic compounds that incorporate peptides, as modulators of GHS-R1a, including antagonists. In a radioligand binding assay using HEK 293 cell membranes, macrocycle **58** (Figure 23) was claimed to have a double-digit nanomolar K_i value toward GHS-R1a,[151] and macrocycle **59** was reported to have a single-digit nanomolar K_i value.[151] However, because no functional activity data were presented in these applications, it is unclear whether or not these compounds are GHS-R1a antagonists.

3.2. Peptidomimetic antagonists

3.2.1. Merck

By direct in vitro screening using rat pituitary primary cell cultures, compound **60** (L 692400, Figure 24), derived from compound **21** (Figure 9), was identified as the first specific GHS-R1a antagonist. This compound alone does not induce GH secretion in vitro, and blocks GH secretion stimulated by compound **21** or MK 0677 (**28**), without inhibiting GH secretion induced by GHRH.[152]

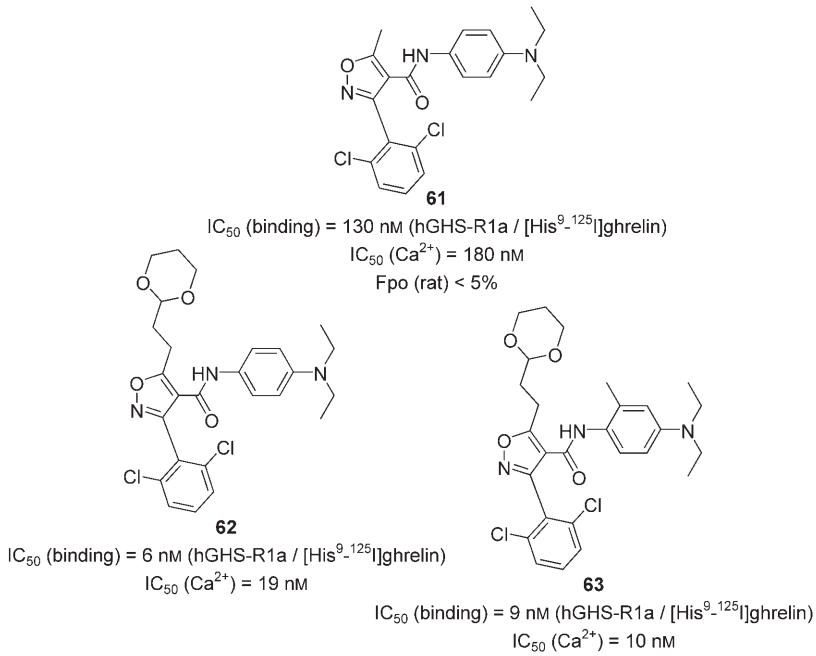
3.2.2 Isoxazole carboxamides from Abbott



60
L 692 400

Figure 24. Peptidomimetic antagonist **60** developed by Merck.

Compound **61** (Figure 25) was first identified by high-throughput screening. Using a GHS-R1a transfected cell model, this compound was confirmed to be a competitive antagonist of the receptor, with modest affinity (IC_{50} = 130 nM) and with an IC_{50} value of 180 nM determined in cellular functional assays.[153] A structure–activity analysis was aimed at enhancing the affinity and functional activity toward GHS-R1a. This study led to the discovery of a new series of antagonist molecules. Compounds **62** and **63**, which belong to this series, display high affinity and antagonistic activity toward GHS-R1a.[154] However, the isoxazole carboxamides in general suffered from poor pharmacokinetic profiles, with less than 5% oral bioavailability in rats.[155]



61
IC_{50} (binding) = 130 nM (hGHS-R1a / [His^{9}-^{125}I]ghrelin)
IC_{50} (Ca^{2+}) = 180 nM
Fpo (rat) < 5%

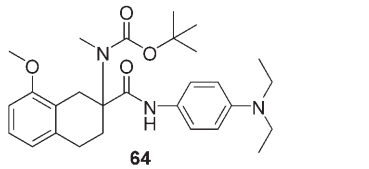
62
IC_{50} (binding) = 6 nM (hGHS-R1a / [His^{9}-^{125}I]ghrelin)
IC_{50} (Ca^{2+}) = 19 nM

63
IC_{50} (binding) = 9 nM (hGHS-R1a / [His^{9}-^{125}I]ghrelin)
IC_{50} (Ca^{2+}) = 10 nM

Figure 25. Isoxazole carboxamides developed by Abbott.

3.2.3 Tetralin carboxamides from Abbott

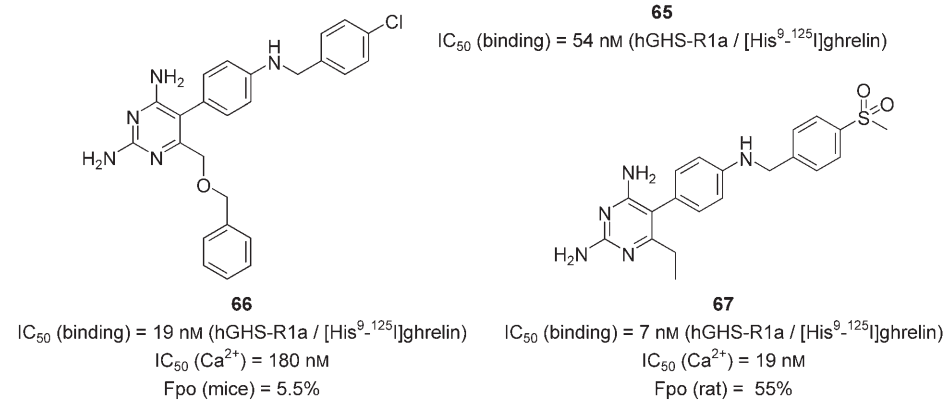
Using the same methodology, Abbott researchers discovered a new family of GHS-R1a antagonists, the tetralin carboxamides.[156,157] One of these, compound **64** (Figure 26), displays an affinity of 16 nM and an IC_{50} value of 29 nM. Furthermore, this compound is selective, showing only weak activity toward a panel of receptors. However, no in vivo effect on food intake or body weight was reported in a rat model. The pharmacokinetic profiles of tetralin carboxamides were better than those for the isoxazole carboxamides (around 20% oral bioavailability in rat), but were still considered inadequate.



64

IC_{50} (binding) = 16 nM (hGHS-R1a / [His^{9}-^{125}I]ghrelin)
IC_{50} (Ca^{2+}) = 29 nM
Fpo (rat) = 19%

Figure 26. Tetralin carboxamide developed by Abbott.



65

IC_{50} (binding) = 54 nM (hGHS-R1a / [His^{9}-^{125}I]ghrelin)

66

IC_{50} (binding) = 19 nM (hGHS-R1a / [His^{9}-^{125}I]ghrelin)
IC_{50} (Ca^{2+}) = 180 nM
Fpo (mice) = 5.5%

67

IC_{50} (binding) = 7 nM (hGHS-R1a / [His^{9}-^{125}I]ghrelin)
IC_{50} (Ca^{2+}) = 19 nM
Fpo (rat) = 55%

Figure 27. The first series of 2,4-diaminopyrimidines developed by Abbott.

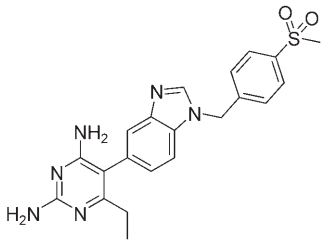
3.2.4. 2,4-Diaminopyrimidines

A series based on the 2,4-diaminopyrimidine scaffold was also explored,[158] but one concern of this diaminopyrimidine chemotype is its inhibitory activity against dihydrofolate reductase (DHFR),[159] a key enzyme in nucleic acid biosynthesis. The undesired activity toward DHFR could be a potential source of toxicity and could also complicate interpretation of pharmacological data. By optimizing the substituents in positions 4 and 6 of the 2,4-diaminopyrimidine core, the authors obtained potent in vitro GHS-R1a antagonists displaying good selectivity (1000-fold) in favor of the receptor over DHFR, such as compound **65** (Figure 27). By using a structure–activity relationship, Abbott obtained the first GHS-R1a antagonist effective in vivo.[160] Administered intraperitoneally, compound **66** causes a decrease in food intake in rats and attenuates body-weight gain following a diet in obese mice. However, its bioavailability of only 5.5% is not sufficient for oral administration. Another compound, **67**, was described as having greater potency and bioavailability (55% by oral administration in rat), but is also a potent inhibitor (IC_{50} = 10 nM) of DHFR.

However, as the crystal structure of the diaminopyrimidine–DHFR complex was solved in 1989,[161] researchers at Abbott undertook docking studies between compound **67** and DHFR with the aim of enhancing the selectivity of their compounds. They compared the binding mode of compound **67** with DHFR and the supposed binding mode of the same compound with GHS-R1a.[155] The GHS-R1a–**67** model was developed based on the dopamine receptor model.[162,163] By comparing those two binding modes, an extra space was noted between the GHS-R1a surface and the benzylamine moiety of compound **67**, compared with the analogous region on the DHFR–**67** complex. By introducing steric hindrance in this part of the molecule, the affinity toward GHS-R1a could be preserved, while supposedly decreasing the affinity for DHFR. An initial series of molecules was synthesized with a benzimidazole core;[155] compounds **68** and **69** (Figure 28) are representative examples. The inhibitory activity of compound **68** toward DHFR relative to that of **67** was diminished (78% inhibition versus 99% at 1 μM), but it is still considerable. The introduction of a cyclic substituent at position 2 of the imidazole (compound **69**), however, enhanced the selectivity; the IC_{50} value for DHFR is in the micromolar range, but the affinity for GHS-R1a is affected.

A second strategy was used to decrease the flexibility of the benzylamine group by replacing the amine function with an amide. Compound **70** (Figure 29) from this series[155] displays an affinity for GHS-R1a similar to that of compound **66** and an affinity toward DHFR of 30 μM. Furthermore, compound **70** has a good pharmacokinetic profile (32% oral bioavailability in rat), and is able to cross the blood–brain barrier.

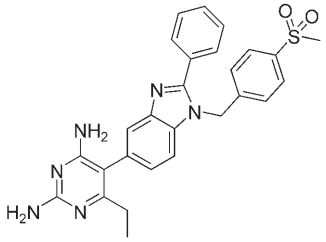
Another series of compounds was synthesized by replacing the amide with a urea group, which is more rigid and less sensitive to enzyme hydrolysis. Compound **71** from this series displays an affinity for GHS-R1a in the nanomolar range and has very good selectivity (IC_{50} (DHFR) > 100 μM). It also presents a



68

IC_{50} (binding) = 4 nM (hGHS-R1a / [His^9-^{125}I]ghrelin)

IC_{50} (Ca^{2+}) = 11 nM

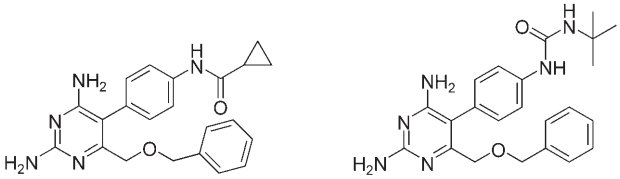


69

IC_{50} (binding) = 16 nM (hGHS-R1a / [His^9-^{125}I]ghrelin)

IC_{50} (Ca^{2+}) = 250 nM

Figure 28. The second series of 2,4-diaminopyrimidines developed by Abbott.



70

IC_{50} (binding) = 66 nM (hGHS-R1a / [His^9-^{125}I]ghrelin)

IC_{50} (Ca^{2+}) = 188 nM

Fpo (rat) = 32%

71

IC_{50} (Ca^{2+}) = 7 nM

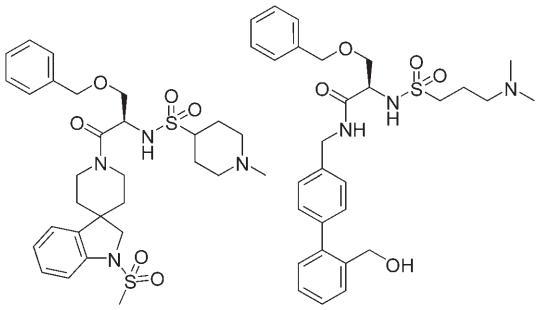
Figure 29. The third series of 2,4-diaminopyrimidines developed by Abbott.

good pharmacokinetic profile, but seems unable to cross the blood–brain barrier.

These latter two compounds have been tested in vivo.[155] In rats, compound **70** decreased food intake within 24 h (50 mg kg^{-1} every 8 h, oral route) and caused a 5% decrease in body weight of obese mice after 14 days of oral treatment (50 mg kg^{-1} twice daily). Compound **71** does not have any effect on food intake or body weight. The authors concluded from this difference that the ability to cross the blood–brain barrier is necessary to block the orexigenic effect of ghrelin.

3.2.5. Elixir Pharmaceuticals

Researchers at Elixir Pharmaceuticals claimed a series of spiroindoline piperidine-based GHS-R1a antagonists.[164] These compounds were based on the spiroindoline piperidine template pioneered by Merck for GHSs. The best analogues were claimed to have K_i values of <100 nM in radioligand binding assays using COS7 cells, and it was determined that the privileged G-protein-coupled receptor (GPCR)-binding spiroindoline piperidine moiety in compound **72** (Figure 30) was not critical for GHS-R1a antagonism. Compound **73**, which features a biaryl replacement, exhibits a double-digit nanomolar K_i value



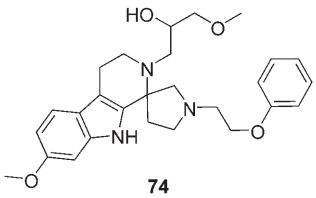
72 73

Figure 30. Peptidomimetic antagonists developed by Elixir Pharmaceuticals.

toward GHS-R1a. No other functional or in vivo biological activity is disclosed in this patent application.

3.2.6 Biovitrum

A series of novel β-carboline spiropyrrolidines have been claimed by Biovitrum to be GHS-R1a antagonists.[165] A total of 234 examples are listed in this application, with K_i values in the range of 10 nM to 5 μM in transfected Chinese hamster ovary (CHO) cell lines. The structure of compound **74** (Figure 31) is representative of this series.



74

K_i (binding) = 69 nM (hGHS-R1a / [His^9-^{125}I]ghrelin)

Figure 31. Peptidomimetic antagonist **74** developed by Biovitrum.

3.2.7. Sumitomo Pharmaceuticals

Researchers from Sumitomo Pharmaceuticals developed a series of oxindole- and benzimidazolone-based GHS-R1a antagonists.[166] Compounds **75** and **76** (Figure 32) are representative of this series. In this application, two compounds, **77** and **78**, were evaluated for their acute effect on food intake in *db*/*db* mice: via intraperitoneal route at a dose of 50 mg kg^{-1}, the cumulative food intake during the 22 h following injection was decreased by 23 and 89% for compounds **77** and **78**, respectively, relative to the average food intake of control animals during the same period.

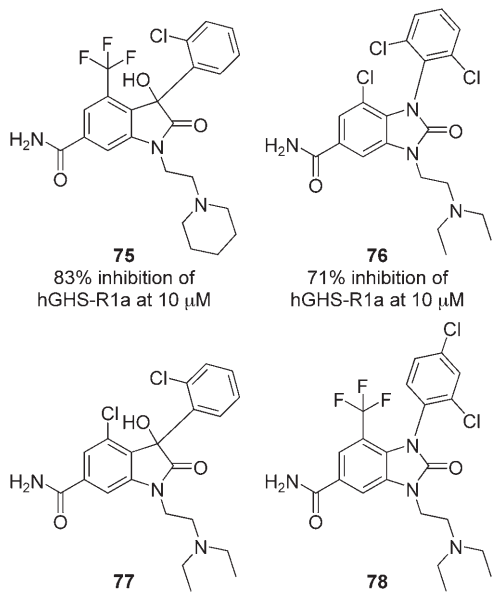


Figure 32. Peptidomimetic antagonists developed by Sumitomo Pharmaceuticals

3.2.8. Amgen

Amgen claimed a series of benzoazepine- and dihydroisoquinoline-based GHS-R1a antagonists (Figure 33).[167] These compounds present some structural similarities with compound **60** from Merck (Figure 24). Compounds **79** and **80** exhibit IC_{50} values from 1 to 10 nM. Numerous structural variations are tolerated, and potent antagonist activities ($IC_{50} < 10$ nM) were obtained.

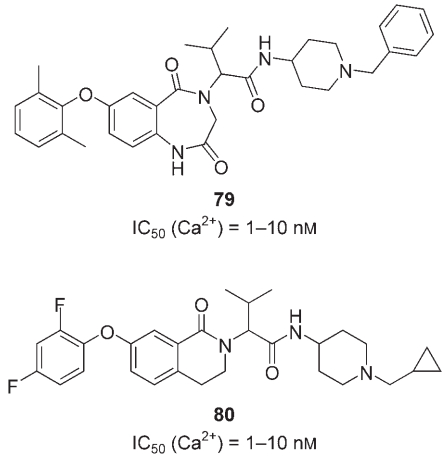


Figure 33. Peptidomimetic antagonists developed by Amgen.

3.2.9. Novo Nordisk

A series of GHS-R1a antagonists, derived from phenylalanine, that can be used for the treatment of obesity or central nervous system (CNS) disorders has been described by Novo Nordisk.[168] Several of these compounds were evaluated in vitro for their capacity to bind and activate the receptor, but no data were disclosed. Compound **81** (Figure 34) decreases food intake in vivo from 10 to 25% when administered via intraperitoneal route in rats, compared with control animals. Neither relative nor absolute stereochemistry of these compounds are specified.

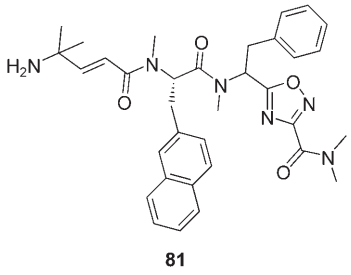


Figure 34. Peptidomimetic antagonist **81** developed by Novo Nordisk.

4. hGHS-R1a Inverse Agonists

GHS-R1a inverse agonists should also be interesting therapeutic tools owing to the reported high constitutive activity of the receptor.[169] Such molecules would be particularly useful in the treatment of obesity.

4.1. Peptide inverse agonists: Merck

By using in vitro screening, researchers from Merck first identified compound **82** (D-Lys^3–GHRP-6, Figure 35), a peptide analogue of GHRP-6 that is able to inhibit GHRP-6-induced GH secretion.[170] Furthermore, via the intracerebroventricular route in mice, it inhibits food intake and body-weight gain.[171] This compound was first described as a GHS-R1a antagonist. Recently, Chan et al. demonstrated that it is, in fact, an inverse agonist, able to inhibit the constitutive activation of phospholipase C (PLC) induced by GHS-R1a.[172] However, this compound has poor affinity for hGHS-R1a[173] and is not selective, binding to the various melanocortin receptor subtypes as well.[174]

His - D-Trp - D-Lys - Trp - D-Phe - Lys - NH_2

82

D-Lys^3–GHRP-6

IC_{50} (binding) = 0.9 mM (hGHSR-1a / [^{35}S]MK 0677)

D-Arg - Pro - Lys - Pro - D-Phe - Gln - D-Trp - Phe - D-Trp - Leu - Leu - NH_2

83

L 756 867 or (D-Arg1, D-Phe5, D-Trp7,9, Leu11)substance P

IC_{50} (IP_3) = 5.2 μM

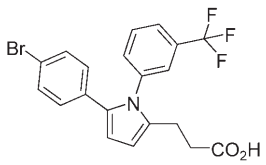
Figure 35. Peptide inverse agonists developed by Merck.

The other compound identified by Merck is compound **83** (L 756 867, Figure 35), derived from subtance P. It inhibits GH secretion induced by compound **21** in rat,[175] and compound-

17-induced GH secretion in pig.[91] Holst et al. characterized **83** as an inverse agonist of GHS-R1a, and not as an antagonist.[169] Indeed, in vitro, this compound is able to inhibit the constitutive production of inositol-1,4,5-trisphosphate (IP_3) induced by GHS-R1a in a dose-dependent manner, but this compound is not selective for hGHS-R1a. It is able to bind to the receptors of several neuropeptides, such as the bombesin receptor,[176] the vasopressin- and gastrin-releasing-peptide receptors[177] and to the interleukin-8 receptor.[178]

4.2. Peptidomimetic inverse agonists: 7TM Pharma

7TM Pharma has disclosed compound **84** (TM 27 810) (Figure 36), a nonpeptidyl inverse agonist of GHS-R1a with an IC_{50} value of 6.5 μM, determined by the inhibition of IP_3 hydrolysis in COS7 cells.[179] Although this compound is much weaker than the previous peptide inverse agonist, it represents a reasonable starting point for further optimization to improve selectivity and oral bioavailability.



84
TM 27 810
IC_{50} (IP_3) = 6.5 μM

Figure 36. Peptidomimetic inverse agonist **84** developed by 7TM Pharma.

Summary

The discovery of new agents that either mimic or modulate the actions of ghrelin via GHS-R1a has attracted considerable interest in recent years. Because endogenous ghrelin appears to play an important role in the long-term regulation of energy balance, GHS-R1a antagonists may be useful in the prevention of weight gain. To realize the full potential of ghrelin antagonism as a novel approach for anti-obesity treatment, GHS-R1a antagonists with better potency, selectivity, and pharmacokinetic properties are needed. GHS-R1a inverse agonists could also play a major role as anti-obesity therapeutic agents. The fields of ghrelin biology and physiology are still rapidly evolving. Further understanding of the complex pathways involving ghrelin will greatly facilitate the drug-discovery process targeting ghrelin and GHS-R1a.

Keywords: drug design · ghrelin · GHS-R1a ligands · growth hormone secretagogues · peptidomimetics

[1] M. Kojima, H. Hosoda, Y. Date, M. Nakazato, H. Matsuo, K. Kangawa, *Nature* **1999**, *402*, 656 – 660.
[2] A. D. Howard, S. D. Feighner, D. F. Cully, J. P. Arena, P. A. Liberator, C. I. Rosenblum, M. Hamelin, D. L. Hreniuk, O. C. Palyha, et al., *Science* **1996**, *273*, 974 – 977.
[3] X. M. Guan, H. Yu, O. C. Palyha, K. K. McKee, S. D. Feighner, D. J. Sirinathsinghji, R. G. Smith, L. H. Van der Ploeg, A. D. Howard, *Brain Res. Mol. Brain Res.* **1997**, *48*, 23 – 29.
[4] R. Yokote, M. Sato, S. Matsubara, H. Ohye, M. Niimi, K. Murao, J. Takahara, *Peptides* **1998**, *19*, 15 – 20.
[5] N. B. Dass, M. Munonyara, A. K. Bassil, G. J. Hervieu, S. Osbourne, S. Corcoran, M. Morgan, G. J. Sanger, *Neuroscience* **2003**, *120*, 443 – 453.
[6] S. Gnanapavan, B. Kola, S. A. Bustin, D. G. Morris, P. McGee, P. Fairclough, S. Bhattacharya, R. Carpenter, A. B. Grossman, M. Korbonits, *J. Clin. Endocrinol. Metab.* **2002**, *87*, 2988 – 2991.
[7] E. Arvat, M. Maccario, L. Di Vito, F. Broglio, A. Benso, C. Gottero, M. Papotti, G. Muccioli, C. Dieguez, F. F. Casanueva, R. Deghenghi, F. Camanni, E. Ghigo, *J. Clin. Endocrinol. Metab.* **2001**, *86*, 1169 – 1174.
[8] F. Broglio, A. Benso, C. Castiglioni, C. Gottero, F. Prodam, S. Destefanis, C. Gauna, A. J. van der Lely, R. Deghenghi, M. Bo, E. Arvat, E. Ghigo, *J. Clin. Endocrinol. Metab.* **2003**, *88*, 1537 – 1542.
[9] R. Fernandez-Fernandez, M. Tena-Sempere, E. Aguilar, L. Pinilla, *Neurosci. Lett.* **2004**, *362*, 103 – 107.
[10] M. Furuta, T. Funabashi, F. Kimura, *Biochem. Biophys. Res. Commun.* **2001**, *288*, 780 – 785.
[11] A. M. Wren, C. J. Small, H. L. Ward, K. G. Murphy, C. L. Dakin, S. Taheri, A. R. Kennedy, G. H. Roberts, D. G. A. Morgan, M. A. Ghatei, S. R. Bloom, *Endocrinology* **2000**, *141*, 4325 – 4328.
[12] A. Inui, *Nat. Rev. Neurosci.* **2001**, *2*, 551 – 560.
[13] T. Akamizu, K. Takaya, T. Irako, H. Hosoda, S. Teramukai, A. Matsuyama, H. Tada, K. Miura, A. Shimizu, M. Fukushima, M. Yokode, K. Tanaka, K. Kangawa, *Eur. J. Endocrinol.* **2004**, *150*, 447 – 455.
[14] M. Tschöp, D. L. Smiley, M. L. Heiman, *Nature* **2000**, *407*, 908 – 913.
[15] M. Nakazato, N. Murakami, Y. Date, M. Kojima, H. Matsuo, K. Kangawa, S. Matsukura, *Nature* **2001**, *409*, 194 – 198.
[16] M. Tschop, C. Weyer, P. A. Tataranni, V. Devanarayan, E. Ravussin, M. L. Heiman, *Diabetes* **2001**, *50*, 707 – 709.
[17] J. C. Weikel, A. Wichniak, M. Ising, H. Brunner, E. Friess, K. Held, S. Mathias, D. A. Schmid, M. Uhr, A. Steiger, *Am. J. Physiol.* **2003**, *284*, E407 – E415.
[18] V. Tolle, M.-H. Bassant, P. Zizzari, F. Poindessous-Jazat, C. Tomasetto, J. Epelbaum, M.-T. Bluet-Pajot, *Endocrinology* **2002**, *143*, 1353 – 1361.
[19] A. Asakawa, A. Inui, T. Kaga, H. Yuzuriha, T. Nagata, M. Fujimiya, G. Katsuura, S. Makino, M. A. Fujino, M. Kasuga, *Neuroendocrinology* **2001**, *74*, 143 – 147.
[20] V. P. Carlini, M. E. Monzon, M. M. Varas, A. B. Cragnolini, H. B. Schioth, T. N. Scimonelli, S. R. de Barioglio, *Biochem. Biophys. Res. Commun.* **2002**, *299*, 739 – 743.
[21] P. Carlini Valeria, M. Varas Mariana, B. Cragnolini Andrea, B. Schioth Helgi, N. Scimonelli Teresa, R. de Barioglio Susana, *Biochem. Biophys. Res. Commun.* **2004**, *313*, 635 – 641.
[22] Y. Masuda, T. Tanaka, N. Inomata, N. Ohnuma, S. Tanaka, Z. Itoh, H. Hosoda, M. Kojima, K. Kangawa, *Biochem. Biophys. Res. Commun.* **2000**, *276*, 905 – 908.
[23] A. Asakawa, A. Inui, T. Kaga, H. Yuzuriha, T. Nagata, N. Ueno, S. Makino, M. Fujimiya, A. Niijima, M. A. Fujino, M. Kasuga, *Gastroenterology* **2001**, *120*, 337 – 345.
[24] A. Inui, A. Asakawa, C. Y. Bowers, G. Mantovani, A. Laviano, M. M. Meguid, M. Fujimiya, *FASEB J.* **2004**, *18*, 439 – 456.
[25] T. L. Peeters, *J. Physiol. Pharmacol.* **2003**, 54, 95 – 103.
[26] K. Fujino, A. Inui, A. Asakawa, N. Kihara, M. Fujimura, M. Fujimiya, *J. Physiol.* **2003**, *550*, 227 – 240.
[27] E. Adeghate, A. S. Ponery, *J. Neuroendocrinol.* **2002**, *14*, 555 – 560.
[28] Y. Date, M. Nakazato, S. Hashiguchi, K. Dezaki, M. S. Mondal, H. Hosoda, M. Kojima, K. Kangawa, T. Arima, H. Matsuo, T. Yada, S. Matsukura, *Diabetes* **2001**, *51*, 124 – 129.
[29] A. Salehi, C. Dornonville de La Cour, R. Hakanson, I. Lundquist, *Reg. Pept.* **2004**, *118*, 143 – 150.
[30] F. Broglio, E. Arvat, A. Benso, C. Gottero, G. Muccioli, M. Papotti, A. J. van der Lely, R. Deghenghi, E. Ghigo, *J. Clin. Endocrinol. Metab.* **2001**, *86*, 5083 – 5086.
[31] M. Murata, Y. Okimura, K. Iida, M. Matsumoto, H. Sowa, H. Kaji, M. Kojima, K. Kangawa, K. Chihara, *J. Biol. Chem.* **2002**, *277*, 5667 – 5674.
[32] O. Ukkola, *Eur. J. Clin. Invest.* **2003**, *33*, 183 – 185.
[33] F. Broglio, C. Gottero, A. Benso, F. Prodam, M. Volante, S. Destefanis, C. Gauna, G. Muccioli, M. Papotti, A. J. van der Lely, E. Ghigo, *Endocrine* **2003**, *22*, 19 – 24.
[34] N. Nagaya, K. Kangawa, *Reg. Pept.* **2003**, *114*, 71 – 77.
[35] M. Enomoto, N. Nagaya, M. Uematsu, H. Okumura, E. Nakagawa, F. Ono, H. Hosoda, H. Oya, M. Kojima, K. Kanmatsuse, K. Kangawa, *Clin. Sci.* **2003**, *105*, 431 – 435.

[36] N. Nagaya, K. Miyatake, M. Uematsu, H. Oya, W. Shimizu, H. Hosoda, M. Kojima, N. Nakanishi, H. Mori, K. Kangawa, *J. Clin. Endocrinol. Metab.* **2001**, *86*, 5854 – 5859.
[37] P. L. Jeffery, A. C. Herington, L. K. Chopin, *J. Endocrinol.* **2002**, *172*, R7 – R11.
[38] M. S. Duxbury, T. Waseem, H. Ito, M. K. Robinson, M. J. Zinner, S. W. Ashley, E. E. Whang, *Biochem. Biophys. Res. Commun.* **2003**, *309*, 464 – 468.
[39] M. Volante, E. Allia, E. Fulcheri, P. Cassoni, E. Ghigo, G. Muccioli, M. Papotti, *Am. J. Pathol.* **2003**, *162*, 645 – 654.
[40] P. Cassoni, C. Ghe, T. Marrocco, E. Tarabra, E. Allia, F. Catapano, R. Deghenghi, E. Ghigo, M. Papotti, G. Muccioli, *Eur. J. Endocrinol.* **2004**, *150*, 173 – 184.
[41] B. B. Bercu, R. F. Walker, *Acta Paediatr.* **1997**, *423*, 102 – 106.
[42] E. Ghigo, E. Arvat, G. Aimaretti, F. Broglio, R. Giordano, F. Camanni, *Bailliere's Clin. Endocrinol. Metab.* **1998**, *12*, 341 – 358.
[43] A. W. Root, F. B. Diamond, Jr., *Endocrine* **2000**, *12*, 137 – 145.
[44] J. D. Veldhuis, W. S. Evans, C. Y. Bowers, S. Anderson, *Endocrine* **2001**, *14*, 45 – 62.
[45] V. L. Fuh, M. A. Bach, *Growth Horm. IGF Res.* **1998**, *8*, 13 – 20.
[46] O. Khorram, *Clin. Obstet. Gynecol.* **2001**, *44*, 893 – 901.
[47] J. S. Strobl, M. J. Thomas, *Pharmacol. Rev.* **1994**, *46*, 1 – 34.
[48] T. Hanada, K. Toshinai, Y. Date, N. Kajimura, T. Tsukada, Y. Hayashi, K. Kangawa, M. Nakazato, *Metab. Clin. Exp.* **2004**, *53*, 84 – 88.
[49] T. Hanada, K. Toshinai, N. Kajimura, N. Nara-Ashizawa, T. Tsukada, Y. Hayashi, K. Osuye, K. Kangawa, S. Matsukura, M. Nakazato, *Biochem. Biophys. Res. Commun.* **2003**, *301*, 275 – 279.
[50] N. M. Neary, C. J. Small, A. M. Wren, J. L. Lee, M. R. Druce, C. Palmieri, G. S. Frost, M. A. Ghatei, R. C. Coombes, S. R. Bloom, *J. Clin. Endocrinol. Metab.* **2004**, *89*, 2832 – 2836.
[51] G. Van den Berghe, *Horm. Res.* **1999**, *51*, 21 – 28.
[52] G. Muccioli, M. Tschop, M. Papotti, R. Deghenghi, M. Heiman, E. Ghigo, *Eur. J. Pharmacol.* **2002**, *440*, 235 – 254.
[53] Y. Hataya, T. Akamizu, K. Takaya, N. K. H. Ariyasu, M. Saijo, K. Moriyama, A. Shimatsu, M. Kojima, K. Kangawa, K. Nakao, *J. Clin. Endocrinol. Metab.* **2001**, *86*, 4552 – 4555.
[54] A. Leal-Cerro, E. Garcia, R. Astorga, F. F. Casanueva, C. Dieguez, *Eur. J. Endocrinol.* **1995**, *132*, 712 – 715.
[55] V. Popovic, A. Leal, D. Micic, H. P. F. Koppeschaar, E. Torres, C. Paramo, S. Obradovic, C. Dieguez, F. F. Casanueva, *Lancet* **2000**, *356*, 1137 – 1142.
[56] G. Aimaretti, C. Baffoni, F. Broglio, J. A. M. Janssen, G. Corneli, R. Deghenghi, A. J. van der Lely, E. Ghigo, E. Arvat, *Clin. Endocrinol.* **2002**, *56*, 765 – 771.
[57] R. Baldelli, X. L. Otero, J. P. Camina, O. Gualillo, V. Popovic, C. Dieguez, F. F. Casanueva, *Endocrine* **2001**, *14*, 95 – 99.
[58] E. Ghigo, G. Aimaretti, E. Arvat, F. Camanni, *Endocrine* **2001**, *15*, 29 – 38.
[59] S. M. Shalet, A. Toogood, A. Rahim, B. M. D. Brennan, *Endocr. Rev.* **1998**, *19*, 203 – 223.
[60] C. Dornonville de La Cour, E. Lindstrom, P. Norlen, R. Hakanson, *Reg. Pept.* **2004**, *120*, 23 – 32.
[61] L. Chang, Y. Ren, X. Liu, W. G. Li, J. Yang, B. Geng, N. L. Weintraub, C. Tang, *J. Cardiovasc. Pharmacol.* **2004**, *43*, 165 – 170.
[62] L. Chang, J. Zhao, G.-z. Li, B. Geng, C.-s. Pan, Y.-f. Qi, C.-s. Tang, *Acta Toxicol. Ther.* **2004**, *25*, 1131 – 1137.
[63] H. Okumura, N. Nagaya, M. Enomoto, E. Nakagawa, H. Oya, K. Kangawa, *J. Cardiovasc. Pharmacol.* **2002**, *39*, 779 – 783.
[64] V. Bodart, M. Febbraio, A. Demers, N. McNicoll, P. Pohankova, A. Perreault, T. Sejlitz, E. Escher, R. L. Silverstein, D. Lamontagne, H. Ong, *Circulation Res.* **2002**, *90*, 844 – 849.
[65] M. Imazio, M. Bobbio, F. Broglio, A. Benso, V. Podio, M. R. Valetto, G. Bisi, E. Ghigo, G. P. Trevi, *Eur. J. Heart Failure* **2002**, *4*, 185 – 191.
[66] F. Broglio, F. Guarracino, A. Benso, C. Gottero, F. Prodam, R. Granata, E. Avogadri, G. Muccioli, R. Deghenghi, E. Ghigo, *Eur. J. Pharmacol.* **2002**, *448*, 193 – 200.
[67] A. Torsello, G. Rossoni, V. Locatelli, V. De Gennaro Colonna, M. Bernareggi, M. Francolini, E. E. Muller, F. Berti, *Endocrine* **2001**, *14*, 109 – 112.
[68] P. Cassoni, M. Papotti, C. Ghe, F. Catapano, A. Sapino, A. Graziani, R. Deghenghi, T. Reissmann, E. Ghigo, G. Muccioli, *J. Clin. Endocrinol. Metab.* **2001**, *86*, 1738 – 1745.
[69] P. Cassoni, M. Papotti, F. Catapano, C. Ghe, R. Deghenghi, E. Ghigo, G. Muccioli, *J. Endocrinol.* **2000**, *165*, 139 – 146.
[70] C. Ghe, P. Cassoni, F. Catapano, T. Marrocco, R. Deghenghi, E. Ghigo, G. Muccioli, M. Papotti, *Endocrinology* **2002**, *143*, 484 – 491.
[71] C. Y. Bowers, J. Chang, F. Momany, K. Folkers, *Mol. Endocrinol. Proc.* **1977**, 287 – 292.
[72] C. Y. Bowers, F. A. Momany, G. A. Reynolds, A. Hong, *Endocrinology* **1984**, *114*, 1537 – 1545.
[73] B. E. Ilson, D. K. Jorkasky, R. T. Curnow, R. M. Stote, *J. Clin. Endocrinol. Metab.* **1989**, *69*, 212 – 214.
[74] C. Y. Bowers, *J. Pediatr. Endocrinol.* **1993**, *6*, 21 – 31.
[75] M. S. Akman, M. Girard, L. F. O'Brien, A. K. Ho, C. L. Chik, *Endocrinology* **1993**, *132*, 1286 – 1291.
[76] R. Deghenghi, M. M. Cananzi, A. Torsello, C. Battisti, E. E. Muller, V. Locatelli, *Life Sci.* **1994**, *54*, 1321 – 1328.
[77] B. P. Imbimbo, T. Mant, M. Edwards, D. Amin, N. Dalton, F. Boutignon, V. Lenaerts, P. Wuthrich, R. Deghenghi, *Eur. J. Clin. Pharmacol.* **1994**, *46*, 421 – 425.
[78] M. A. Bednarek, S. D. Feighner, S.-S. Pong, K. K. McKee, D. L. Hreniuk, M. V. Silva, V. A. Warren, A. D. Howard, L. H. Y. Van der Ploeg, J. V. Heck, *J. Med. Chem.* **2000**, *43*, 4370 – 4376.
[79] A. Torsello, C. Ghe, E. Bresciani, F. Catapano, E. Ghigo, R. Deghenghi, V. Locatelli, G. Muccioli, *Endocrinology* **2002**, *143*, 1968 – 1971.
[80] M. Matsumoto, H. Hosoda, Y. Kitajima, N. Morozumi, Y. Minamitake, S. Tanaka, H. Matsuo, M. Kojima, Y. Hayashi, K. Kangawa, *Biochem. Biophys. Res. Commun.* **2001**, *287*, 142 – 146.
[81] K. Kangawa, M. Kojima, H. Hosoda, H. Matsuo, Y. Minamitake (Japan), WO 2001007475, **2001**.
[82] M. Bednarek (Merck), WO 2001092292, **2001**.
[83] R. Deghenghi, *Growth Horm. Secretagogues* **1999**, 19 – 24.
[84] V. Guerlavais, D. Boeglin, D. Mousseaux, C. Oiry, A. Heitz, R. Deghenghi, V. Locatelli, A. Torsello, C. Ghe, F. Catapano, G. Muccioli, J.-C. Galleyrand, J.-A. Fehrentz, J. Martinez, *J. Med. Chem.* **2003**, *46*, 1191 – 1203.
[85] J. Martinez, J.-A. Fehrentz, V. Guerlavais (Zentaris), WO 2001096300, **2001**.
[86] D. Mousseaux, L. Le Gallic, J. Ryan, C. Oiry, D. Gagne, J.-A. Fehrentz, J.-C. Galleyrand, J. Martinez, *Br. J. Pharmacol.* **2006**, *148*, 350 – 365.
[87] F. Broglio, F. Boutignon, A. Benso, C. Gottero, F. Prodam, E. Arvat, C. Ghe, F. Catapano, A. Torsello, V. Locatelli, G. Muccioli, D. Boeglin, V. Guerlavais, J. A. Fehrentz, J. Martinez, E. Ghigo, R. Deghenghi, *J. Endocrinol. Invest.* **2002**, *25*, RC26 – RC28.
[88] T. C. Somers, K. A. Elias, R. G. Clark, R. S. McDowell, M. S. Stanley, J. P. Burnier, T. E. Rawson (Genentech), WO 9615148, **1996**.
[89] K. Raun, B. S. Hansen, N. L. Johansen, H. Thogersen, K. Madsen, M. Ankersen, P. H. Andersen, *Eur. J. Endocrinol.* **1998**, *139*, 552 – 561.
[90] M. Ankersen, N. L. Johansen, K. Madsen, B. S. Hansen, K. Raun, K. K. Nielsen, H. Thogersen, T. K. Hansen, B. Peschke, J. Lau, B. F. Lundt, P. H. Andersen, *J. Med. Chem.* **1998**, *41*, 3699 – 3704.
[91] T. K. Hansen, M. Ankersen, B. S. Hansen, K. Raun, K. K. Nielsen, J. Lau, B. Peschke, B. F. Lundt, H. Thogersen, N. L. Johansen, K. Madsen, P. H. Andersen, *J. Med. Chem.* **1998**, *41*, 3705 – 3714.
[92] M. Ankersen, T. K. Hansen, I. Ahnfelt-Ronne, A. M. Kappelgaard, *Drug Discovery Today* **1999**, *4*, 497 – 506.
[93] M. Ankersen (Novo Nordisk), WO 9936431, **1999**.
[94] M. B. Andersen, B. S. Hansen, K. Raun, S. Tullin, L. Thim (Novo Nordisk), WO 2001056592, **2001**.
[95] B. Peschke, B. S. Hansen, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1295 – 1298.
[96] M. Ankersen, B. S. Hansen, T. K. Hansen, J. Lau, B. Peschke, K. Madsen, N. L. Johansen, *Eur. J. Med. Chem.* **1999**, *34*, 783 – 790.
[97] M. Ankersen, K. K. Nielsen, T. K. Hansen, K. Raun, B. S. Hansen, *Eur. J. Med. Chem.* **2000**, *35*, 487 – 497.
[98] B. Peschke, M. Ankersen, T. K. Hansen, B. S. Hansen, J. Lau, K. K. Nielsen, K. Raun, *Eur. J. Med. Chem.* **2000**, *35*, 599 – 618.
[99] B. Peschke, M. Ankersen, M. Bauer, T. K. Hansen, B. S. Hansen, K. K. Nielsen, K. Raun, L. Richter, L. Westergaard, *Eur. J. Med. Chem.* **2002**, *37*, 487 – 501.
[100] R. G. Smith, K. Cheng, W. R. Schoen, S. S. Pong, G. Hickey, T. Jacks, B. Butler, W. W. S. Chan, L. Y. P. Chaung, et al., *Science* **1993**, *260*, 1640 – 1643.
[101] K. Cheng, W. W. S. Chan, B. Butler, L. Wei, W. R. Schoen, M. J. Wyvratt, Jr., M. H. Fisher, R. G. Smith, *Endocrinology* **1993**, *132*, 2729 – 2731.

[102] M.-H. Chen, D. B. R. Johnston, R. P. Nargund, A. A. Patchett, J. R. Tata, L. Yang (Merck), WO 9413696, **1994**.
[103] A. A. Patchett, R. G. Smith, M. J. Wyvratt, *Pharm. Biotechnol.* **1998**, *11*, 525 – 554.
[104] G. Hickey, T. Jacks, F. Judith, F. Taylor, W. R. Schoen, D. Krupa, P. Cunningham, J. Clark, R. G. Smith, *Endocrinology* **1994**, *134*, 695 – 701.
[105] K. H. Leung, D. A. Cohn, R. R. Miller, G. A. Doss, R. A. Stearns, R. E. S. Simpson, W. P. Feeney, S.-H. L. Chiu, *Drug Metab. Dispos.* **1996**, *24*, 753 – 760.
[106] W. R. Schoen, D. Ok, R. J. DeVita, J. M. Pisano, P. Hodges, K. Cheng, W. W. S. Chan, B. S. Butler, R. G. Smith, et al., *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1117 – 1122.
[107] R. J. DeVita, W. R. Schoen, G. A. Doldouras, M. H. Fisher, M. J. Wyvratt, Jr., K. Cheng, W. W. S. Chan, B. S. Butler, R. G. Smith, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1281 – 1286.
[108] R. J. DeVita, W. R. Schoen, M. H. Fisher, A. J. Frontier, J. M. Pisano, M. J. Wyvratt, Jr., K. Cheng, W. W. S. Chan, B. S. Butler, et al., *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2249 – 2254.
[109] L. Chu, H. Mrozik, M. H. Fisher, J. E. Brown, K. Cheng, W. W. S. Chan, W. R. Schoen, M. J. Wyvratt, B. S. Butler, R. G. Smith, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2245 – 2250.
[110] T. Jacks, G. Hickey, F. Judith, J. Taylor, H. Chen, D. Krupa, W. Feeney, W. Schoen, D. Ok, et al., *J. Endocrinol.* **1994**, *143*, 399 – 406.
[111] P. Lin, J. M. Pisano, W. R. Schoen, K. Cheng, W. W. S. Chan, B. S. Butler, R. G. Smith, M. H. Fisher, M. J. Wyvratt, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3237 – 3242.
[112] R. P. Nargund, K. H. Barakat, K. Cheng, W. W. S. Chan, B. R. Butler, R. G. Smith, A. A. Patchett, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1265 – 1270.
[113] A. A. Patchett, R. P. Nargund, J. R. Tata, M. H. Chen, K. J. Barakat, D. B. Johnston, K. Cheng, W. W. Chan, B. Butler, G. Hickey, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 7001 – 7005.
[114] M.-H. Chen, M. G. Steiner, A. A. Patchett, K. Cheng, L. Wei, W. W. S. Chan, B. Butler, T. M. Jacks, R. G. Smith, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2163 – 2168.
[115] D. C. Dean, R. P. Nargund, S.-S. Pong, L.-Y. P. Chaung, P. Griffin, D. G. Melillo, R. L. Ellsworth, L. H. T. Van der Ploeg, A. A. Patchett, R. G. Smith, *J. Med. Chem.* **1996**, *39*, 1767 – 1770.
[116] T. Jacks, R. Smith, F. Judith, K. Schleim, E. Frazier, H. Chen, D. Krupa, D. Hora, Jr., R. Nargund, et al., *Endocrinology* **1996**, *137*, 5284 – 5289.
[117] Z. Ye, Y. Gao, R. K. Bakshi, M.-H. Chen, S. P. Rohrer, S. D. Feighner, S.-S. Pong, A. D. Howard, A. Blake, E. T. Birzin, L. Locco, R. M. Parmar, W. W. S. Chan, J. M. Schaeffer, R. G. Smith, A. A. Patchett, R. P. Nargund, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 5 – 8.
[118] B. L. Palucki, S. D. Feighner, S. S. Pong, K. K. McKee, D. L. Hreniuk, C. Tan, A. D. Howard, L. H. Y. Van der Ploeg, A. A. Patchett, R. P. Nargund, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1955 – 1957.
[119] B. A. Lefker, P. A. Carpino, L. C. Pan, K. L. Chidsey-Frink, P. DaSilva-Jardine, F. M. Dicapua, D. A. Griffith, W. A. Hada, Jr., J. K. Inthavongsay, S. K. Lewis, F. M. Mangano, M. C. Murray, O. Ng, J. G. Owens, C. M. Pirie, C. R. Rose, N. I. Ryan, J. R. Schafer, J. L. Tucker, A. S. Wright, M. P. Zawistoski, D. D. Thompson, *Growth Hormone Secretagogues in Clinical Practice (2nd International Symposium on GH Secretagogues)* **1998**, pp. 107–120.
[120] P. A. Carpino, B. A. Lefker, S. M. Toler, L. C. Pan, J. R. Hadcock, E. R. Cook, J. N. DiBrino, A. M. Campeta, S. L. DeNinno, K. L. Chidsey-Frink, W. A. Hada, J. Inthavongsay, F. M. Mangano, M. A. Mullins, D. F. Nickerson, O. Ng, C. M. Pirie, J. A. Ragan, C. R. Rose, D. A. Tess, A. S. Wright, L. Yu, M. P. Zawistoski, P. A. DaSilva-Jardine, T. C. Wilson, D. D. Thompson, *Bioorg. Med. Chem.* **2003**, *11*, 581 – 590.
[121] L. C. Pan, P. A. Carpino, B. A. Lefker, J. A. Ragan, S. M. Toler, J. C. Pettersen, D. O. Nettleton, O. Ng, C. M. Pirie, K. Chidsey-Frink, B. Lu, D. F. Nickerson, D. A. Tess, M. A. Mullins, D. B. MacLean, P. A. DaSilva-Jardine, D. D. Thompson, *Endocrine* **2001**, *14*, 121 – 132.
[122] P. A. Carpino, B. A. Lefker, S. M. Toler, L. C. Pan, J. R. Hadcock, M. C. Murray, E. R. Cook, J. N. Di Brino, S. L. De Ninno, K. L. Chidsey-Frink, W. A. Hada, J. Inthavongsay, S. K. Lewis, F. M. Mangano, M. A. Mullins, D. F. Nickerson, O. Ng, C. M. Pirie, J. A. Ragan, C. R. Rose, D. A. Tess, A. S. Wright, L. Yu, M. P. Zawistoski, J. C. Pettersen, P. A. DaSilva-Jardine, T. C. Wilson, D. D. Thompson, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3279 – 3282.
[123] D. A. Griffith, B. S. Bronk (Pfizer Products), EP 1002802, **2000**.
[124] P. A. Carpino, D. A. Griffith, B. A. Lefker (Pfizer), WO 9858947, **1998**.
[125] P. A. Carpino (Pfizer Products), JP 2000072771, **2000**.
[126] P. Huang, G. H. Loew, H. Funamizu, M. Mimura, N. Ishiyama, M. Hayashida, T. Okuno, O. Shimada, A. Okuyama, S. Ikegami, J. Nakano, K. Inoguchi, *J. Med. Chem.* **2001**, *44*, 4082 – 4091.
[127] H. Funamizu, N. Ishiyama, S. Ikegami, T. Okuno, K. Inoguchi, P. Huang, G. H. Loew (Kaken Pharmaceutical), WO 9909991, **1999**.
[128] N. Ishiyama, H. Ishige, M. Mimura, T. Okuno, K. Ukai, T. Kiyofuji, S. Tauchi, K. Inoguchi, P. Huang, G. H. Loew (Kaken Pharmaceutical), WO 2000048623, **2000**.
[129] J. A. Dodge, K. L. Hauser, M. L. Heiman, S. A. Jones, C. A. Alt, H. U. Bryant, J. D. Cohen, J. D. Copp, K. J. Fahey, W. H. Gritton, L. N. Jungheim, J. H. Kennedy, C. W. Lugar III, B. S. Muehl, A. D. Palkowitz, A. M. Ratz, G. A. Rhodes, R. L. Robey, D. E. Seyler, T. A. Shepherd, K. J. Thrasher, W. G. Trankle (Eli Lilly), WO 9908699, **1999**.
[130] D. E. Seyler, J. A. Dodge, J. J. Osborne, K. L. Cox, D. Viswanath, A. F. Wilmot, M. J. Keaton, M. L. Heiman, H. U. Bryant, G. B. Cutler, Jr., *Drug Dev. Res.* **2000**, *49*, 260 – 265.
[131] J. A. Dodge, B. Evers, L. N. Jungheim, B. S. Muehl, G. Ruehter, K. J. Thrasher (Eli Lilly), WO 2002032888, **2002**.
[132] J. A. Dodge, P. A. Hipskind (Eli Lilly), EP 761220, **1997**.
[133] R. F. Kauffman, A. D. Palkowitz (Eli Lilly), WO 9908697, **1999**.
[134] J. Robl, J. A. Tino, A. S. Hernandez, J. J. Li, J. Li, S. G. Swartz (Bristol–Myers Squibb), WO 2000054729, **2000**.
[135] J. J. Li (Bristol–Myers Squibb), WO 2000024398, **2000**.
[136] J. J. Li, J. A. Tino (Bristol–Myers Squibb), WO 2001085695, **2001**.
[137] R. G. Smith, P. R. Griffin, Y. Xu, A. G. A. Smith, K. Liu, J. Calacay, S. D. Feighner, C. S. Pong, D. Leong, A. Pomes, K. Cheng, L. H. T. Van der Ploeg, A. D. Howard, J. Schaeffer, R. J. Leonard, *Biochem. Biophys. Res. Commun.* **2000**, *276*, 1306 – 1313.
[138] S. Tullin, B. S. Hansen, M. Ankersen, J. Moller, K. A. Von Cappelen, L. Thim, *Endocrinology* **2000**, *141*, 3397 – 3402.
[139] J. Nagamine, R. Nagata, H. Seki, N. Nomura-Akimaru, Y. Ueki, K. Kumagai, M. Taiji, H. Noguchi, *J. Endocrinol.* **2001**, *171*, 481 – 489.
[140] T. Tokunaga, W. E. Hume, T. Umezome, K. Okazaki, Y. Ueki, K. Kumagai, S. Hourai, J. Nagamine, H. Seki, M. Taiji, H. Noguchi, R. Nagata, *J. Med. Chem.* **2001**, *44*, 4641 – 4649.
[141] T. Tokunaga, T. Umezome, W. E. Hume, R. Nagata, K. Okazaki, Y. Ueki, K. Kumagai (Sumitomo Pharmaceuticals), WO 2000010975, **2000**.
[142] D. L. Drazen, S. C. Woods, *Curr. Opin. Clin. Nutr. Metab. Care* **2003**, *6*, 621 – 628.
[143] O. Ukkola, *J. Endocrinol. Invest.* **2004**, *27*, 96 – 98.
[144] H. Zhao, *Drug Dev. Res.* **2005**, *65*, 50 – 54.
[145] H. E. Bays, *Obes. Res.* **2004**, *12*, 1197 – 1211.
[146] D. E. Cummings, K. Clement, J. Q. Purnell, C. Vaisse, K. E. Foster, R. S. Frayo, M. W. Schwartz, A. Basdevant, D. S. Weigle, *Nat. Med.* **2002**, *8*, 643 – 644.
[147] A. Delparigi, M. Tschop, M. L. Heiman, A. D. Salbe, B. Vozarova, S. M. Sell, J. C. Bunt, P. A. Tataranni, *J. Clin. Endocrinol. Metab.* **2002**, *87*, 5461 – 5464.
[148] R. Deghenghi (Zentaris), WO 2002008250, **2002**.
[149] H. A. Halem, J. E. Taylor, J. Z. Dong, Y. Shen, R. Datta, A. Abizaid, S. Diano, T. Horvath, P. Zizzari, M.-T. Bluet-Pajot, J. Epelbaum, M. D. Culler, *Eur. J. Endocrinol.* **2004**, *151*, S71-S75.
[150] P. Zizzari, H. Halem, J. Taylor, J. Z. Dong, R. Datta, M. D. Culler, J. Epelbaum, M. T. Bluet-Pajot, *Endocrinology* **2005**, *146*, 3836 – 3842.
[151] P. Deslongchamps, Y. Dory, L. Ouellet, G. Villeneuve, M. Ramaseshan, D. Fortin, M. Peterson, H. Hoveyda, S. Beaubien, E. Marsault (Tranzyme Pharma), WO 2005012332, **2005**.
[152] K. Cheng, W. W. Chan, B. Butler, L. Wei, R. G. Smith, *Horm. Res.* **1993**, *40*, 109 – 115.
[153] B. Liu, G. Liu, Z. Xin, M. D. Serby, H. Zhao, V. G. Schaefer, H. D. Falls, W. Kaszubska, C. A. Collins, H. L. Sham, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5223 – 5226.
[154] Z. Xin, H. Zhao, M. D. Serby, B. Liu, V. G. Schaefer, D. H. Falls, W. Kaszubska, C. A. Colins, H. L. Sham, G. Liu, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1201 – 1204.
[155] Z. Xin, M. D. Serby, H. Zhao, C. Kosogof, B. G. Szczepankiewicz, M. Liu, BoLiu, C. W. Hutchins, K. A. Sarris, E. D. Hoff, H. D. Falls, C. W. Lin, C. A. Ogiela, C. A. Collins, M. E. Brune, E. N. Bush, B. A. Droz, T. A. Fey, V. E.

Knourek-Segel, R. Shapiro, P. B. Jacobson, D. W. A. Beno, T. M. Turner, H. L. Sham, G. Liu, *J. Med. Chem.* **2006**, *49*, 4459 – 4469.
[156] H. Zhao, Z. Xin, G. Liu, V. G. Schaefer, H. D. Falls, W. Kaszubska, C. A. Collins, H. L. Sham, *J. Med. Chem.* **2004**, *47*, 6655 – 6657.
[157] H. Zhao, Z. Xin, J. R. Patel, L. T. J. Nelson, B. Liu, B. G. Szczepankiewicz, V. G. Schaefer, H. D. Falls, W. Kaszubska, C. A. Collins, H. L. Sham, G. Liu, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1825 – 1828.
[158] B. Liu, M. Liu, Z. Xin, H. Zhao, M. D. Serby, C. Kosogof, L. T. J. Nelson, B. G. Szczepankiewicz, W. Kaszubska, V. G. Schaefer, H. D. Falls, C. W. Lin, C. A. Collins, H. L. Sham, G. Liu, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1864 – 1868.
[159] F. M. Sirotnak, W. D. Ensminger, J. J. Burchall, J. A. Montgomery, *Folate Antagonists as Therapeutic Agents, Vol. 2*, Academic Press, Orlando, **1984**, p. 303.
[160] M. D. Serby, H. Zhao, B. G. Szczepankiewicz, C. Kosogof, Z. Xin, B. Liu, M. Liu, L. T. J. Nelson, W. Kaszubska, H. D. Falls, V. Schaefer, E. N. Bush, R. Shapiro, B. A. Droz, V. E. Knourek-Segel, T. A. Fey, M. E. Brune, D. W. A. Beno, T. M. Turner, C. A. Collins, P. B. Jacobson, H. L. Sham, G. Liu, *J. Med. Chem.* **2006**, *49*, 2568 – 2578.
[161] R. J. Griffin, M. A. Meek, C. H. Schwalbe, M. F. G. Stevens, *J. Med. Chem.* **1989**, *32*, 2468 – 2474.
[162] C. Hutchins, *Endocr. J.* **1994**, *2*, 7 – 23.
[163] H. Geneste, W. Amberg, G. Backfisch, A. Beyerbach, W. M. Braje, J. Delzer, A. Haupt, C. W. Hutchins, L. L. King, D. R. Sauer, L. Unger, W. Wernet, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1934 – 1937.
[164] A. Napper, P. Distefano, M. A. Navia, J. O. Saunders, R. Curtis, J. Luly, J.-F. Pons, R. J. Thomas, T. Coulter, B. J. Geesaman (Elixir Pharmaceuticals), WO 2005097788, **2005**.
[165] P. Brandt, U. Bremberg, R. Crossley, M. Graffner Nordberg, A. Jenmalm Jensen, E. Ringberg, T. Ward (Biovitrum), WO 2005048916, **2005**.
[166] R. Nagata, T. Tokunaga, T. Takasaki, J. Nagamine, M. Taiji (Sumitomo Pharmaceuticals), WO 2005035498, **2005**.
[167] X. Chen, X. Chen, R. V. Connors, K. Dai, Y. Fu, J. C. Jaen, Y.-J. Kim, L. Li, M. E. Lizarzaburu, J. T. Mihalic, S. J. Shuttleworth (Amgen), WO 2006020959, **2006**.
[168] K. Raun, L. B. K. Knudsen, E. J. H. Carlsen, B. Peschke, J. Lau, K. Rimwall (Novo Nordisk), WO 2005112903, **2005**.
[169] B. Holst, A. Cygankiewicz, T. H. Jensen, M. Ankersen, T. W. Schwartz, *Mol. Endocrinol.* **2003**, *17*, 2201 – 2210.
[170] K. Cheng, W. W. Chan, A. Barreto, Jr., E. M. Convey, R. G. Smith, *Endocrinology* **1989**, *124*, 2791 – 2798.
[171] A. Asakawa, A. Inui, T. Kaga, G. Katsuura, M. Fujimiya, M. A. Fujino, M. Kasuga, *Gut* **2003**, *52*, 947 – 952.
[172] C.-B. Chan, P.-K. Leung, H. Wise, C. H. K. Cheng, *FEBS Lett.* **2004**, *577*, 147 – 153.
[173] M. Traebert, T. Riediger, S. Whitebread, E. Scharrer, H. A. Schmid, *J. Neuroendocrinol.* **2002**, *14*, 580 – 586.
[174] H. B. Schioth, R. Muceniece, J. E. S. Wikberg, *Neuropeptides* **1997**, *31*, 565 – 571.
[175] K. Cheng, L. Wei, L. Y. Chaung, W. W. S. Chan, B. Butler, R. G. Smith, *J. Endocrinol.* **1997**, *152*, 155 – 158.
[176] P. J. Woll, E. Rozengurt, *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 1859 – 1863.
[177] A. C. MacKinnon, U. Tufail-Hanif, C. D. Lucas, D. Jodrell, C. Haslett, T. Sethi, *Br. J. Cancer* **2005**, *92*, 522 – 531.
[178] M. B. Jarpe, C. Knall, F. M. Mitchell, A. M. Buhl, E. Duzic, G. L. Johnson, *J. Biol. Chem.* **1998**, *273*, 3097 – 3104.
[179] B. H. Lange, T. W. Schwartz, T. M. Frimurer, O. Rist (7TM Pharma), WO 2004056869, **2004**.

Received: January 18, 2007
Revised: April 5, 2007
Published online on May 22, 2007

