Rational Design, Discovery, and Synthesis of a Novel Series of Potent Growth Hormone Secretagogues[⊥]

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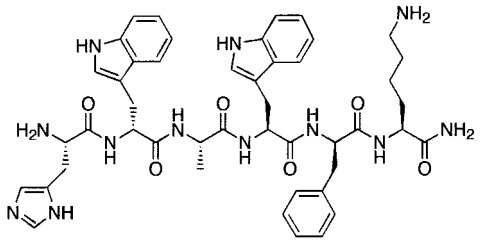
In the joint experimental and computational efforts reported here to obtain novel chemical entities as growth hormone secretagogues (GHSs), a small database of peptides and non-peptides known to have GHS activity was used to generate and assess a 3D pharmacophore for this activity. This pharmacophore was obtained using a systematic and efficient procedure, "DistComp", developed in our laboratory. The 3D pharmacophore identified was then used to search 3D databases to explore chemical structures that could be novel GHSs. A number of these were chosen for synthesis and assessment of their ability to release growth hormone (GH) from rat pituitary cells. Among the compounds tested, those with a benzothiazepin scaffold were discovered with micromolar activity. To facilitate lead optimization, a second program, a site-dependent fragment QSAR procedure was developed. This program calculates a library of chemical and physical properties of "fragments" or chemical components in a known pharmacophore and determines which, if any, of these properties are important for the observed activity. The combined use of the 3D pharmacophore and the results of the site-dependent fragment QSAR analysis led to the discovery and synthesis of a novel series of potent GHSs, a number of which had nanomolar in vitro activity.

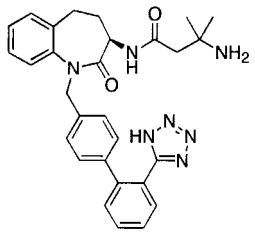
Introduction

Growth hormone (GH), a 191 amino acid peptide that is synthesized and stored in the pituitary gland, is the primary anabolic hormone of the body. It is released from the pituitary by two distinct pathways. The first is through the action of the hypothalamic hormones, growth hormone releasing hormone (GHRH) which stimulates release and by the action of somatostatin, which is inhibitory. In the second pathway, distinct from that of GHRH, peptides structurally unrelated to GHRH such as the hexapeptide His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 (GHRP-6) were found to stimulate GH release. In addition to stimulation of growth, GH is known to have a number of basic effects on the metabolic processes, e.g. stimulation of protein synthesis and free fatty acid mobilization and to cause a switch in energy metabolism from carbohydrate to fatty acid metabolism. Promising use has been made of recombinant human GH for the treatment of GH deficient children and adults, and the potential clinical benefit of GH replacement therapy is being established.[1]

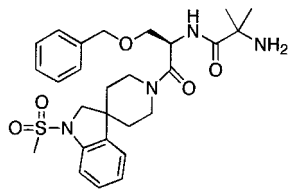
Since the discovery of the peptidyl GH secretagogue GHRP-6 (**1**, Figure 1) by Bowers and Momany et al.,[2,3] GH secretagogues have received considerable attention in the last several years with the hope for more physiological GH release by oral administration than by subcutaneous injection of GH itself.[4,5] Significant progress has been made toward identifying small molecules that mimic the mechanism of action and in vivo



1 (GHRP-6)



2 (L-692,429)



3 (MK-0677)

Figure 1. Known GH secretagogues (**1**) GHRP-6; (**2**) L-692,-429; and (**3**) MK-0677.

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[⊥] Dedicated to Dr. Gilda H. Loew on the occasion of her 70th birthday.

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properties of GHRP-6. Examples are the benzolactam L-692,429[6,7] (**2**, Figure 1) and the spiropiperidine-based MK-0677[8] (**3**, Figure 1). Experiments in rat primary pituitary cells indicated that L-692,429 and MK-0677 are mechanistically indistinguishable from the hexapeptide GHRP-6 and, hence, are peptidomimetic agonists.[6,9] Subsequently, a G-protein coupled receptor in pituitary and hypothalamus that functions in GH release was cloned and shown to be the target of the GH secretagogues.[10] This receptor is distinct from both the GHRH and somatostatin receptors. Competitive binding studies with the GH secretagogue receptor have provided direct evidence to show that GHRP-6, L-692,429, and MK-0677 are agonist mimetics of one another.[11] A recent study using site-directed mutagenesis and molecular modeling also classified the binding of peptide and non-peptide agonists in the context of its receptor environment.[12] These results led to the conclusion that all peptide and non-peptide ligands shared a common binding domain in transmembrane (TM) region 3 of the GH secretagogue receptor, although identical disposition of all agonists at the binding site may not be required.[12] In addition to L-692,429 and MK-0677, some other GH secretagogues reported in the literature include camphor-based compounds,[13] quinazolinones,[14] and quite a number of potent peptidyl analogues of GHRP-6,[15−20] including KP-102 (GHRP-2[20]). Furthermore, the recent discovery of an endogenous agonist of the GHS receptor, ghrelin,[21] provides a basis for elucidating the physiological functions of GHSs including GH secretion. While several different chemical entities are undergoing preclinical and clinical studies, it is still important to continue to design, synthesize, and assess novel nonpeptidyl GH secretagogues for potential clinical use.

To this end, in the joint experimental and computational efforts reported here, a small database of peptides and non-peptides known to have GHS activity was used to develop and assess a 3D pharmacophore for this activity. This pharmacophore was generated using a systematic and efficient procedure, DistComp, developed in our laboratory.[22] The 3D pharmacophore identified was then used to search 3D databases to discover chemical entities that could be novel GH releasing agonists. A number of these were chosen for synthesis and assessment of their ability to release GH from rat pituitary cells. Several promising lead compounds were discovered with micromolar activity. To facilitate lead optimization, a second program, site-dependent fragment QSAR procedure was developed. This program calculates a library of chemical and physical properties of "fragments" or chemical components in a known pharmacophore and determines which, if any, of these properties are important for the observed activity. The combined use of the 3D pharmacophore and the results of the site-dependent fragment QSAR analysis together with medicinal chemistry efforts and SAR studies led to the discovery of a novel series of potent GH secretagogues, a number of which had nanomolar in vitro activity. Further modifications of these potent in vitro agonists led to the discovery of compounds with enhanced bioavailability and in vivo activity. These compounds to be described in later publications are promising candidates for clinically useful GH replacement therapy.

Table 1. Compounds Chosen for 3D Pharmacophore Development

compound	sequence	activity EC_{50} (nM)[a]
	A. Peptides	
GHRP-6	His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2	5.0
[D-Lys^6]GHRP-6	His-D-Trp-Ala-Trp-D-Phe-D-Lys-NH_2	100
KP102	D-Ala-D-βNal-Ala-Trp-D-Phe-Lys-NH_2	0.2
KP102-OH	D-Ala-D-βNal-Ala-Trp-D-Phe-Lys-OH	1.0
KP102 (N-5)	D-Ala-D-βNal-Ala-Trp-D-Phe-OH	10
KP102 (Tu-4)	D-Ala-D-Phe-Ala-Phe-D-Phe-Lys-NH_2	10
[Val^3]GHRP-6	His-D-Trp-Val-Trp-D-Phe-Lys-NH_2	>1000

compound	activity EC_{50} (nM)
B. Non-peptdes	
MK-0677	1.3[b]
L-164080	3.0[c]
G7134	0.1[d]
G7220	1.8[e]

[a] For a direct comparison, activities of listed peptides were measured at Kaken. [b] Reference 8. [c] Reference 17. [d] Reference 18. [e] Reference 19.

Computer-Aided Rational Design

The computational design strategy for the development of novel GH secretagogues was implemented in three stages: (1) conceptual stage, 3D pharmacophore development; (2) exploratory stage, database search and lead generation; and (3) optimization stage, development of QSAR for refinement.

3D Pharmacophore Development. Although a specific G-protein coupled GHS receptor has recently been identified,[9,10] it is still difficult to extract precise structural information for a ligand design other than the importance of a protonable amine. Thus, a ligand-based computational design strategy was used. The objective of these computational studies was to identify and characterize the molecular determinants of GHS activity using a set of known potent peptidyl and nonpeptidyl agonists. Specifically, six peptidyl and four nonpeptidyl agonists were chosen. They are listed in Table 1, together with their EC_{50}s for the release of GH from rat pituitary cells. Also included in this study as negative control was a peptide, [Val^3]GHRP-6, found to be devoid of such activity, as also shown in Table 1. The structures of these compounds are shown in Figure 2a,b. These agonists were used to develop 3D pharmacophores, which represent the three-dimensional arrangements of specific functional groups essential for activity. To this end, an in-house program, DistComp,[22] was used to systematically determine whether there is any common spatial arrangement of key functional groups shared by the known GH secretagogues that are absent in the inactive analogue. And then the resulting 3D pharmacophore, representing a particular spatial arrangement of atoms or functional groups common to the agonists, can be used to search databases to discover novel GH secretagogues that satisfy these requirements. These candidate GH secretagogues can then be acquired or synthesized and assessed for their ability to release GH.

The search for the form in which flexible molecules such as peptides bind to receptors is a challenging task because many low-energy conformations are accessible and they coexist in equilibrium. The complexity increases enormously when several diverse families of fairly flexible molecules are included, and the goal is to

Table 2. Conformational Libraries of Peptides and Non-peptides Used To Develop the 3D Pharmacophore

compound	no. of conformers $\Delta E \leq 6$ kcal/mol
A. Peptides	
GHRP-6	563
[D-Lys^6]GHRP-6	505
KP102	105
KP102−OH	243
KP102(N5)	162
KP102(Tu4)	165
[Val^3]GHRP-6	216
B. Non-peptides	
MK-0677	228
L164,080	1073
G7134	216
G7220	168

identify the common geometric arrangements of moieties that are determinants of receptor recognition or activation because all low-energy conformations of each molecule should be included in analysis. A novel computer program, DistComp, has thus been developed[22] in our laboratory to perform systematic and automated comparisons of molecular conformations in different molecules for the determination of 3D pharmacophores. DistComp provides a procedure for identifying common spatial arrangements of selected moieties in a given set of molecules. No prior assumption of an active conformation is necessary. There is also no need for a rigid template. To achieve this goal, conformational libraries consisting of a set of unique conformers for each compound used are required as input to the DistComp program. All these low-energy conformations are then considered in the systematic search for a 3D pharmacophore.

Another key input central to this procedure is the selection of sets of common functional moieties assumed to be important for recognition or activation. The validity of these candidate recognition or activation sites is then assessed by the program. Specifically, for each hypothetical set of recognition or activation moieties selected, the program systematically determines whether any common 3D relationships among them exist in active analogues but are absent in inactive ones. Each set of proposed chemical moieties that satisfies this requirement, together with the common spatial arrangements identified, comprise candidate 3D pharmacophores.

As mentioned, conformational libraries consisting of a set of unique conformers for each compound used are also required as input to the DistComp program. Thus, prior to use of DistComp, the conformational libraries of each of the selected molecules (Table 1) were generated by a procedure of repeated cycles of high (900 K) and low (300 K) temperature molecular dynamics combined with energy minimization of molecular structures.[23] These cycles were continued until no new low-energy conformers were generated. All these calculations were performed using QUANTA4.0/CHARMm (Molecular Simulations, Inc., San Diego, CA). The conformations obtained for each compound were then clustered into unique families by a set of torsional angles in the molecule with a window of 60°. The results of the conformational search are summarized in Table 2. Shown in this table are the number of unique conformations found for each analogue within 6 kcal/mol in energy from the lowest energy. We see from this table that there are many low-energy conformers within 6 kcal/mol from the lowest energy in each molecule, not only in the peptides but also in the nonpeptidyl agonists. All these low-energy conformations were used as input to the DistComp program and considered in the systematic search for a 3D pharmacophore.

The first use of DistComp was to generate a 3D pharmacophore common to all six peptidyl agonists but absent in the inactive analogue using the set of six pharmacophoric sites (A)−(F) shown in Figure 2a. Among the sites (A)−(F) in the figure, site (A) represents the N-terminal C alpha carbon, which is directly linked to a protonated amine and a small hydrophobic group. Sites (B) and (E) are proton-accepting groups. Sites (C), (D), and (F) are aromatic rings. Figure 3 shows an overlay of the six peptidyl agonists with these six pharmacophoric sites superimposed. The conformations used in this superimposition (Figure 3) are the lowest energy conformer of each analogue that satisfied the requirements of the pharmacophore. The energy of this conformer relative to the lowest energy in each agonist is given in Table 3. There is no low-energy conformer of the inactive analogue [Val^3]GHRP-6 (**5**, Figure 2a) that shares this common peptide pharmacophore. This is because that in the bioactive conformation of GHRP-6, the methyl moiety of Ala^3 is close to the aromatic ring of Trp^4 (~3 Å). A direct replacement of the Ala with Val in this bioactive conformation results in significant steric conflicts. Therefore, [Val^3]GHRP-6 is not tolerated by the bioactive conformation of GHRP-6. This finding provided support for the validity of the peptide pharmacophore identified as well as an explanation for why [Val^3]GHRP-6 is inactive.

The next computational effort was to use the DistComp program to determine if a common 3D pharmacophore exists among the peptidyl and nonpeptidyl agonists. To this end, the four nonpeptidyl agonists shown in Figure 2b, together with their conformational libraries, were added to the input compound list for DistComp. The results indicated that when a subset of the peptide pharmacophoric sites (A−C, F), shown in Figure 2b, were used, a common 3D pharmacophore was identified. This 3D pharmacophore, common to both peptidyl and nonpeptidyl GH secretagogues, is shown in Figure 4. Shown in this figure are the four pharmacophoric sites and the common distances found between them. Figure 5 shows the overlay of the nonpeptidyl agonists, MK-0677 (**3**),[8] L-164,080 (**10**),[17] G7134 (**11**),[18] and G7220 (**12**)[19] with the peptide agonist GHRP-6. We see in this figure that there is a good superimposition of the four pharmacophoric sites despite the structural diversity of these active compounds. The energies ΔE of each conformer used in the overlay relative to the lowest energy of the molecule are shown in Table 3.

Database Search, Discovery, and Synthesis of Lead Compound. The 3D pharmacophore common to both peptidyl and nonpeptidyl GH secretagogues developed (Figure 4) was used to search databases to discover diverse and novel leads that complied with the 3D requirements of this pharmacophore. Because it is a "through space" 3D pharmacophore, rather than a "through bonds" 2D pharmacophore, compounds with

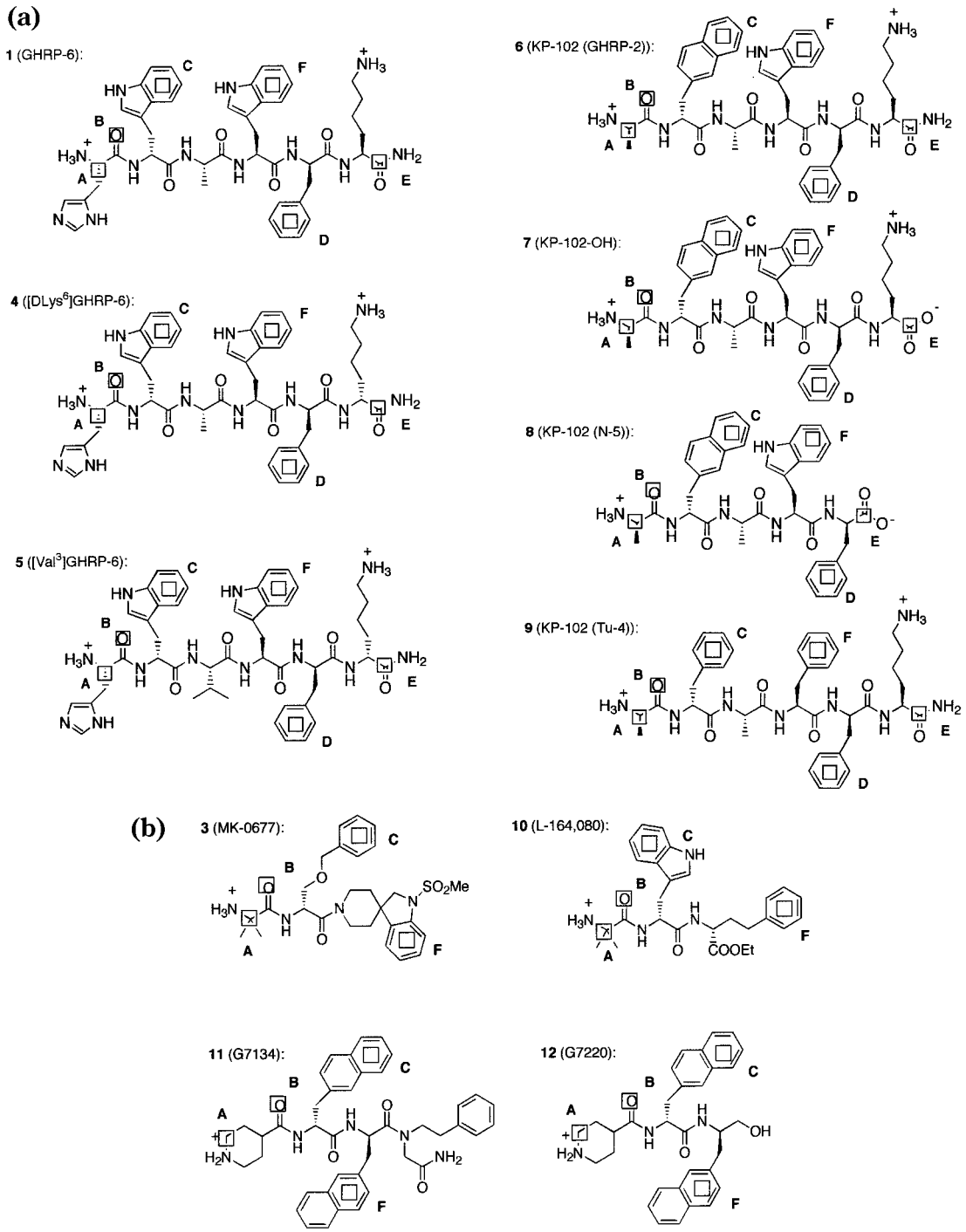


Figure 2. Known GH secretagogues selected for 3D pharmacophore studies: (a) six peptidyl agonists and one inactive peptide used as a negative control. Also shown in this figure are the six pharmacophoric sites (A−F) used for determination of a common 3D pharmacophore in peptidyl agonists and (b) four nonpeptidyl agonists. Also shown in this figure are the four pharmacophoric sites (A−C,F) used for determination of a common 3D pharmacophore for peptidyl and nonpeptidyl agonists.

entirely different molecular scaffolds may be explored to comply with it. The 3D databases used for search included MDDR (Molecular Design Drug Report) and NCI (National Cancer Institute) Databases using ISIS (MDL Information Systems, Inc., San Leandro, CA). Compounds obtained from database search were then evaluated and modified using the following criteria. (1) Each candidate compound was computationally assessed to verify that the 3D pharmacophore requirements were met by a low-energy conformer. (2) Since our goal was to discover novel and diverse new leads, scaffold novelty and chemical diversity as well as conformational rigidity were important in selecting compounds.

If the overlay of a candidate compound with the known agonists, such that all the pharmacophoric sites were superimposed, demonstrated a significant portion of the candidate compound occupying a region unoccupied by the known active compounds, this compound was then modified to eliminate the extra region, if its scaffold was of particular interest. In this process, synthetic feasibility was imposed for a more practical design. The criterion that we used in the compound modification step included the compliance with the 3D

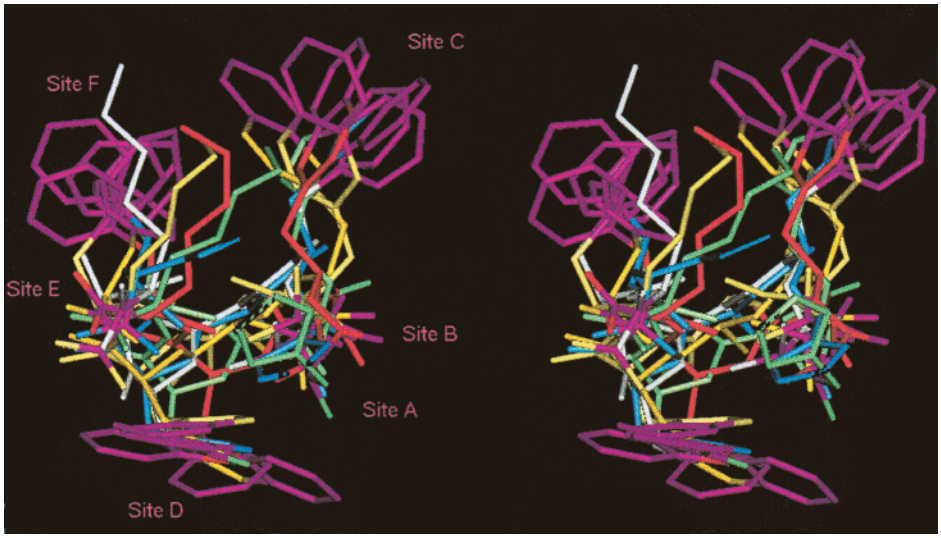


Figure 3. Stereoview showing the superimposition of the six peptides using the pharmacophoric sites (purple) shown in Figure 2a: (**1**) GHRP-6, green; (**4**) [DLys^6]GHRP-6, blue; (**6**) KP-102, yellow; (**7**) KP-102-OH, red; (**8**) KP-102(N-5), white; and (**9**) KP-102(Tu-4), lemon .

Table 3. Energy of the "Bioactive Conformation" Relative to the Lowest Energy in Each Agonist

compound	ΔE (kcal/mol)
GHRP-6	1.7
[D-Lys^6]GHRP-6	3.6
KP102	3.1
KP102−OH	5.3
KP102(N5)	2.6
KP102(Tu4)	3.5
G7134	3.2
G7220	4.0
MK-0677	2.1
L164080	2.4

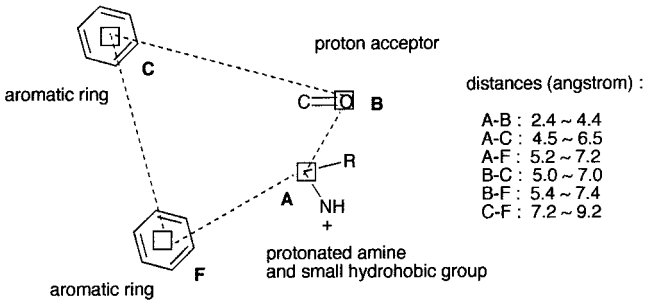


Figure 4. Common 3D pharmacophore for peptidyl and nonpeptidyl GH secretagogues. Shown in this figure are the four pharmacophoric sites and the common distances between them determined by DistComp. This 3D pharmacophore was used for rational design of novel GH secretagogues.

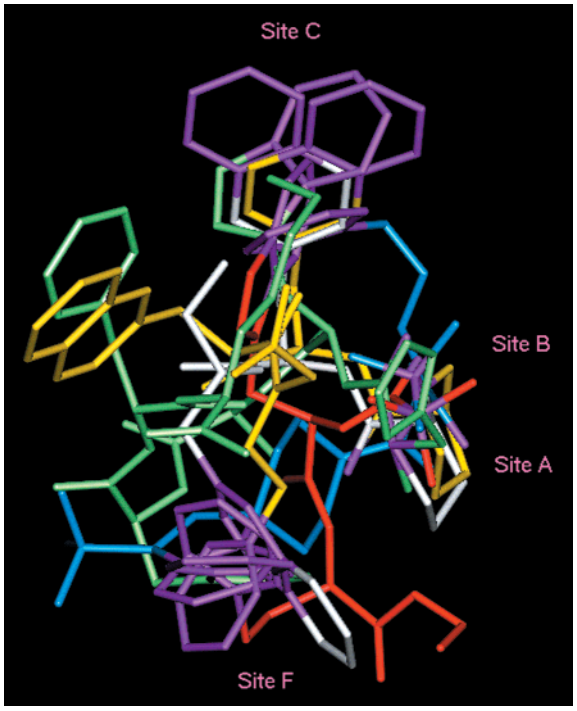


Figure 5. Overlay of four nonpeptidyl agonists, MK-0677 (**3**, blue), L164,080 (**10**, red), G7134 (**11**, yellow), and G7220 (**12**, white), with the peptide agonist GHRP-6 (**1**, green) using the pharmacophoric sites (purple) shown in Figure 4.

pharmacophore (Figure 4) and ease of synthesis. Using these strategies, several candidates were selected for synthesis and biological evaluation. Among these compounds, an initial new lead **16a** (Figure 6) with micromolar activity in GH release in rat pituitary cells was successfully discovered.

Development of a Novel Site-Dependent Fragment QSAR Program for Lead Optimization. The goal of the computational studies in this stage was to provide a rational basis for lead optimization to enhance the activity of the initial lead. To this end, a novel site-dependent fragment QSAR method was developed to explore whether there were any correlations between the activity and the physical and electronic properties of each pharmacophoric site that comprises the 3D pharmacophore. If so, the desired site-dependent properties could then be used as additional criteria for optimizing and refining compounds discovered on the basis of conformity to the 3D pharmacophore. This site-dependent fragment QSAR method was developed to further characterize compounds already determined to conform to a given 3D pharmacophore defined in a specific geometric arrangement of selected functional groups. To this end, the most useful capability built into the site-dependent fragment QSAR analysis was the identification and calculation of potentially relevant properties of each pharmacophoric site (i.e. site-depend-

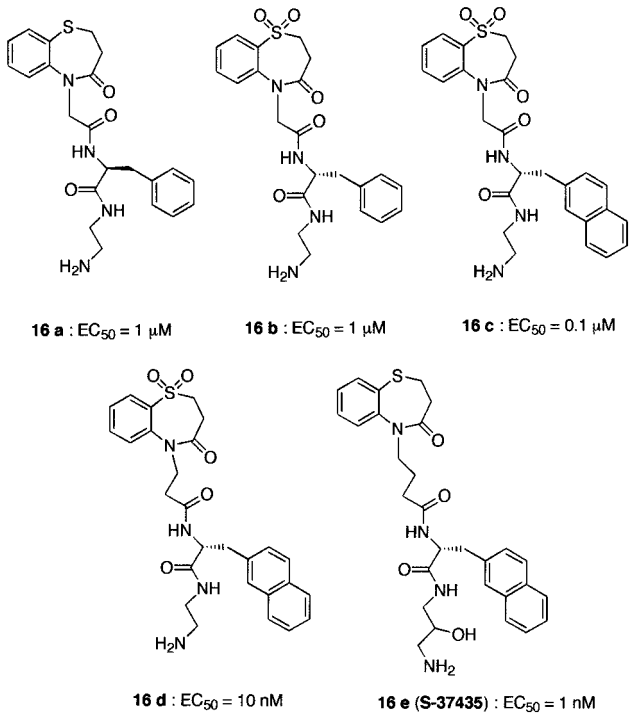


Figure 6. Chemical structures and GHS activities of novel benzothiazepin compounds.

ent properties) rather than the properties of the entire molecule. These properties could then be used in a regression analysis to identify the ones that modulate activity. Among the properties calculated for each site were the following: (1) regional net charge; (2) polarizability; (3) free energy of solvation; (4) van der Waals volume; (5) hydrophobicity; (6) proton donating capability, defined as the partial charge on the proton donor; and (7) proton accepting capability, defined as the partial charge on the proton acceptor. The definition of the pharmacophoric sites or fragments already identified by Distcomp was an important input to the site-dependent fragment QSAR analysis. These pharmacophoric sites are molecular fragments capable of making binding interactions such as amines, phenyl, or carbonyl groups and their substituents or connecting groups.

The results of the site-dependent fragment QSAR analysis for the six peptidyl agonists (Figure 2a) indicated that the hydrophobicities of the sites (C), (E), and (F) in Figure 2a were favored for activity, while the overall hydrophobicity of the entire molecule was, however, unfavorable. Therefore, to increase the GH secretion activity of compounds that agree with the peptide pharmacophore, hydrophobic (C), (E), and (F) sites but hydrophilic nature of the whole molecule were preferred. These results provided a useful guide for the modification of specific pharmacophoric sites for enhancing GHS activities.

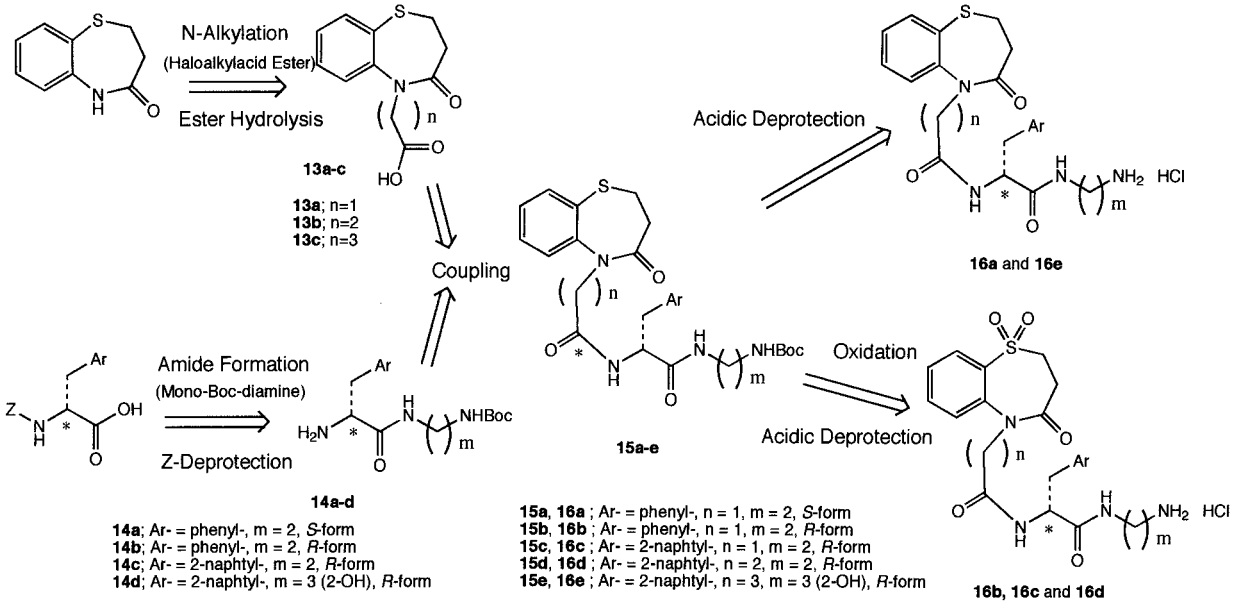
Chemistry

The lead compound **16a** was designed from the result of database searching using the strategy described above, and the extra regions, comparing to the known GH secretagogues in the overlay, were eliminated as much as possible. The syntheses of **16a** and its various analogues (**16b**−**e**) were fairly straightforward as shown in Scheme 1. Typical intermediates **13a**−**c** were coupled to several aromatic amino acid derivatives **14a**−**d** to give **15a**−**e**. The desired products **16a** and **16e** were afforded by an acidic deprotection. **16b**, **16c,** and **16d** were derived through an oxidation step before the deprotection, as shown in Scheme 1.

Results and Discussion on the Novel Benzothiazepin Compounds

As discussed above, the modeling and database search studies led to the discovery and synthesis of the initial lead **16a** (Figure 6). A complete conformational study of this molecule indicated that it fits well to the 3D pharmacophore shown in Figure 4. The four pharmacophoric sites (A−C, F), shown in Figure 7, conform to those in Figure 4. An overlay of this molecule with MK-0677 is shown in Figure 8, in which a low-energy conformation of **16a** (0.6 kcal/mol relative to the lowest energy) has a good superimposition with MK-0677.

Scheme 1. Preparation of Novel GH Secretagogues (**16a**−**e**)



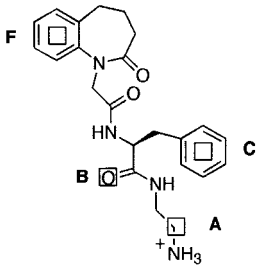


Figure 7. The four pharmacophoric sites in compound **16a** that are common to the pharmacophore shown in Figure 4.

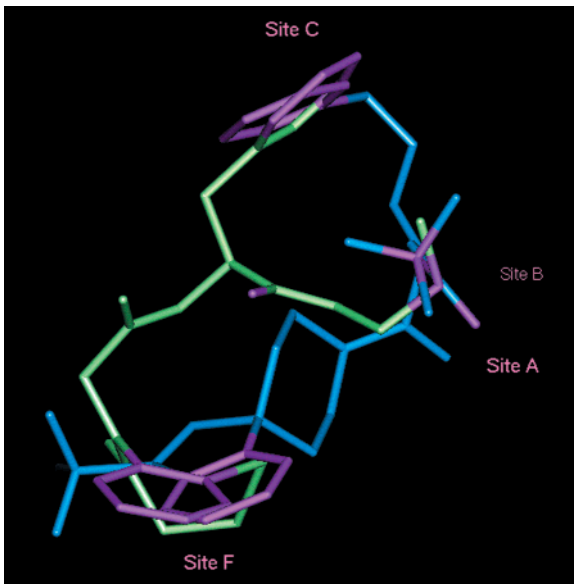


Figure 8. Overlay of compound **16a** (green) with MK-0677 (blue).

Also shown in Figure 6 are some analogues of **16a** and their GHS activities. **16b** differs from **16a** in the chiral configuration and the oxidation state of the sulfur atom. **16b** was synthesized with the considerations that a *R*-phenylalanine moiety (**16b**) would be more resistant to proteases than the *S*-configuration (**16a**) and that a sulfone group (**16b**) could be more stable in vivo than sulfur (**16a**). The modeling study indicated that both *R*- and *S*-chiral centers in this scaffold can conform to the 3D pharmacophore in Figure 4. In fact, **16b** showed similar activity to **16a** ($EC_{50} = 1\ \mu M$). A replacement of the phenyl ring in **16b** with a more hydrophobic naphthalene ring resulted in **16c** with a 10-fold increase in activity. This change was made based on the results of the site-dependent fragment QSAR analysis described earlier which suggested that the hydrophobicity of this ring, which is the site (C) in Figure 7 and in the pharmacophore (Figure 4), was favored for activity. A further modification of **16c** with the linkage one atom longer between the benzothiazepin and the phenylalanine moieties led to **16d** with another 10-fold increase in activity. A complete conformational study of an analogue similar to **16d** (compound not shown) demonstrated that with this longer linkage the molecule can still conform well to the 3D pharmacophore shown in Figure 4. To further improve potency, **16e** was synthesized to introduce a hydroxyl group to the diamine moiety thereby increasing the solubility of the molecule. Indeed, **16e** showed potent GHS activity ($EC_{50} = 1$ nM), consistent with the results of the site-dependent fragment QSAR studies which suggested that enhancing the hydrophilic nature of the whole molecule was favorable for activity. In addition, several analogues similar to **16e** were synthesized which retained nanomolar potency (data not shown).

The discovery of the initial lead (**16a**) and its analogues (**16b**−**e**) provided some new insights for the development of nonpeptidyl agonists: (1) the *N*-terminal moiety in peptidyl agonists (Figure 2a) can be replaced by a fairly simple aliphatic diamine; (2) a reversed peptide backbone, as in **16a**−**e**, is well-tolerated suggesting that side chain groups, not backbone amides, make important interactions with the receptors in these GHS compounds; and (3) compounds **16a**−**e** are potent GHSs with a novel benzothiazepin scaffold. The lead discovery strategy reported in this study is distinct from the usual approach of lead identification via high-throughput screens. The present study illustrates the successful use of 3D pharmacophore models and fragment QSAR analyses in drug design.

Conclusion

The computer-aided drug design strategies used in the present study have provided a crucial basis for the discovery of novel nonpeptidyl GH secretagogues incorporating a benzothiazepin scaffold. An in-house program DistComp was used to develop a 3D pharmacophore common to some known peptidyl and nonpeptidyl GH secretagogues. Database searches using this pharmacophore together with strategies used for compound evaluation and modification led to the discovery of a promising lead. A site-dependent fragment QSAR method was developed for fragmental property refinement. Compound modifications using these results as a guide led to the discovery and synthesis of agonists with nanomolar in vitro activity. The studies presented here provided an important rational basis for the development of novel potent peptidomimetics.

Experimental Section

Chemistry (General). Column chromatography was performed on silica gel 60 (Merck) unless otherwise noted. Mass spectra were recorded using a JEOL JMS-HX110A mass spectrometer. Proton magnetic resonance (^{1}H NMR: 270 MHz) spectra were recorded using a JEOL JNM EX-270 spectrometer and reported in ppm (δ) downfield from the internal standard tetramethylsilane.

General Procedure for the Preparation of 13a−c. 4-(4-Oxo-2,3-dihydro-1,5-benzothiazepin-5-yl)butanoic Acid (13c). To a mixture of 125 mg (0.70 mmol) of 4-oxo-2,3-dihydro-1,5-benzothiazepin and 280 mg (2.0 mmol) of K_2CO_3 in 3 mL of DMF was added at room temperature a solution of 195 mg (1.0 mmol) of 4-bromobutanoic acid ethyl ester in 2 mL of DMF. After the mixture was stirred overnight, the solvent was removed under vacuum, and then the resulting residue was diluted with 2 mL of EtOH. To this solution was added 2 mL of 1 N NaOH at 0 °C. The mixture was allowed to warm to room temperature, stirred for 3 h, and then concentrated under reduced pressure. The resulting residue was partitioned between 0.1 N HCl (15 mL) and EtOAc (15 mL), while cooling on ice. The organic layer was washed with brine (2 × 10 mL), dried over anhydrous sodium sulfate, filtered, and concentrated to dryness. The residue was chromatographed ($CHCl_3$:MeOH = 100:1) to isolate an ester form of **13c** (160 mg, 85%). Then to a solution of this ester in 5 mL of MeOH was added 1 mL of 1 N NaOH at 0 °C. After being stirred at 0 °C for 3 h, the reaction mixture was adjusted to pH 2 with 1 N HCl and then concentrated. The residue was diluted with 10 mL of

water and extracted with EtOAc (2 × 20 mL). The organic layer was dried (Na_2SO_4), filtered, and then concentrated to give **13c** (102 mg, 79%): ^{1}H NMR ($CDCl_3$) δ: 1.83 (m, 2H), 2.53 (m, 4H), 3.35 (m, 2H), 3.53 (m, 1H), 4.40 (m, 1H), 7.28 (m, 2H), 7.45 (m, 1H), 7.63 (d, J = 7.6 Hz, 1H); MS (FAB) m/z 266 $(M + H)^+$.

2-(4-Oxo-2,3,-dihydro-1,5-benzothiazepin-5-yl)ethanoic Acid (13a). A procedure similar to that above for **13c** employing 2-bromoethanoic acid methyl ester, instead of 4-bromobutanoic acid ethyl ester, gave **13a** (97%):^{1}H NMR ($CDCl_3$) δ: 2.68 (bs, 2H), 3.40 (bs, 2H), 4.06 (d, J = 14.5 Hz, 1H), 4.94 (d, J = 14.8 Hz, 1H), 7.05 (bs, 1H), 7.33 (m, 3H), 7.63 (d, J = 7.6 Hz, 1H); MS (FAB) m/z 238 $(M + H)^+$.

3-(4-Oxo-2,3-dihydro-1,5-benzothiazepin-5-yl)propionic Acid (13b). Similarly to the N-alkylation procedure for **13c** employing 2-propenoic acid *tert*-butyl ester, instead of 4-bromobutanoic acid ethyl ester, the ester intermediate was prepared to give the ester form of **13b**. The title compound **13b** was obtained by acidic (TFA) hydrolysis (85%): ^{1}H NMR ($CDCl_3$) δ: 2.58 (m, 3H), 2.88 (m, 1H), 3.35 (m, 2H), 3.70 (m, 1H), 4.53 (m, 1H), 7.30 (m, 2H), 7.48 (m, 1H), 7.61 (d, J = 7.9 Hz, 1H); MS (FAB) m/z 252 $(M + H)^+$.

General Procedure for the Preparation of 14a−d. *N*-[3-(tert-Butoxycarbonylamino)-2-hydroxypropyl]-2-(*R*)-amino-3-(naphthalen-2-yl)propionamide (14d). To a mixture of 1.1 g (3.1 mmol) of Cbz-D-naphthylalanine and 500 mg (2.6 mmol) of 1,1-dimethylethyl-*N*-(3-amino-2-hydroxypropyl)carbamate in 15 mL of DMF were added 530 mg (3.9 mmol) of HOBt and 720 mg (3.8 mmol) of EDC successively under cooling on ice. The mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was poured into 150 mL of saturated $NaHCO_3$. The resulting precipitate was collected by filtration and dried under reduced pressure to give 1.43 g of white powder. Then a solution of 1.43 g of the obtained powder in 15 mL of DMF was stirred under 4 atm of hydrogen in the presence of 400 mg of palladium-on-charcoal (10%: w/w) at 35 °C for 4 d. The reaction mixture was filtered to remove the catalyst, and the filtrate was concentrated by evaporation. The resulting residue was crystallized with *n*-hexane/EtOAc (1:1) to give 970 mg (2.5 mmol, 98%) of **14d**: ^{1}H NMR ($CDCl_3$) δ: 1.43 (s, 9H), 1.80 (bm, 3H), 3.16 (m, 6H), 3.74 (m, 2H), 5.17 (bs, 1H), 7.35 (d, J = 8.6 Hz, 1H), 7.46 (m, 2H), 7.65 (s, 1H), 7.80 (m, 4H); MS (FAB) m/z 388 $(M + H)^+$.

***N*-[2-(tert-Butoxycarbonylamino)ethyl]-2-(*S*)-amino-3-phenylpropionamide (14a).** A procedure similar to that above for **14d** employing Cbz-L-phenylalanine and 1,1-dimethylethyl-*N*-(2-aminoethyl)carbamate, instead of Cbz-D-naphthylalanine and 1,1-dimethylethyl-*N*-(3-amino-2-hydroxypropyl)-carbamate, gave **14a** (57%): ^{1}H NMR ($CDCl_3$) δ: 1.43 (s, 9H), 1.56 (bs, 2H), 2.70 (s, 1H), 3.25 (m, 3H), 3.35 (m, 2H), 3.63 (m, 1H), 4.87 (s, 1H), 7.28 (m, 5H), 7.52 (bs, 1H); MS (FAB) m/z 308 $(M + H)^+$.

***N*-[2-(tert-Butoxycarbonylamino)ethyl]-2-(*R*)-amino-3-phenylpropionamide (14b).** A procedure similar to that above for **14a** employing Cbz-D-phenylalanine, instead of Cbz-L-phenylalanine, gave **14b** (67%):^{1}H NMR ($CDCl_3$) δ: 1.43 (s, 9H), 1.69 (bs, 2H), 2.73 (s, 1H), 3.25 (m, 3H), 3.35 (m, 2H), 3.60 (m, 1H), 4.87 (s, 1H), 7.28 (m, 5H), 7.52 (bs, 1H); MS (FAB) m/z 308 $(M + H)^+$.

***N*-[2-(tert-Butoxycarbonylamino)ethyl]-2-(*R*)-amino-3-(naphthalen-2-yl)propionamide (14c).** A procedure similar to that above for **14b** employing Cbz-D-naphthylalanine, instead of Cbz-D-phenylalanine, gave **14c** (67%):^{1}H NMR ($CDCl_3$) δ: 1.43 (s, 9H), 1.56 (bs, 2H), 2.88 (s, 1H), 3.28 (m, 2H), 3.39 (m, 3H), 3.68 (m, 1H), 4.91 (bs, 1H), 7.36 (dd, J = 13.5, 13.5 Hz, 1H), 7.46 (m, 2H), 7.59 (bs, 1H), 7.65 (s, 1H), 7.80 (m, 3H); MS (FAB) m/z 356 $(M + H)^+$.

General Coupling Procedure for the Preparation of 15a−e. *N*-[1-(*R*)-(3-*tert*-Butoxycarbonylamino-2-hydroxypropylcarbamoyl)-2-(naphthalen-2-yl)ethyl]-4-(4-oxo-2,3-dihydro-1,5-benzothiazepin-5-yl)butyramide (15e). To a stirring solution of 210 mg (0.54 mmol) of **14d** in 3 mL of DMF were added 79 mg (0.30 mmol) of **13c**, 92 mg (0.68 mmol) of HOBt, and 125 mg (0.67 mmol) of EDC at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was poured into 40 mL of saturated aqueous sodium hydrogen carbonate. The resulting precipitate was collected by filtration and then dissolved in 5 mL of EtOAc. This solution was dried over anhydrous sodium sulfate, filtered, and then concentrated to dryness. Chromatography of the residue using $CHCl_3$/MeOH (100:1) gave **15e** (240 mg, 92%): ^{1}H NMR ($CDCl_3$) δ: 1.42 (s, 9H), 1.70 (m, 1H), 1.93 (m, 1H), 2.15 (m, 1H), 2.38 (m, 3H), 2.73 (m, 1H), 3.22 (m, 9H), 3.70 (m, 1H), 4.35 (m, 1H), 4.83 (m, 1H), 5.30 (m, 1H), 6.22 (m, 1H), 7.03 (m, 1H), 7.23 (m, 1H), 7.40 (m, 5H), 7.60 (d, J = 7.6 Hz, 1H), 7.67 (s, 1H), 7.82 (m, 3H); MS (FAB) m/z 635 $(M + H)^+$.

***N*-[1-(*S*)-(2-*tert*-Butoxycarbonylaminoethylcarbamoyl)-2-phenylethyl]-2-(4-oxo-2,3-dihydro-1,5-benzothiazepin-5-yl)acetamide (15a).** A coupling procedure similar to that above for **15e** employing **14a** and **13a**, instead of **14d** and **13c**, gave **15a** (93%): ^{1}H NMR ($CDCl_3$) δ: 1.43 (s, 9H), 2.63 (m, 2H), 3.20 (m, 8H), 4.05 (d, J = 16.5 Hz, 1H), 4.55 (d, J = 16.2 Hz, 1H), 4.75 (m, 1H), 5.70 (m, 1H), 6.99 (m, 5H), 7.28 (m, 1H), 7.45 (m, 2H), 7.65 (d, J = 7.6 Hz, 1H); MS (FAB) m/z 555 $(M + H)^+$.

***N*-[1-(*R*)-(2-*tert*-Butoxycarbonylaminoethylcarbamoyl)-2-phenylethyl]-2-(1,1,4-trioxo-2,3-dihydro-1,5-benzothiazepin-5-yl)acetamide (15b).** A coupling procedure similar to that above for **15a** employing **14b**, instead of **14a**, gave **15b** (96%): ^{1}H NMR ($CDCl_3$) δ: 1.43 (s, 9H), 2.68 (m, 2H), 3.22 (m, 8H), 4.05 (d, J = 15.5 Hz, 1H), 4.55 (d, J = 15.5 Hz, 1H), 4.75 (m, 1H), 5.70 (m, 1H), 6.98 (m, 5H), 7.29 (m, 1H), 7.40 (m, 2H), 7.65 (d, J = 7.6 Hz, 1H); MS (FAB) m/z 555 $(M + H)^+$.

***N*-[1-(*R*)-(2-*tert*-Butoxycarbonylaminoethylcarbamoyl)-2-(naphthalen-2-yl)ethyl]-2-(1,1,4-trioxo-2,3-dihydro-1,5-benzothiazepin-5-yl)acetamide (15c).** A coupling procedure similar to that above for **15b** employing **14c**, instead of **14b**, gave **15c** (98%): ^{1}H NMR ($CDCl_3$) δ: 1.43 (s, 9H), 2.61 (m, 2H), 3.29 (m, 8H), 4.00 (d, J = 16.8 Hz, 1H), 4.55 (d, J = 16.2 Hz, 1H), 4.85 (m, 1H), 5.70 (m, 1H), 7.07 (m, 3H), 7.40 (m, 6H), 7.62 (m, 1H), 7.75 (m, 1H); MS (FAB) m/z 577 $(M + H)^+$.

***N*-[1-(*R*)-(2-*tert*-Butoxycarbonylaminoethylcarbamoyl)-2-(naphthalen-2-yl)ethyl]-3-(1,1,4-trioxo-2,3-dihydro-1,5-benzothiazepin-5-yl)propionamide (15d).** A coupling procedure similar to that above for **15c** employing **13b**, instead of **13a**, gave **15d** (98%): ^{1}H NMR ($CDCl_3$) δ: 1.38 (s, 9H), 2.45 (m, 4H), 3.13 (m, 8H), 3.60 (m, 1H), 4.55 (m, 2H), 6.65 (m, 1H), 7.50 (m, 13H); MS (FAB) m/z 591 $(M + H)^+$.

General Deprotection Procedure for the Preparation of 16a and e. *N*-[1-(*R*)-(3-Amino-2-hydroxypropylcarbamoyl)-2-(naphthalen-2-yl)ethyl]-4-(4-oxo-2,3-dihydro-1,5-benzothiazepin-5-yl)butyramide Hydrochloride (16e). To a stirring solution of 80 mg (0.13 mmol) of **15e** in 2 mL of EtOAc was added 1 mL of 4 N HCl under cooling on ice−water and then stirring was continued at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure. To the oily residue was added 5 mL of ether, and the precipitated product was collected by filtration and dried under reduced pressure to give **16e** (69 mg, 92%) as a white solid: ^{1}H NMR (DMSO-d_6) δ: 1.43 (m, 2H), 2.05 (m, 2H), 2.43 (m, 3H), 2.85 (m, 2H), 3.23 (m, 6H), 3.72 (bs, 1H), 4.00 (bs, 1H), 4.55 (m, 1H), 5.55 (bs, 1H), 7.24 (t, J = 7.8 Hz, 1H), 7.40 (m, 5H), 7.58 (d, J = 7.3 Hz, 1H), 7.80 (m, 6H), 8.14 (d, J = 7.3 Hz, 1H), 8.23 (m, 1H); MS (FAB) m/z 535 $(M + H)^+$; HRFABMS calcd for $C_{29}H_{35}N_4O_4S$ (MH^+), 535.2379; found, 535.2391.

***N*-[1-(*S*)-(2-Aminoethylcarbamoyl)-2-phenylethyl]-2-(4-oxo-2,3-dihydro-1,5-benzothiazepin-5-yl)acetamide Hydrochloride (16a).** White solid: 93% from **15a**: ^{1}H NMR (DMSO-d_6) δ: 2.43 (m, 2H), 2.82 (m, 3H), 3.09 (m, 1H), 3.32 (m, 4H), 3.84 (m, 2H), 4.57 (bs, 2H), 7.23 (m, 7H), 7.40 (m, 1H), 7.59 (d, J = 6.3 Hz, 1H), 7.97 (bs, 2H), 8.33 (m, 2H); MS (FAB) m/z 427 $(M + H)^+$.

General Oxidation and Deprotection for the Preparation of 16b, c, and d. *N*-[1-(*R*)-(2-Aminoethylcarbamoyl)-2-phenylethyl]-2-(1,1,4-trioxo-2,3-dihydro-1,5-benzothiazepin-5-yl)acetamide Hydrochloride (16b). To a stirring solution of 110 mg (0.20 mmol) of **15b** in 2 mL of CH_2Cl_2 was added a solution of 195 mg (0.0.5 mmol) of MCPBA in 4 mL of CH_2Cl_2 under cooling on ice−water, and then stirring was continued at room temperature for 3 h. The reaction mixture was poured into 30 mL of saturated $NaHCO_3$ under cooling on ice−water, and then the organic layer was extracted by EtOAc (2 × 30 mL), dried over Na_2SO_4, filtered, and evaporated. Chromatography of the residue using $CHCl_3$/MeOH (100:1) gave an oil (110 mg, 94%). Then the similar procedure to this oil for preparing **16e** gave **16b** (445 mg, 90%) as a white solid: ^{1}H NMR (DMSO-d_6) δ: 2.45 (m, 1H), 2.60 (m, 1H), 2.83 (m, 3H), 3.11 (m, 1H), 3.33 (m, 3H), 3.83 (m, 2H), 4.55 (m, 2H), 7.27 (m, 6H), 7.58 (m, 1H), 7.75 (m, 1H), 7.90 (d, J = 7.6 Hz, 1H), 7.98 (bs, 2H), 8.26 (bs, 1H), 8.52 (d, J = 8.3 Hz, 1H); MS (FAB) m/z 459 $(M + H)^+$.

***N*-[1-(*R*)-(2-Aminoethylcarbamoyl)-2-(naphthalen-2-yl-)ethyl]-2-(1,1,4-trioxo-2,3-dihydro-1,5-benzothiazepin-5-yl)acetamide Hydrochloride (16c).** White solid: 79% from **15c**: ^{1}H NMR (DMSO-d_6) δ: 2.53 (m, 2H), 3.50 (m, 9H), 4.68 (m, 2H), 7.63 (m, 13H), 8.38 (m, 1H), 8.60 (d, J = 8.6 Hz, 1H); MS (FAB) m/z 509 $(M + H)^+$.

***N*-[1-(*R*)-(2-Aminoethylcarbamoyl)-2-(naphthalen-2-yl-)ethyl]-3-(1,1,4-trioxo-2,3-dihydro-1,5-benzothiazepin-5-yl)propionamide Hydrochloride (16d).** White solid: 85% from **15d**: ^{1}H NMR (DMSO-d_6) δ: 2.33 (m, 4H), 2.85 (m, 3H), 3.60 (m, 7H), 4.49 (m, 1H), 7.48 (m, 7H), 7.78 (m, 6H), 8.31 (bs, 2H); MS (FAB) m/z 509 $(M + H)^+$.

In Vitro Assay of GH Release from Rat Pituitary Cells. Compounds **16a**−**e** were evaluated by in vitro testing for the efficacy and potency to release growth hormone (GH) in rat primary anterior pituitary cells. Preparation of rat primary anterior pituitary cells was essentially the same as described by Chen et al.[24] Namely, rats were sacrificed by decapitation, and then the pituitary was quickly removed. The anterior pituitaries were digested with 0.2% collagenase, 0.2% hyarulonidase, and 200 U/mL DNase I in Hank's balanced salt solution. The cells were resuspended in Dulbecco's Modified Eagle's medium containing 7.5% horse serum, 5.0% fetal calf serum, 1.0% nonessential amino acids, 100 U/mL penicillin, and 100 μg/mL streptomycin and then adjusted to 1.0 × 10^5 cells/mL. The suspension (0.5 mL) was placed in each ell of 48-well trays and cultured for 3 days before the experiments.

On the experiments, cells were washed twice with the above medium containing 20 mM HEPES (pH 7.4). GH release was initiated by addition of the medium containing 20 mM HEPES and a test compound. Incubation was carried out for 15 min at 37 °C, and then released GH into the medium was measured by a standard radioimmunoassay (RIA) procedure. EC_{50} values were reported based upon the maximum GH level which resulted from 10^{-8} M KP-102.

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Supporting Information Available: Combustion analyses and HPLC data for peptides (**4**−**9**), which were prepared by a conventional solid-phase synthesis with using a peptide synthesizer (PSSM8, SHIMADZU Co., Kyoto, Japan), except for GHRP-6 which was purchased from BACHEM AG (Product No. H-9990.0005) and used as received. This material is available free of charge via the Internet at http://pubs.acs.org.

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