Oxindole Derivatives as Orally Active Potent Growth Hormone Secretagogues

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Received August 7, 2001

A series of substituted oxindole derivatives was synthesized and evaluated for growth hormone (GH) releasing activity using cultured rat pituitary cells. (+)-6-Carbamoyl-3-(2-chlorophenyl)-(2-diethylaminoethyl)-4-trifluoromethyloxindole (SM-130686, **37S**) was found to have potent activity (EC_{50} = 3.0 nM), while the other enantiomer **37R** had reduced activity. The absolute configuration of **37S** was confirmed by X-ray crystallographic analysis. Compound **37S** showed a good pharmacokinetic profile in rats with 28% oral bioavailability at 10 mg/kg and excellent in vivo activity as evidenced by a significant weight gain after 4 days of oral administration at 10 mg/kg twice a day. Compound **37S** displaced the binding of ^{35}S-MK-677 to human GHS-R with an IC_{50} value of 1.2 ± 0.2 nM.

Introduction

Growth hormone (GH), a 191-amino acid hormone, has important roles such as promotion of growth and acceleration of metabolic processes such as lipolysis and protein synthesis.[1] GH is released episodically from the pituitary gland, and its release is regulated by two hypothalamic hormones, growth hormone releasing hormone (GHRH), and somatostatin. GHRH stimulates GH release, whereas somatostatin inhibits GH release.[1] Recombinant human growth hormone (rGH) has been used for the treatment of GH deficiency in children over the past several decades.[2] In addition, it has shown beneficial effects in the treatment of patients with burns, bone fractures, and Turner's syndrome.[1,3,4] rGH also has a potential in reversing several effects of aging in the elderly.[5] However, the use of rGH has limitations such as its high cost, and it must be administered by injection due to its lack of oral efficacy. Therefore, there have been many efforts to identify orally active GH releasing agents.

In 1977, Bowers and co-workers reported that series of peptides derived from Met-enkephalin released GH from the pituitary gland[6] and later discovered that these growth hormone releasing peptides (GHRPs) stimulated the secretion of GH via a mechanism distinct from GHRH.[7] This discovery opened the possibility that these relatively small peptides, so-called GH secretagogues (GHSs),[8] might be alternatives to rGH. After continuous investigations in Bowers' group,[9] hexapeptide GHRP-6 (His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2) was identified as a potent GHS (EC_{50} = 10 nM) in 1984.[10,11] Although GHRP-6 was proved to have excellent activity and specificity not only in animal models[11,12] but also in human subjects,[13] its peptidyl nature limited its clinical use.[14]

Encouraged by the result that a small molecule such as GHRP-6 could stimulate the release of endogenous GH, many research groups have initiated studies for identifying peptidomimetic and nonpeptidic GHSs with improved pharmacokinetic properties. So far, several small molecule GHSs have been reported (Chart 1).[15,16] Among them, L-692,429 and MK-677 are noted as extensively studied GHSs. In 1993, a nonpeptidyl benzolactam compound L-692,429 (EC_{50} = 60 nM) was reported from Merck's group.[17] Although L-692,429 became the first nonpeptidyl GHS to be evaluated in humans,[18] its oral bioavailability in dog was less than 5%.[19] The spiropiperidine compound MK-677 was reported as the first orally active GHS in 1995.[20] MK-677 had strong activity in vitro (EC_{50} = 1.3 nM) and elevated GH levels in beagles after oral doses as low as 0.125 mg/kg.[20] Furthermore, in 1996, the same group also succeeded in cloning the GHS receptor (GHS-R).[21] The GHS-R was found to be a G-protein coupled receptor and expressed in the pituitary gland and in other areas.[21,22] Thus, it is now clear that synthetic GHSs release GH through the GHS-R.

These reports stimulated us to search for a new GHS by means of a GH releasing assay using cultured rat pituitary cells.[23] Herein, we report the preparation and biological activities of the nonpeptidyl oxindole GHS **37S** (SM-130686) and related analogues.[24] These oxindole compounds are orally active and have a novel structure totally different from other GHSs reported so far.

A recent major advance in this field was the discovery of an endogenous ligand for the GHS-R. In 1999, Kangawa et al. isolated a novel acylated peptide, designated as ghrelin, from the stomach, which had strong affinity to the GHS-R and stimulated GH release after intravenous administration to rats.[25] This discov-

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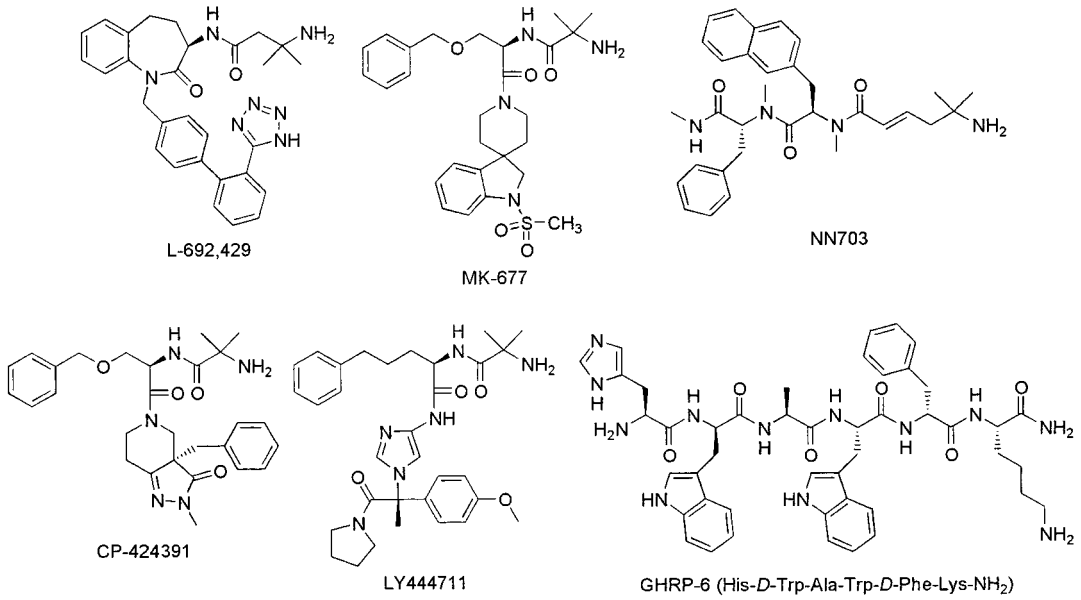
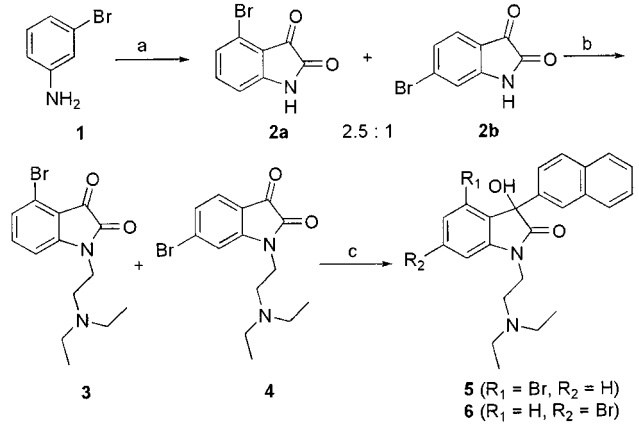
10.1021/jm0103763 CCC: $20.00
Published on Web 11/16/2001

Chart 1. Structures of Small Molecule GHSs

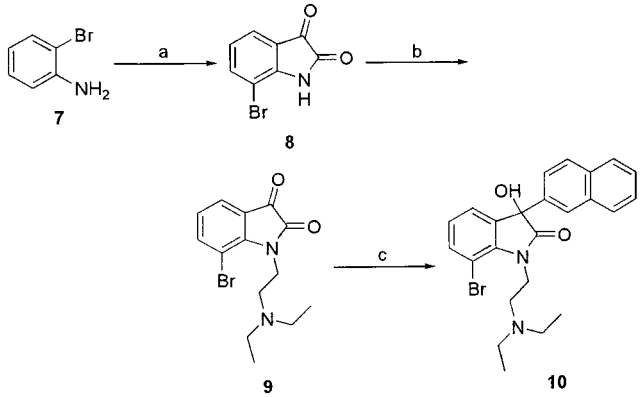


Scheme 1[a]



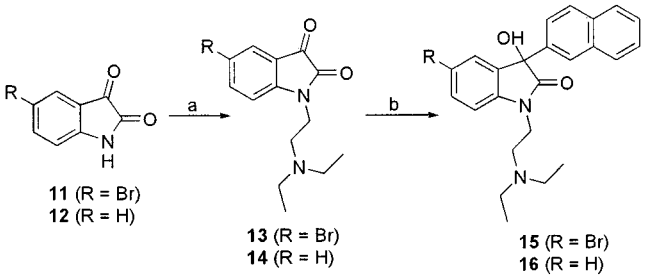
[a] Reagents and conditions: (a) 1. $NH_2OH{\cdot}HCl$, $CCl_3CHO{\cdot}H_2O$, Na_2SO_4, HCl, H_2O, reflux, 2. H_2SO_4, 80 °C, 64% (2 steps); (b) NaH, $Cl(CH_2)_2NEt_2{\cdot}HCl$, DMF, 60 °C, 38% for **3**, 15% for **4**; (c) 2-naphthylMgBr, THF, rt, 48% for **5**, 62% for **6**.

Scheme 2[a]



[a] Reagents and conditions: (a) 1. $NH_2OH{\cdot}HCl$, $CCl_3CHO{\cdot}H_2O$, Na_2SO_4, HCl, H_2O, reflux, 2. H_2SO_4, 80 °C, 41% (2 steps); (b) NaH, $Cl(CH_2)_2NEt_2{\cdot}HCl$, DMF, 60 °C, 38%; (c) 2-naphthylMgBr, THF, rt, 56%.

Scheme 3[a]



[a] Reagents and conditions: (a) NaH, $Cl(CH_2)_2NEt_2{\cdot}HCl$, DMF, 60 °C, 31% for **13**, 64% for **14**; (b) 2-naphthylMgBr, THF, rt, 56% for **15**, 42% for **16**.

ery could unveil as yet unidentified new therapeutic uses of SM-130686 as well as other GHSs.

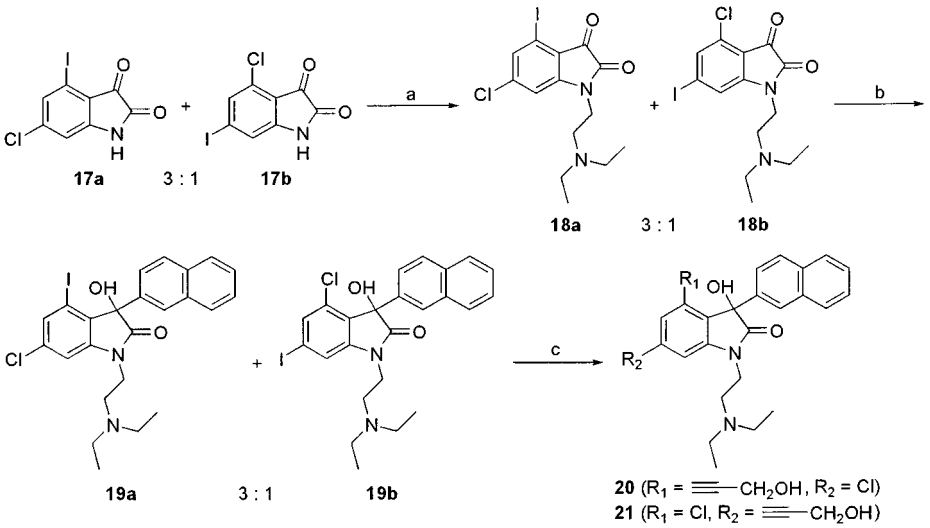
Chemistry

Compounds **5** and **6** were synthesized as shown in Scheme 1. The key intermediary isatins were prepared according to the Sandmeyer procedure. Reaction of *m*-bromoaniline with chloral hydrate in the presence of hydroxylamine followed by heating in concentrated sulfuric acid gave an inseparable regioisomeric mixture of **2a** and **2b** in a 2.5:1 ratio in 64% yield. *N*-Alkylation of the mixture with 2-diethylaminoethyl chloride, using sodium hydride as a base, in DMF afforded **3** and **4** in 38% and 15% yield, respectively, which were easily separated by silica gel column chromatography. Addition of 2-naphthylmagnesium bromide to either **3** or **4** afforded 4-bromooxindole **5** or 6-bromooxindole **6** in 48% and 62% yield, respectively.

In a similar way, regioisomeric 7-bromooxindole **10**, 5-bromooxindole **15**, and unsubstituted oxindole **16** were synthesized from *o*-bromoaniline **7**, commercially available 5-bromoisatin **11** and isatin **12**, respectively, according to Schemes 2 and 3.

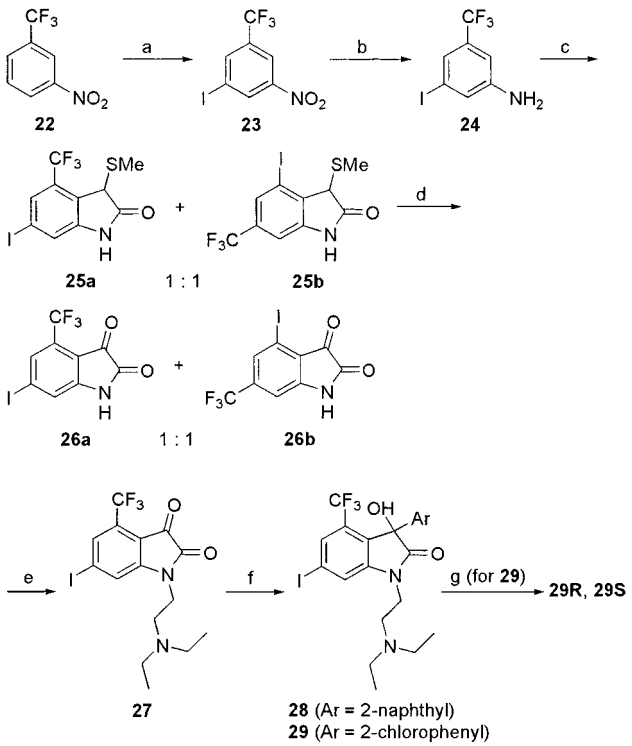
Scheme 4 depicts the preparation of **20** and **21**. An inseparable mixture of *N*-alkylated oxindoles **19a** and **19b** were obtained in a 3:1 ratio from a 3:1 mixture of **17a** and **17b**[26] by a method similar to the synthesis of **5**. Sonogashira coupling[27] of a mixture of **19a** and **19b** with propargyl alcohol in the presence of a catalytic

Scheme 4[a]



[a] Reagents and conditions: (a) NaH, $Cl(CH_2)_2NEt_2$·HCl, DMF, 60 °C, 49%; (b) 2-naphthylMgBr, THF, rt, 79%. (c) propargyl alcohol, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, 50 °C, 44% for **20**, 16% for **21**.

Scheme 5[a]



[a] Reagents and conditions: (a) NIS, H_2SO_4, 50 °C, 21%; (b) $SnCl_2$·H_2O, EtOH, reflux 80%; (c) 1. SO_2Cl_2, $MeSCH_2CO_2Et$, Et_3N, CH_2Cl_2 −78 °C then rt, 2. 1 N HCl, Et_2O, rt, 86% (2 steps); (d) $CuCl_2$, CuO, acetone, rt, 77%; (e) NaH, $Cl(CH_2)_2NEt_2$·HCl, DMF, 60 °C, 27%; (f) for **28**, 2-naphthylMgBr, THF, rt, 78%; for **29**, 2-chlorophenylMgBr, toluene, Et_2O, rt, 55%; (g) optical resolution, Chiralpak OD.

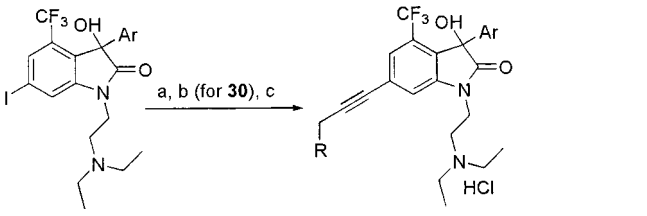
amount of dichlorobistriphenylphosphine palladium and copper iodide in a mixed solvent of triethylamine and THF gave separable oxindoles **20** and **21** in 44% and 16% yield, respectively.

Scheme 5 illustrates the synthesis of a series of 6-iodo-4-trifluoromethyloxindoles. For the synthesis of 3-iodo-5-trifluoromethylaniline **24**, we utilized the Gasmann reaction,[28] since the cyclization of **24** using the Sandmeyer procedure appeared to be difficult owing to the electron deficient nature of the aromatic ring. Regiospecific iodination of 3-trifluoromethylnitrobenzene **22** was accomplished by a modification of Olah's method[29] using *N*-iodosuccimide in concentrated sulfuric acid to provide **23** in 21% yield, which was then reduced to **24** by treatment with $SnCl_2$ in refluxing ethanol in 80% yield. Addition of sulfuryl chloride, ethyl methylthioacetate, and triethylamine successively to **24** at −78 °C in dichloromethane gave a mixture of 3-methylthiooxindoles **25a** and **25b**, which was then oxidized by cupric chloride and cupric oxide to generate the inseparable isatins **26a** and **26b** in a 1:1 ratio in 81% overall yield. After *N*-alkylation of the mixture of **26a** and **26b**, the desired 6-iodo-4-trifluoromethylisatin **27** and the other regioisomer, 4-iodo-6-trifluoromethylisatin, were isolated in 27% and 29% yield, respectively, by silica gel column chromatography. Addition of 2-naphthylmagnesium bromide or 2-chlorophenylmagnesium bromide to **27** provided oxindole **28** or **29**, respectively. These oxindoles served as versatile intermediates for derivatives with a substituent at the C-6 position. To examine the stereochemical requirement at the C3 position, optical resolution of **29** into **29R** and **29S** using preparative HPLC on a chiral stationary column was performed.

Scheme 6 shows the preparation of C-6 substituted derivatives. Compounds **30**−**33** were obtained using Sonogashira coupling of **28** or **29** with the corresponding alkynes. In the synthesis of **30**, subsequent deprotection of the silyl group was performed by treatment with aqueous hydrogen fluoride in acetonitrile.

Scheme 7 depicts the synthesis of C-6 carbamoyl compounds. Compounds **28** and **29** were treated with zinc cyanide in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium in DMF at 80 °C followed by hydrolysis of the resulting nitriles **34** and **35** with potassium hydroxide in anhydrous *tert*-butyl alcohol at 50 °C[30] to give **36** and **37**, respectively. Similarly, both enantiomers of **37**, **(+)-37S** and **(−)-37R**, were obtained from **(+)-29S** and **(−)-29R**, respectively. The absolute configuration at the C-3 position of **(+)-**

Scheme 6[a]

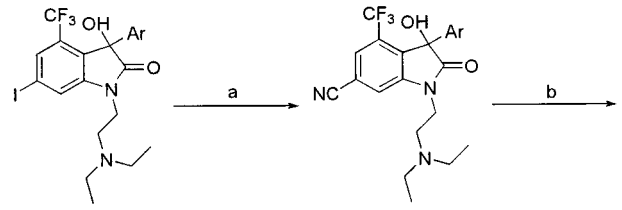


28 or **29**

30 (R = OH, Ar = 2-naphthyl)
31 (R = CH_2CON-$(CH_2)_2O(CH_2)_2$-, Ar = 2-naphthyl)
32 (R = CH_2CONH_2, Ar = 2-naphthyl)
33 (R = CH_2CON-$(CH_2)_2O(CH_2)_2$-, Ar = 2-chlorophenyl)

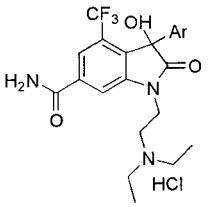
[a] Reagents and conditions: (a) acetylene derivatives (1-*tert*-butyldimethylsilyloxy-2-propyne for **30**, 4-morpholinocarbonyl-1-butyne for **31** and **33**, 4-carbamoyl-1-butyne for **32**), $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, THF, 50 °C; (b) aq HF, CH_3CN, rt; (c) HCl, dioxane, rt, 66% for **30** (3 steps), 56% for **31** (2 steps), 48% for **32** (2 steps), 61% for **33** (2 steps).

Scheme 7[a]



28 (Ar = 2-naphthyl)
29 (Ar = 2-chlorophenyl)
29R
29S

34 (Ar = 2-naphthyl)
35 (Ar = 2-chlorophenyl)
35R
35S



36 (Ar = 2-naphthyl)
37 (Ar = 2-chlorophenyl)
37R
37S

[a] Reagents and conditions: (a) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 60 °C, 80% for **34**, 64% for **35**, 60% for **35R**, 71% for **35S**; (b) 1. powdered KOH, *tert*-butyl alcohol, 50 °C, 2. HCl, dioxane rt, 95% for **36** (2 steps), quant. for **37** (2 steps), quant. for **37R** (2 steps), quant. for **37S** (2 steps).

37S was confirmed to be *S* by X-ray crystallographic analysis of nitrile compound **(+)-35S** as shown in Figure 1.

Results and Discussion

Structure−Activity Relationships. In the course of our study aimed at generating an orally active GH secretagogue, we found that an oxindole derivative in our chemical library showed activity in the in vitro GH releasing assay using rat pituitary cells.[23] After initial modifications of that derivative, we found that 6-bromo-1-diethylaminoethyl-3-(2-naphthyl)oxindole **6** exhibited reproducible activity with an EC_{50} of 380 nM. Since the chemical structure of **6** is totally different from any other known GHSs, we were encouraged to synthesize various derivatives of **6** and evaluated them for their GH releasing activity, the results of which are summarized in Table 1.

In the beginning of the study, we prepared unsubstituted oxindole **16** and the mono bromo substituted

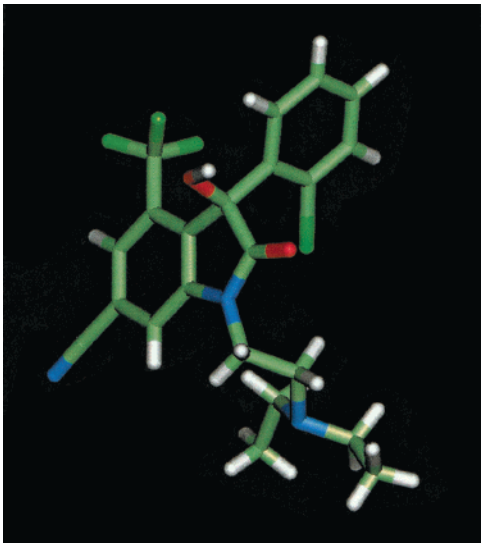
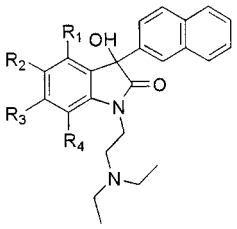


Figure 1. X-ray structure of **35S**

Table 1. Growth Hormone Releasing Activities of the Oxindole Derivatives[a]

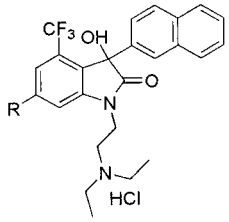


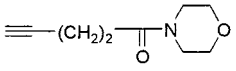
compound	R_1	R_2	R_3	R_4	EC_{50} (nM)[b]	clogP
5	Br	H	H	H	77	5.12
15	H	Br	H	H	> 1000	5.23
6	H	H	Br	H	380	5.12
10	H	H	H	Br	> 1000	4.79
16	H	H	H	H	> 1000	4.22
20	≡—CH_2OH	H	Cl	H	> 1000	5.05
21	Cl	H	≡—CH_2OH	H	310	5.05
30	CF_3	H	≡—CH_2OH	H	310	5.47

[a] The growth hormone releasing activities were measured in rat pituitary cells. [b] The values were means of more than two independent experiments and were normalized for MK-677 (1.3 nM).

oxindoles (**5**, **10**, **15**). 5- or 7-Bromooxindoles (**15**, **10**) and **16** resulted in reduced potency, whereas 4-bromooxindole **5** increased activity (EC_{50} = 77 nM), compared to **6**. This indicated that both the C-4 and C-6 positions could be further modified. The major goals of the modifications were not only to increase the activity but also to reduce the lipophilicity, since the lead compound **6** had a relatively high clogP value (5.12), which would be unfavorable for an oral bioavailability.[31] A versatile intermediate for introducing a hydrophilic substituent into either the C-4 or C-6 position would be the corresponding iodoisatin derivatives, which could readily be functionalized using Pd catalyzed coupling reactions. Although such iodo-derivatives are generally not easily accessible, we fortunately had a mixture of 6-chloro-4-iodoisatin **17a** and 4-chloro-6-iodoisatin **17b**[26] in hand. First of all, we prepared propargyl alcohol derivatives **20** and **21** using the mixture of **17a** and **17b**. Compound **20** showed only weak activity (EC_{50} > 1000

Table 2. Growth Hormone Releasing Activities of the Oxindole Derivatives[a]



compound	R	EC_{50} (nM)[b]	clogP
31		30 ± 13	6.03
32	$-C\equiv C-(CH_2)_2CONH_2$	30 ± 23	5.70
36	$CONH_2$	18 ± 10	4.47

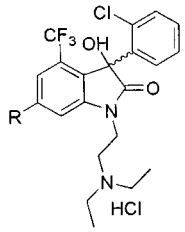
[a] The growth hormone releasing activities were measured in rat pituitary cells. [b] The values were means of more than two independent experiments and were normalized for MK-677 (1.3 nM).

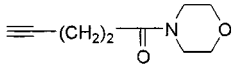
nM), whereas compound **21** retained the activity (EC_{50} = 310 nM), indicating that introduction of a hydrophilic group into the C6 region could be tolerated. Thus, 4-chloro-6-iodoisatin could serve as a key intermediate for the structure and activity relationship (SAR) study. However, we found that 4-chloro-6-iodoisatin and even 4-chloro-6-iodooxindole were difficult to separate from the regioisomeric by simple column chromatography, so that it could not be used for that purpose. Next, we prepared 1-diethylaminoethyl-6-iodo-4-trifluoromethylisatin **27**. Fortunately, this was easily separated from the isomer 1-diethylaminoethyl-4-iodo-6-trifluoromethylisatin, and more importantly, compound **30** showed comparable activity (EC_{50} = 310 nM) to **21**. These results encouraged us to investigate 6-substituted-4-trifluoromethyloxindole derivatives using 1-diethylaminoethyl-6-iodo-4-trifluoromethylisatin **27** as an intermediate.

Table 2 summarizes the GH releasing activity of the 6-substituted series. First, we utilized Sonogashira coupling to introduce various hydrophilic groups through a triple bond linkage into the oxindole ring. Amide derivatives **31** (EC_{50} = 30 nM) and **32** (EC_{50} = 30 nM) showed enhanced activities compared to **30** and provided a 10-fold increase in potencies. Next, we synthesized carbamoyl compound **36**, since we calculated that a carbamoyl group attached directly to the oxindole ring would reduce the clogP value. For example, **31** had a clogP value with 6.03, whereas the clogP value of **36** is 4.47. Gratifyingly, **36** also displayed the most potent activity (EC_{50} = 18 nM) among the 6-substituted derivatives tested.

The naphthyl group is relatively lipophilic and we replaced the naphthyl ring with other less lipophilic aromatic rings. Among several aromatic rings, 2-chlorophenyl was found to be optimal in terms of potency and reducing the clogP value. We found that 2-chlorophenyl derivative **33** exhibited more potent GH releasing activity (EC_{50} = 3.7 nM, clogP = 5.40) than **31** as shown in Table 3. Similarly to the 2-naphthyl series, 6-carbamoyl compound **37** showed the strong activity (EC_{50} = 3.5 nM, clogP = 3.83). Each enantiomer of 6-carbamoyl compound **37** was prepared and (+)-isomer **37S** was found to retain the potency (EC_{50} = 3.0 nM).

Table 3. Growth Hormone Releasing Activities of the Oxindole Derivatives[a]



compound	R	EC_{50} (nM)[b]	clogP
33		3.7 ± 1.1	5.40
37	$CONH_2$	3.5	
37S	$CONH_2$	3.0 ± 1.6 (6.3 ± 3.4)[c]	3.83
37R	$CONH_2$	210	

[a] The growth hormone releasing activities were measured in rat pituitary cells. [b] The values were normalized for MK-677 (1.3 nM). [c] Unnormalized value.

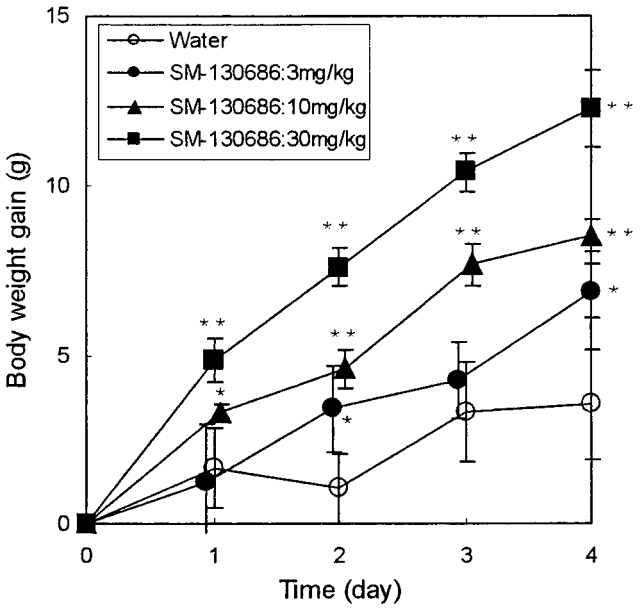


Figure 2. Effect of SM-130686 on the body weight gain. SM-130686 was orally administered twice a day every day for 4 days in normal female rats. Data are represented as means ± SEM (n = 6). *P < 0.025; *P < 0.005; **P < 0.01, Shirley-Williams test (vs 0 mg/kg treatment group).

On the other hand, (−)-isomer **37R** showed reduced potency (EC_{50} = 210 nM). The absolute configuration of the more active isomer was revealed to be "*S*" as determined by the X-ray crystallographic analysis of precursor **35S**.

In Vivo Efficacy and Pharmacokinetics. Compound **37S** was selected and was evaluated for in vivo activity. Administration of **37S** orally increased the weight in a dose dependent manner after 4 days in rats. When **37S** was administered at 3, 10, and 30 mg/kg twice a day, the body weight gains were 6.9, 8.9, and 12.3 g, respectively, whereas the distilled water administered group showed a weight gain of 3.5 g (Figure 2). In addition to the significant effect on the weight gain, **37S** was found to show excellent profile in continuous studies. The body weight gain in the **37S** administered

group was almost equivalent to the fat free mass gain. After administration of **37S** at 10 mg/kg orally twice a day for 9 days in rats, there were significant increases in both the body weight and the lean body mass (19.5 ± 2.1 and 18.0 ± 7.5 g, respectively).[32] This result indicates that **37S** promotes growth by accelerating anabolic effects through GH secretion and is intriguing because endogenous GHS ghrelin was reported to increase the fat mass gain.[33]

In a series of in vitro studies, **37S** was proven to have the same mechanism of action in terms of GH release as the other GHSs. It displaced the binding of [^{35}S]MK-677[34] to human GHS-R[21] expressed in CHO cells in a dose-dependent manner with an IC_{50} value of 1.2 ± 0.2 nM. The in vitro specificity of **37S** was evaluated in over 50 receptor and enzyme assays including adrenergic, angiotensin, bradykinin, cholecystokinin, dopamine, GABA, histamine, muscarinic, nicotinic acetylcholine, opiate, serotonin somatostatin, and vasopressin receptors, and no significant binding was observed at 1 μM.

Compound **37S** was evaluated in a rat pharmacokinetic study. After an oral dose of 10 mg/kg to rats, C_{max} was 274 ng/mL with a T_{max} of 0.4 h and the bioavailability was 28%. When intravenously administered to rats (5 mg/kg), Cl, Vd, and $t_{1/2}$ were 119 mL/min/kg, 9.9 L/kg and 1.5 h, respectively.

Conclusion

We have successfully synthesized a new class of GH secretagogue oxindole derivatives. Among them, **37S** (SM-130686) showed the same range of potencies as MK-677 in vitro, although the structure was different from that of MK-677. Compound **37S** (SM-130686) also increased the body weight gain after oral administration to rats. Recently, an endogenous ligand for GHS-R, ghrelin, was identified and showed strong GH releasing activity.[25] More recently, adenosine was discovered as another endogenous ligand for GHS.[35] Although Ca^{2+} concentration in the cells was elevated by adenosine similarly to the case of ghrelin, it does not exhibit any GH releasing property in pituitary cells.[35] Thus the complexity of the GHS-R has emerged, and unknown roles of GHS-R by these natural ligands may soon be elucidated. Further study indicated that **37S** (SM-130686) might be a partial agonist to GHS-R,[32] and the more detailed pharmacological evaluation of **37S** and its derivatives is now in progress in our laboratory.

Experimental Section

General Notes. Melting points were measured on either a Thomas-Hoover or a Yanaco melting point apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a JEOL GX-270 or Bruker AVANCE 400 spectrometers using tetramethylsilane as an internal standard. LC mass spectra were obtained on a PE SCIEX API 150EX spectrometer. Elemental analyses, low-resolution mass spectra, and high-resolution mass spectra were obtained from Sumitomo Analytical Center, Inc. Thin-layer chromatography and flash column chromatography was performed on silica gel glass-backed plates (5719, Merck & Co.) and silica gel 60 (230−400 or 70−230 mesh, Merck & Co.), respectively. Optical rotations were measured on a JASCO DIP-370. Optical purity was determined by HPLC using Hitachi L 6000 pump and L 4000 UV detector with a chiral column (Chiralpak AD, Daicel).

4-Bromoisatin (2a) and 6-Bromoisatin (2b). To a solution of trichloroacetaldehyde monohydrate (15.8 g, 95.5 mmol) in H_2O (200 mL) were successively added sodium sulfate (21.8 g, 153 mmol), 3-bromoaniline (**1**, 8.00 mL, 73.5 mmol) in a mixture of H_2O (75 mL) and 36% HCl (6.5 mL, 78.0 mmol), and a solution of hydroxylamine hydrochloride (19.0 g, 273 mmol) in H_2O (85 mL) with vigorous stirring. After the addition was completed, the reaction mixture was heated at reflux for 10 min and allowed to cool to room temperature. The precipitate formed was collected by filtration, washed with H_2O, and then dried in vacuo to yield the crude isonitrosoacetanilide. This product was added portion-wise to rapidly stirred concentrated sulfuric acid (250 mL) at a rate to keep the reaction temperature between 50 and 70 °C. The resulting solution was stirred at 80 °C for 10 min and allowed to cool to room temperature. The cooled mixture was poured carefully onto crushed ice (ca. 900 g). The mixture was allowed to stand for 1 h. The orange precipitate was collected by filtration, washed with H_2O, and then dried to yield a 2.5:1 mixture of **2a** and **2b** (10.6 g, 64%). **2a**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 11.17 (brs, 1 H), 7.46 (dd, 1 H, J = 8.0, 8.0 Hz), 7.22 (dd, 1 H, J = 0.7, 8.0 Hz), 6.89 (dd, 1 H, J = 0.7, 8.0 Hz). **2b**: ^{1}H NMR (270 MHz, $CDCl_3$) δ 11.10 (brs, 1 H), 7.47 (d, 1 H, J = 7.9 Hz), 7.26 (dd, 1 H, J = 1.7, 7.9 Hz), 7.08 (d, 1 H, J = 1.7 Hz). MS m/z 225 (M^+).

1-(2-Diethylaminoethyl)-4-bromoisatin (3) and 1-(2-Diethylaminoethyl)-6-bromoisatin (4). To a solution of a 2.5:1 mixture of **2a** and **2b** (2.25 g, 9.95 mmol) in DMF (30 mL) was added sodium hydride (60% in oil, 960 mg, 24.0 mmol) with stirring at 0 °C, followed by addition of 2-diethylaminoethyl chloride hydrochloride (2.05 g, 11.9 mmol). The mixture was stirred at 60 °C for 2 h and cooled to room temperature. Water was then added, and the mixture was extracted with a mixture of toluene and ethyl acetate (1:1). The organic phase was washed with brine, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (AcOEt) to give **3** (1.22 g, 38%) and **4** (478 mg, 15%). **3**: R_f 0.21 (AcOEt); LC-Ms m/z 327 (M^+ + 3), 325 (M^+ + 1); ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.44 (d, 1 H, J = 7.7 Hz), 7.25 (dd, 1 H, J = 1.5, 7.7 Hz), 7.17 (d, 1 H, J = 1.5 Hz), 3.77 (t, 2 H, J = 6.6 Hz), 2.69 (t, 2 H, J = 6.6 Hz), 2.55 (q, 4 H, J = 7.1 Hz), 0.97 (t, 6 H, J = 7.1 Hz); HRMS calcd for $C_{14}H_{18}BrN_2O_2$ 325.0552, found 325.0580. **4**: R_f 0.42 (AcOEt); mp 62−64 °C; LC-MS m/z 327 (M^+ + 3), 325 (M^+ + 1); ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.39 (dd, 1 H, J = 7.8, 7.8 Hz), 7.23 (d, 1 H, J = 7.8 Hz), 6.90 (d, 1 H, J = 7.8 Hz), 3.80 (t, 2 H, J = 6.8 Hz), 2.68 (t, 2 H, J = 6.8 Hz), 2.56 (q, 4 H, J = 7.1 Hz), 0.97 (t, 6 H, J = 7.1 Hz); Anal. ($C_{14}H_{17}BrN_2O_2$): C, H, N.

4-Bromo-1-(2-diethylaminoethyl)-3-hydroxy-3-(2-naphthyl)oxindole (5). To a solution of **3** (205 mg, 0.631 mmol) in anhydrous THF (3.0 mL) was added freshly prepared 1.0 N 2-naphthylmagnesium bromide in THF (1.2 mL, 1.20 mmol) at room temperature. The reaction mixture was stirred overnight. Saturated aqueous $NaHCO_3$ was added, and the mixture was extracted with ethyl acetate, and then washed with brine. The organic layer was dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$−MeOH, 60:1, then 30:1) to give the title compound (134 mg, 48%): mp 158−162 °C; LC-MS m/z 455 (M^+ + 3), 453 (M^+ + 1); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.00 (d, 1 H, J = 1.6 Hz), 7.75 ∼ 7.85 (m, 3 H), 7.44 ∼ 7.50 (m, 2 H), 7.34 (dd, 1 H, J = 2.0, 8.6 Hz), 7.19 ∼ 7.30 (m, 2 H), 6.97 (dd, 1 H, J = 1.0, 7.6 Hz), 3.61 ∼ 3.93 (m, 2 H), 2.72 (t, 2 H, J = 6.9 Hz), 2.57 (q, 4 H, J = 7.1 Hz), 0.97 (t, 6 H, J = 7.1 Hz); HRMS calcd for $C_{24}H_{26}BrN_2O_2$ 453.1178, found 453.1167.

6-Chloro-1-(2-diethylaminoethyl)-3-hydroxy-4-(3-hydroxy-1-propynyl)-3-(2-naphthyl)oxindole (20) and 4-Chloro-1-(2-diethylaminoethyl)-3-hydroxy-6-(3-hydroxy-1-propynyl)-3-(2-naphthyl)oxindole (21). To a mixture of **19a** and **19b** (198 mg, 0.370 mmol) and dichlorobis(triphenylphosphine)palladium (II) (33.8 mg, 0.0482 mmol) in a mixed solvent of triethylamine (1.0 mL) and tetrahydrofuran (3.0 mL) were added propargyl alcohol (0.04 mL, 0.687 mmol) and copper(I) iodide (8.4 mg, 0.0441 mmol). The mixture was stirred at 50 °C for 3 h. After cooling, water was added and the mixture was extracted with ethyl acetate. The organic phase was dried

over magnesium sulfate and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$−MeOH, 20:1) to give **20** (75.0 mg, 44%) and **21** (27.3 mg, 16%). **20**: R_f 0.18 ($CHCl_3$−MeOH, 10:1); MS *m*/*z* 462 (M^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.04 (d, 1 H, J = 3.3 Hz), 7.74 ∼ 7.82 (m, 3 H), 7.44 ∼ 7.48 (m, 2 H), 7.35 (dd, 1 H, J = 1.8, 8.7 Hz), 7.06 (d, 1 H, J = 2.0 Hz), 6.98 (d, 1 H, J = 2.0 Hz), 4.15 (s, 2 H), 3.74 ∼ 3.90 (m, 2 H), 2.68 ∼ 2.82 (m, 2 H), 2.62 (q, 4 H, J = 7.1 Hz), 0.99 (t, 6 H, J = 7.1 Hz). For elemental analysis, HCl salt of **20** was prepared by treating **20** with 4 N HCl in dioxane followed by concentration to dryness: Anal. ($C_{27}H_{28}Cl_2N_2O_3 \cdot {}^1/_2H_2O \cdot {}^1/_2C_4H_8O_2$): C, H, N. **21**: R_f 0.11 ($CHCl_3$−MeOH, 10:1); mp 172−173 °C; MS *m*/*z* 462 (M^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.99 (d, 1 H, J = 1.7 Hz), 7.75 ∼ 7.84 (m, 3 H), 7.44 ∼ 7.50 (m, 2 H), 7.36 (dd, 1 H, J = 1.8, 8.7 Hz), 7.11 (d, 1 H, J = 1.2 Hz), 6.96 (d, 1 H, J = 1.2 Hz), 4.50 (s, 2 H), 3.83 (t, 2 H, J = 6.3 Hz), 2.71 ∼ 2.85 (m, 2 H), 2.65 (q, 4 H, J = 7.2 Hz), 0.99 (t, 6 H, J = 7.2 Hz); Anal. ($C_{27}H_{27}ClN_2O_3 \cdot {}^1/_3CH_4O$): C, H, N.

3-Iodo-5-nitrobenzotrifluoride (23). To a mixture of *N*-iodosuccimide (33.7 g, 150 mol) and concentrated sulfuric acid (100 mL) was added dropwise a solution of 3-nitrobenzotrifluoride (**22**, 13.3 mL, 100 mol) in concentrated sulfuric acid (46 mL). After the addition was completed, the reaction mixture was heated at 50 °C for 6 h and poured into crushed ice. The mixture was extracted with ethyl acetate. The organic phase was washed with aqueous Na_2SO_3, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (hexane−ethyl acetate, 1:0, then 200:1) to give the title compound (6.65 g, 21%): MS *m*/*z* 317 (M^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.76 (s, 1 H), 8.47 (s, 1 H), 8.28 (s, 1 H); HRMS calcd for $C_7H_3F_3INO_2$ 316.9161, found 316.9150.

3-Iodo-5-trifluoromethylaniline (24). To a solution of **23** (6.92 g, 21.8 mmol) in EtOH (150 mL) was added tin(II) chloride hydrate (15.3 g, 67.8 mmol), and the solution was heated at reflux for 1 h. After cooling to room temperature, the mixture was poured into water, basified with $NaHCO_3$, and extracted with ethyl acetate. The organic phase was washed with brine, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (hexane−ethyl acetate, 10:1) to give the title compound (5.03 g, 80%): mp 37−38 °C; MS *m*/*z* 287 (M^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.29 (s, 1 H), 7.17 (s, 1 H), 6.83 (s, 1 H), 3.87 (brs, 2 H); HRMS calcd for $C_7H_5F_3IN$ 286.9419, found 286.9438.

6-Iodo-3-methylthio-4-trifluoromethyloxindole (25a) and 4-Iodo-3-methylthio-6-trifluoromethyloxindole (25b). To a solution of **24** (2.00 g, 6.98 mmol) in dichloromethane (35 mL) cooled to −78 °C was added sulfuryl chloride (0.68 mL, 8.36 mmol). The solution was stirred for 30 min. To the mixture was then added ethyl methylthioacetate (1.17 mL, 9.09 mol), and stirring was continued for further 2 h at −78 °C. After triethylamine (2.43 mL, 17.4 mol) was added, the mixture was allowed to warm to room temperature, treated with an excess amount of 1 N HCl, and stirred overnight. The organic phase was separated, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (hexane−ethyl acetate, 4:1) to give a 1:1 mixture of **25a** and **25b** (2.44 g, 86%): MS *m*/*z* 373 (M^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.88 (brs, 1 H), (7.72 (s), 7.65 (s), 1 H), (7.44 (s), 7.13 (s), 1 H), (4.33 (s), 4.14 (s), 1 H), (2.09 (s), 2.05 (s), 3 H); HRMS calcd for $C_{10}H_7F_3INOS$ 372.9245, found 372.9211.

6-Iodo-4-trifluoromethylisatin (26a) and 4-Iodo-6-trifluoromethylisatin (26b). To a solution of a 1:1 mixture of **25a** and **25b** (66.5 g, 178 mmol) in acetone (730 mL) were added copper(II) chloride (27.5 g, 205 mmol) and copper(II) oxide (16.4 g, 205 mmol). The mixture was stirred at room temperature for 3 h. To the resulting mixture were added 1 N HCl and ethyl acetate. The organic phase was separated, washed with 1 N HCl, and then brine, dried over magnesium sulfate, and concentrated. The resulting solid was filtered and washed with dichloromethane to give a 1:1 mixture of **26a** and **26b** (46.6 g, 77%): MS *m*/*z* 341 (M^+); ^{1}H NMR (270 MHz, DMSO-d_6) δ (11.34 (s), 11.26 (s), 1 H), (7.77 (s), 7.68 (s), 1 H), (7.55 (s), 7.08 (s), 1 H); HRMS calcd for $C_9H_3F_3INO_2$ 340.9161, found 340.9164.

1-(2-Diethylaminoethyl)-6-iodo-4-trifluoromethylisatin (27). The title compound was prepared from a 1:1 mixture of **26a** and **26b** by the method described in the preparation of **3**. Purification was carried out by silica gel column chromatography (hexane−ethyl acetate, 3:1, then 1:1): R_f 0.13 (hexane−AcOEt, 1:1); mp 108−111 °C dec; MS *m*/*z* 440 (M^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.71 (s, 1 H), 7.68 (s, 1 H), 3.80 (t, 2 H, J = 6.1 Hz), 2.69 (t, 2 H, J = 6.1 Hz), 2.55 (q, 4 H, J = 7.1 Hz), 0.97 (t, 6 H, J = 7.1 Hz); Anal. ($C_{15}H_{16}F_3IN_2O_2$): C, H, N.

3-(2-Chlorophenyl)-1-(2-diethylaminoethyl)-3-hydroxy-6-iodo-4-trifluoromethyloxindole (29). To a solution of **27** (4.96 g, 11.3 mmol) in a mixed solvent of diethyl ether (81 mL) and toluene (27 mL) was added dropwise freshly prepared 0.64 N 2-chlorophenylmagnesium bromide in ether (18.0 mL, 11.5 mol) at room temperature. The mixture was stirred for 20 min. Saturated aqueous $NaHCO_3$ was added, and the mixture was extracted with ethyl acetate. The organic phase was washed with saturated aqueous $NaHCO_3$, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$−MeOH, 100:1, then 50:1) to give the title compound (3.45 g, 55%): mp 160−162 °C; MS *m*/*z* 552 (M^+); ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.09 (d, 1 H, J = 6.8 Hz), 7.61 (s, 1 H), 7.53 (s, 1 H), 7.41 (dd, 1 H, J = 6.8, 6.8 Hz), 7.21 ∼ 7.32 (m, 2 H), 3.97 ∼ 4.11 (m, 1 H), 3.68 ∼ 3.78 (m, 1 H), 2.55 ∼ 2.84 (m, 6 H), 1.03 (t, 6 H, J = 7.1 Hz); Anal. ($C_{21}H_{21}ClF_3IN_2O_2$): C, H, N.

(+)-3-(2-Chlorophenyl)-1-(2-diethylaminoethyl)-3-hydroxy-6-iodo-4-trifluoromethyloxindole (29S) and (−)-3-(2-Chlorophenyl)-1-(2-diethylaminoethyl)-3-hydroxy-6-iodo-4-trifluoromethyloxindole (29R). Each enantiomer of racemic **29** was separated using preparative HPLC on a Chiralpak OD with 6% 2-propanol/hexane as an eluent. The enantiomeric excesses were determined by using HPLC on a Chiralpak AD with 10% 2-propanol/hexane as an eluent at a flow rate of 0.5 mL/min. The (+)-enantiomer eluted at the retention time of 12.7 min, and the (−)-enantiomer eluted at the retention time of 16.3 min. The spectral properties of the title compounds were identical with those of **29**. **29S**: mp 63−67 °C; $[\alpha]_D$ +56.8 (c = 0.26, MeOH); 89% ee. **29R**: mp 61−64 °C; $[\alpha]_D$ −60.0 (c = 0.33, MeOH); 91% ee.

3-(2-Chlorophenyl)-6-cyano-1-(2-diethylaminoethyl)-3-hydroxy-4-trifluoromethyloxindole (35). A mixture of **29** (2.97 g, 5.37 mmol), 60% zinc cyanide (926 mg, 4.73 mmol), and tetrakis(triphenylphosphine)palladium (0) (630 mg, 0.545 mmol) in DMF (30 mL) was stirred at 60 °C for 3 h. After cooling, saturated aqueous $NaHCO_3$ was added, and the mixture was extracted with a mixture of toluene and ethyl acetate (1:1). The organic phase was washed with saturated aqueous $NaHCO_3$, dried over magnesium sulfate, and concentrated. The residue was purified by silica gel column chromatography (chloroform−methanol, 80:1, then 50:1) to give the title compound (1.56 g, 64%): mp 188−189 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.07 (d, 1 H, J = 7.6 Hz), 7.57 (s, 1 H), 7.40 ∼ 7.47 (m, 2 H), 7.33 (ddd, 1 H, J = 1.5, 7.6, 7.6 Hz), 7.24 (dd, 1 H, J = 1.5, 7.9 Hz), 4.03 ∼ 4.13 (m, 1 H), 3.73 ∼ 3.83 (m, 1 H), 2.59 ∼ 2.78 (m, 6 H), 1.01 (t, 6 H, J = 7.1 Hz); Anal. ($C_{22}H_{21}ClF_3N_3O_2 \cdot {}^1/_3H_2O$): C, H, N.

3-(2-Chlorophenyl)-6-carbamoyl-1-(2-diethylaminoethyl)-3-hydroxy-4-trifluoromethyloxindole (37). To a solution of **35** (1.52 g, 3.36 mmol) in *tert*-butyl alcohol (70 mL), warmed to 50 °C, was added powdered KOH (ca. 5.0 g). The mixture was stirred at 50 °C for 1 h and passed through a celite pad. The celite was washed with THF, and the filtrate was concentrated. The residue was partitioned between water and ethyl acetate, and the organic phase was separated. The organic phase was dried over magnesium sulfate and concentrated, which was treated with 4 N HCl in dioxane followed by concentration to dryness to give the title compound (1.66 g, 98%): mp 213−217 °C; MS *m*/*z* 469 (M^+); ^{1}H NMR (270 MHz, DMSO-d_6) δ 10.41 (brs, 1 H), 8.33 (brs, 1 H), 8.15 (s, 1

H), 8.08 (d, 1 H, $J = 7.3$ Hz), 7.84 (s, 1 H), 7.77 (brs, 1 H), 7.48 (ddd, 1 H, $J = 1.7$, 7.6, 7.6 Hz), 7.11 ~ 7.40 (m, 4 H), 4.16 ~ 4.36 (m, 2 H), 3.25 ~ 3.51 (m, 6 H), 1.26 (t, 6 H, $J = 7.3$ Hz); Anal. ($C_{22}H_{24}Cl_2F_3N_3O_3 \cdot H_2O$): C, H, N.

(+)-3-(2-Chlorophenyl)-6-cyano-1-(2-diethylaminoethyl)-3-hydroxy-4-trifluoromethyloxindole (35S). The title compound was prepared from **29S** by the method described in the preparation of **35**. The spectral properties of the title compound were identical with those of **35**. The enantiomeric excess was determined by using HPLC on a Chiralpak AD with 10% 2-propanol/hexane as an eluent at a flow rate of 0.5 mL/min. The title compound eluted at the retention time of 13.2 min: mp 170−172 °C; $[\alpha]_D$ +76.5 ($c = 0.31$, MeOH); 90% ee.

(+)-3-(2-Chlorophenyl)-6-carbamoyl-1-(2-diethylaminoethyl)-3-hydroxy-4-trifluoromethyloxindole (37S). The title compound was prepared from **35S** by the method described in the preparation of **37**. The spectral properties of the title compound were identical with those of **37**. The enantiomeric excess was determined by using HPLC on a Chiralpak AD with 10% 2-propanol/hexane as an eluent at a flow rate of 1.0 mL/min. The title compound eluted at the retention time of 17.9 min: mp 168−172 °C; $[\alpha]_D$ +81.4 ($c = 0.41$, MeOH); 97% ee.

GH Releasing Assay In Vitro: Rat Pituitary Cell Assay. Anterior pituitary cells were obtained from 7 week-old male Wistar/ST rats. The tissue was washed three times with HBSS (Hank's balanced salt solution), cut into small pieces, and transferred to the isolation buffer (100 μL/lobe) containing 0.8% collagenase and 0.2% DNase type I. The mixture was incubated for less than 25 min at 37 °C. The cells were collected and washed three times with the culture medium; DMEM (Dulbecco's modified Eagle's medium) was supplemented with 5% horse serum, 2.5% fetal calf serum, 1% nonessential amino acids, 1% penicillin, and 1% streptomycin. Cells were resuspended in the above medium and brought to a density of 1.0×10^5 cell/mL. The cells (200 μL/well) were seeded onto a 96 well-plate (Nunc, Denmark) and cultured for 5 days at 37 °C under 5% CO_2.

Following the culture period the cells were washed once with the stimulation buffer (the culture medium containing 25 mM HEPES, pH = 7.3) and then incubated for 90 min at 37 °C under 5% CO_2. This buffer was then replaced with the fresh stimulation buffer containing test compound followed by incubation for 15 min at 37 °C under 5% CO_2. The medium was collected and GH concentration measured.

GH concentration was measured by the double-antibody RIA method. Rabbit anti-rat GH antibody was purchased from Biogenesis, Poole, England. GH concentration was expressed in terms of the NIH-RP-2 standard. The intra- and interassay coefficients of variation were below 11% and 13% with a 1 ng/mL limit of quantitation. EC_{50} values of the test compounds were calculated as the concentration inducing half-maximal stimulation with the following scheme: GH content = $E_{max} \times$ concentration of test compound/(EC_{50} + concentration of test compound) + basal GH content.

In Vivo Study: Effect of 37S (SM-130686) on the Body Weight Gain. Female 11 week-old F344/N rats were used in this study. Rats were randomly allocated to three treatment groups, each containing six animals. Compound **37S** (SM-130686) was orally administrated twice a day to each rat (at 10 a.m. and 4 p.m.) for 4 days (starting from day 0). The rats were weighed every morning just before administration of the compound.

GHS Binding Activity. Crude membranes were isolated from CHO cells stably expressing the human GHS-R 1A. These were suspended in binding buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 2.5 mM EDTA, pH = 7.4) to a concentration of 1 mg protein/mL. These membranes (25 μg protein/well) were mixed with ^{35}S-MK-677 (100 000 dpm/well), with or without different concentrations (0.1−1000 nM) of **37S** (SM-130686), and the binding buffer up to a total volume of 250 μL. Nonspecific binding was obtained by adding 500 nM of cold MK-677. The membranes were incubated at 25 °C for 60 min, and the bound radioligand was separated from free radioligand by washing with the binding buffer through a GF/B filter. The radioactivity on the filters was counted in β-plate scintillation counter (Amersham Pharmacia Biotech, England).

Acknowledgment. We are grateful to Ms. N. Okahara-Mitsuhata and Ms. H. Ikeda for technical assistance and to Dr. K. Yanagi for discussing concerning X-ray crystallography.

Supporting Information Available: Experimental procedures and spectral data for **6**, **8**−**10**, **13**−**19**, **28**, **30**−**34**, **35R**, **36**, and **37R**, elemental analyses, and X-ray crystallographic analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0103763