Discovery of a Novel and Potent Human and Rat β_3-Adrenergic Receptor Agonist, [3-[(2*R*)-[[(2*R*)-(3-Chlorophenyl)-2-hydroxyethyl]amino]propyl]-1*H*-indol-7-yloxy]acetic Acid

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In search for potent and selective β_3-adrenergic receptor (β_3-AR) agonists as potential drugs for the treatment of type II diabetes and obesity, a novel series of 1-(3-chlorophenyl)-2-aminoethanol derivatives were prepared and evaluated for their biological activity at human β_1-, β_2-, and β_3-ARs and rat β_3-AR expressed in Chinese hamster ovary (CHO) cells. Replacement of the right-hand side (RHS, benzene ring) in the 'first generation' β_3-AR agonists BRL 37344 and CL 316243 with a 1*H*-indole ring gave compound 31 with unique pharmacological properties among β_3-AR agonists. Initial *in vitro* assays showed that 31 possesses modest rat and human β_3-ARs agonistic activity. Introduction of various substituent into the indole nucleus of 31 afforded a number of compounds with good β_3-ARs agonistic activity. In particular, 90 having a carboxylic acid functionality at the 7-position of the indole nucleus showed the most potent human β_3-AR agonistic activity. Finally, optical resolution of 90 led to the identification of the most promising compound, [3-[(2*R*)-[[(2*R*)-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]-1*H*-indol-7-yloxy]acetic acid (96, AJ-9677). This compound exhibited potent human β_3-AR agonistic activity (EC_{50}=0.062 nM, IA=116%) with 210- and 103-fold selectivity over human β_2-AR and β_1-AR, respectively. Compound 96 also exhibited potent rat β_3-AR agonistic activity (EC_{50}=0.016 nM, IA=110%). Moreover, repeated oral administration of 96 inhibited body weight gain and significantly decreased glucose, insulin, free fatty acid, and triglyceride concentrations in plasma in KK-A^y/Ta mice. On the basis of this pharmacological profile, 96 entered clinical development as a drug for the treatment of type II diabetes and obesity.

Key words β_3-adrenergic receptor agonist; AJ-9677; indole; type II diabetes; obesity

β-Adrenergic receptors (β-ARs) have been sub-classified as β_1- and β_2-ARs since 1967.[1] This sub-classification has led to the development of β_1- and β_2-AR antagonists and/or agonists which have been useful for the treatment of cardiovascular diseases and asthma. In early 1980s, a third β-AR, initially referred to as "atypical"[2] and later called β_3-AR has been found in a number of species, including man,[3—6] and in 1989, the human,[7] rat,[8] and mouse[9] β_3-ARs were first cloned and characterized. Like other β-ARs, β_3-ARs belong to the G-protein-coupled receptor family that includes receptors with a common structure consisting of seven transmembrance-spanning connected by interhelical loops.[10] The β_3-AR is located on the surface of both white and brown adipocytes and is known to be stimulated by endogenous catecholamines, adrenalin, and noradrenaline. It has also been reported that β_3-AR receptor plays a significant role in regulating lipolysis and thermogenesis in rodent and human adipose tissues.[11,12] Moreover, studies of β_3-AR mRNA demonstrated that, β_3-ARs exist in human heart, gall bladder, gastrointestinal tract, prostate, and urinary bladder detrusor tissue in addition to adipocytes.[13—16] Since the discovery of β_3-AR, a number of laboratories have been engaged in developing potent and selective β_3-AR agonists for the treatment of various metabolic and gastrointestinal diseases such as obesity, non-insulin dependent (Type-II) diabetes, irritable bowel syndrome, and urinary frequency and incontinence.[17—21] Early β_3-AR agonists (the "first generation" of potent and selective rat β_3-AR agonists) such as BRL 37344 (BRL 35135),[22—24] CL 316243,[25—27] and SR 58611A,[28] having a 3-chlorophenyl moiety in the left-hand side (LHS) and a carboxylic acid or an ester functionality in the right-hand side (RHS) as shown in Chart 1, have been reported to be effective anti-obesity and anti-diabetic agents in rodents.[29] These compounds, through their potent agonistic activity for the β_3-AR, stimulate lipolysis and energy expenditure and cause only a slight increase in heart rate (β_1-AR activity) and weak muscle tremor (β_2-AR activity). Unfortunately, some of these early β_3-AR agonists developed for the treatment of metabolic disorders failed to produce similar effects in humans, due to a lack of selectivity and/or potency, or poor pharmacokinetics.[30] Because of structural differences between human and rat β_3-ARs with regard to the amino acid sequences of each receptor, activity at the rat β_3-AR could not effectively predict that at the human β_3-AR.[31] Thus, a new generation of human β_3-AR agonists with minimal side effects associated with activation of human β_1- and β_2-ARs has long been needed.

A new generation of β_3-AR agonists currently under preclinical development is represented by the tetrahydroisoquinoline **1**,[32—34] SB 226552,[35,36] L 755507,[37] L 770644,[38,39] and LY-377604[40] (Chart 1). These compounds having both potent and full agonistic activity, and high selectivity at the human β_3-AR were, in primary screening assays, evaluated in Chinese hamster ovary (CHO) cells expressing the cloned human β_3-AR. This method of evaluation was believed to be more accurate in predicting the effects that can be expected in humans. However, despite all these recent developments there is still not a single therapy available for the treatment of type II diabetes, obesity, frequent urination, and other related diseases. With the exception of the tetrahy-

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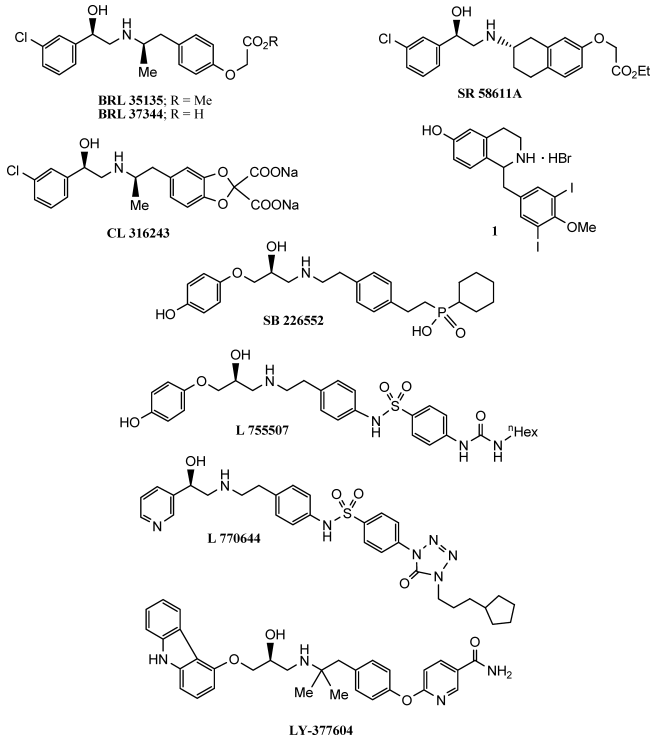


Chart 1. Structures of Some β_3-AR Agonists

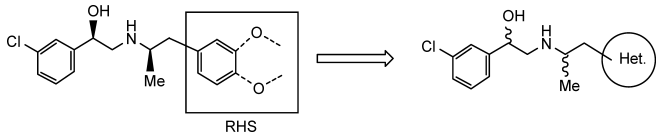
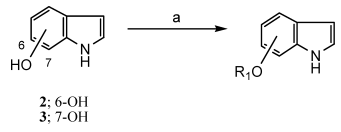


Chart 2. Design of Novel β_3-AR Agonists

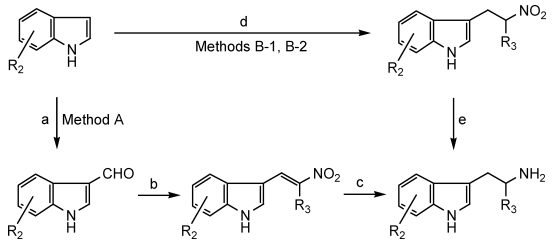
droisoquinoline β_3-AR agonist **1**, a number of papers have described compounds in either the arylethanolamine or aryloxypropanolamine class. Among these analogues, a great deal of structural diversity has appeared in the RHS portion of these analogues distal to the arylethanolamine or aryloxypropanolamine pharmacophore, suggesting a permissive interaction with the receptor in this portion of the molecule.[12)]

To discover a novel class of potent and selective β_3-AR agonists, we have synthesized phenylethanolamine derivatives bearing several heterocycles in the RHS of BRL 37344 and CL 316243 (see Chart 2); while keeping the LHS 3-chlorophenyl moiety intact as this portion of the molecule, found in a number of other highly selective rat β_3-AR agonists, is important for β_3-AR agonistic activity. From the structure–activity relationship (SAR) studies of these derivatives, we have found the novel and selective β_3-AR, 1-(3-chlorophenyl)-2-[(1*H*-indol-3-yl)propyl]aminoethanol (**31**), as a lead compound with moderate β_3-AR agonistic activity (rat spontaneous colonic contraction[41)]: $EC_{50}=22.9\pm3.1$ nM) and weak β_1- and β_2-ARs agonistic activity (inhibition of rat uterus spontaneous motility[41)]: $EC_{50}=577.3\pm149.4$ nM and enhancement of spontaneous beating in guinea-pig isolated right atria[24)]: $EC_{50}=>10000$ nM, respectively). Furthermore, **31** showed more potent human β_3-AR agonistic activity than SR 58611A and CL 316243. To improve the potency and selectivity of **31**, we focused our efforts on the introduction of various substituents into the indole nucleus and its side-chain at the 3-position. It is well-known that the β_3-AR agonist BRL 37344 essentially requires an (*R*,*R*)-configuration at the benzyl position with a hydroxy and an adjacent methyl groups at the amino group to enhance its agonistic activity for rat β_3-AR.[42)] Thus, optical resolution performed on selected compound and further SAR studies of **31** led to the discovery of the optically active [3-[(2*R*)-[[(2*R*)-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]-1*H*-indol-7-yloxy]acetic acid (**96**, AJ-9677). This compound showed the most potent human and rat β_3-ARs agonistic activity with low affinity for human β_1- and β_2-ARs.



Reagents and conditions: (a) R_1-Br, K_2CO_3, KI, acetone or DMF, reflux or 90 °C/CH_2=CHCN, triton B, reflux.

Chart 3. Synthesis of 6- or 7-Substituted 1*H*-Indoles



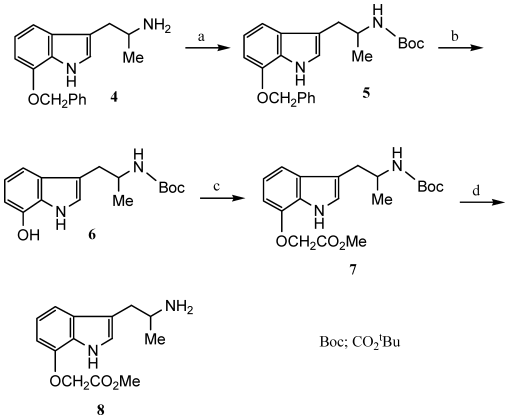
Reagents and conditions: (a) $POCl_3$, DMF; (b) $MeNO_2$ or $EtNO_2$, AcOH, reflux; (c) $LiAlH_4$, THF; (d) $HC(=CH_2)NO_2$ or $MeC(=CH_2)NO_2$, benzene; (e) Raney Ni, H_2, EtOH (B-1), Fe, NH_4Cl, aqueous EtOH, reflux (B-2).

Chart 4. Synthesis of Tryptamine Derivatives (1)

In this paper, we describe the synthesis and SAR studies of a novel series of 1-(3-chlorophenyl)-2-aminoethanol derivatives and investigate their biological activity at human β_1-, β_2-, and β_3-ARs and rat β_3-AR.

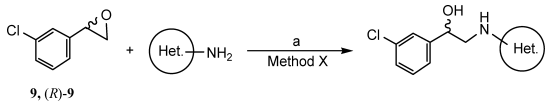
Chemistry

The starting substituted 1*H*-indoles are commercially available or can be prepared according to the literature. Methyl (1*H*-indol-6-yloxy)acetate and 7-ethoxy-, 7-propoxy-, 7-isopropoxy-, and 7-(2-ethoxyethoxy)-1*H*-indoles were prepared by reaction of the commercially available 6- and 7-hydroxy-1*H*-indoles (**2** and **3**, respectively) with the corresponding alkylhalides or substituted alkyl halides in the presence of K_2CO_3 and KI. Treatment of **3** with acrylonitrile in the presence of triton B at refluxing temperature gave the 7-(2-cyanoethoxy)-1*H*-indole in 89% yield (Chart 3). The requisite intermediate tryptamine derivatives are commercially available or can be prepared from substituted 1*H*-indoles using usual procedures described in the literature. In general, 3-formyl-1*H*-indoles obtained by Vilsmeier reaction[43,44)] ($POCl_3$/DMF) of the substituted 1*H*-indoles or the commercially available 3-formyl-1*H*-indoles were treated with nitromethane or nitroethane in AcOH to produce nitroolefins in good yields. Conjugated nitroolefins were reduced by $LiAlH_4$ to directly give substituted tryptamines although the yields were moderate to low (Chart 4, method A). Another synthetic route to the substituted tryptamines[45,46)] was as follows



Reagents and conditions: (a) Boc_2O, MeOH; (b) Pd/C, H_2 EtOH; (c) $ClCH_2CO_2Me$, K_2CO_3, KI, acetone; (d) aqueous HCl, EtOH.

Chart 5. Synthesis of Tryptamine Derivatives (2)



Reagents and conditions: (a) MeOH.

Chart 6. Synthetic Route to Aminoethanol Derivatives (1)

(Chart 4, method B): treatment of substituted 1*H*-indoles with nitroethylene[47] or 2-nitro-1-propene[47] produced from nitro-alcohols provided the corresponding 3-(2-nitroethyl- or 2-nitropropyl)-1*H*-indoles, which were hydrogenated over Raney-Ni in EtOH (method B-1) or reduced with Fe in the presence of NH_4Cl in refluxing aqueous EtOH (method B-2) to give the desired tryptamine derivatives in moderate to good yields.

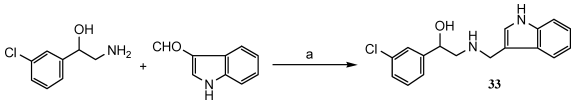
The methyl [3-(2-aminopropyl)-1*H*-indol-7-yloxy]acetate (**8**) was prepared from the 3-(2-aminopropyl)-7-benzyloxy-1*H*-indole (**4**), which was obtained by method A from the 7-benzyloxy-1*H*-indole.[48—51] Protection of **4** by a *tert*-butoxycarbonyl (Boc) group followed by hydrogenation of the resultant **5** over Pd on carbon gave the 7-hydroxytryptamine **6**. Reaction of **6** with methyl chloroacetate in the presence of K_2CO_3 and KI in refluxing acetone, followed by deprotection of the resultant **7** using aqueous HCl produced the desired tryptamine **8** (Chart 5).

Most of the compounds listed in Tables 1—5 (**24**—**91** except **33**, **45**—**53**, **84**, **90**), were prepared by nucleophilic addition of alkyl amines bearing various heterocycles, tryptophan derivatives, or tryptamine derivatives to the racemic 3-chlorostyrene oxide (**9**) or its optical isomer[25,52—57] (*R*)-**9** in MeOH (Chart 6, method X).

The 1-(3-chlorophenyl)-2-[(1*H*-indol-3-yl)methyl]aminoethanol (**33**) was prepared by reductive alkylation. Treatment of the 2-amino-1-(3-chlorophenyl)ethanol[58,59] with 3-formyl-1*H*-indole, followed by reduction of the intermediate imine with $NaBH_4$ provided **33** in only 15% yield (Chart 7).

The 1*H*-indole derivatives **45**—**49** (Table 2), having carbamoyl, hydroxymethyl, and carboxylic acid functionalities, respectively were obtained by amidation with ammonia, reduction with $LiBH_4$, or alkaline hydrolysis of **43** and/or **44**, which were derived from the D- or L-tryptophan methyl esters and **9** using method X, respectively (Chart 8, method Y).

The optically active 1-(3-chlorophenyl)-2-[2-(1*H*-indol-3-

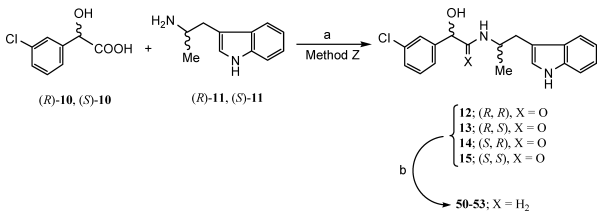


Reagents and conditions: (a) (i) $NaHCO_3$, MeOH; (ii) $NaBH_4$.

Chart 7. Synthesis of 1*H*-Indole Derivative **33**

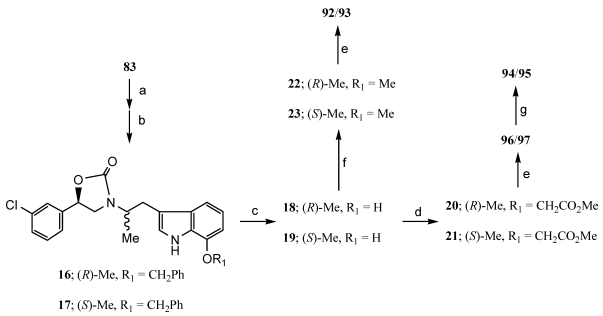
Reagents and conditions: (a) 28% aqueous NH_4OH, EtOH; (b) $NaBH_4$, LiCl, EtOH, THF; (c) NaOH, aqueous MeOH.

Chart 8. Synthetic Route to Aminoethanol Derivatives (2)



Reagents and conditions: (a) benzotirazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP), DMF, room temperature; (b) BH_3-THF, THF, reflux.

Chart 9. Synthetic Route to Optically Active Tryptamine Derivatives



Reagents and conditions: (a) *N*,*N*′-carbonyldiimidazole; (b) separation by silica gel column chromatography; (c) Rh/C, H_2; (d) $ClCH_2CO_2Me$, K_2CO_3, KI; (e) aqueous NaOH; (f) MeI, K_2CO_3, KI; (g) $SOCl_2$, MeOH.

Chart 10. Synthesis of Optically Active Tryptamine Derivatives

yl)propyl]aminoethanols (**50**—**53**) listed in Table 3 were synthesized by treatment of the optically active 3-chloromandelic acids[60] (*R*)-**10** and (*S*)-**10** with the optically active α-methyltryptamines[61] (*R*)-**11** and (*S*)-**11** using benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) as a coupling reagent, followed by reduction of the amide group of the optically active mandelamides **12**—**15** with borane in refluxing THF solution (Chart 9).

Protection of the 2-aminoethanol moiety of the 7-benzyloxy-1*H*-indole derivative **83** with a carbonyl group, and successive separation of the resultant 1,3-oxazolidinone derivative as diastereomeric mixture using silica gel column chromatography afforded the optical isomers **16** and **17** having *R*- and *S*-configurations at the α-methyl group, respectively. To avoid removal of the 3-chlorine atom in the benzene ring of **16** and **17**, catalytic hydrogenation of **16** and **17** over Pd on carbon in the presence of chlorobenzene or Rh on carbon in EtOH was carried out and the corresponding 7-hydroxy-1*H*-indole derivatives **18** and **19** were obtained. Treatment of the

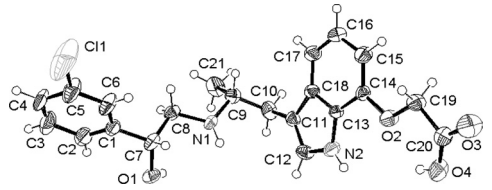


Fig. 1. An ORTEP Drawing of **96**, Representing Heavy Atoms as 50% Probability Ellipsoids and H Atoms as Spheres of Arbitrary Radius

resulting products **18** and **19** with methyl chloroacetate in the presence of K_2CO_3 and KI, followed by alkaline hydrolysis of the methyl (1*H*-indol-7-yloxy)acetates **20** and **21** with aqueous NaOH produced simultaneous ester hydrolysis and deprotection of the 2-aminoethanol moiety giving the desired optically active (1*H*-indol-7-yloxy)acetic acids **96** and **97**. The corresponding methyl esters **94** and **95** of **96** and **97** were obtained in good yields by treatment of **96** and **97** with $SOCl_2$ in MeOH. In a manner similar to that described for the preparation of **96** and **97** from **18** and **19**, methylation of **18** and **19** with MeI and successive alkaline hydrolysis of **22** and **23** afforded the optically active 7-methoxy-1*H*-indoles **92** and **93** (Chart 10).

The absolute configurations of the optically active 7-benzyloxy-1*H*-indole derivative **17** [(*R*,*S*)-configuration] and **96** [(*R*,*R*)-configuration] were confirmed by single-crystal X-ray analysis, and the ORTEP diagram of **96** is shown in Fig. 1.

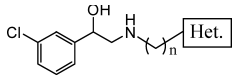
Results and Discussion

In order to expedite the identification of potent β_3-AR agonists, we focused our efforts on optimizing the RHS moiety of BRL 37344 and CL 316243, while keeping the LHS 3-chlorophenyl moiety intact. First, we examined the 1-(3-chlorophenyl)-2-aminoethanol derivatives **24**—**38** bearing a variety of heterocycles for their inhibition of rat proximal colon spontaneous contraction (rat β_3-AR agonistic activity)[41] and ability to increase cAMP levels in CHO cells expressing cloned human β_3-AR at concentration of 10^{-7} M (human β_3-AR agonistic activity), and compared the results to those of reference compounds SR 58611A and CL 316243. As illustrated in Table 1, the first generation of β_3-AR agonists (SR 58611A and CL 316243) exhibited potent rat β_3-AR agonistic activity. Compounds **24**—**27** having a pyrrole, thiophene, and pyridine rings, respectively, were essentially inactive or had weak agonistic activity at the rat and/or human β_3-AR. However, the 1-(3-chlorophenyl)-2-[2-(benzo[*b*]thiophen-3-yl)ethyl]aminoethanol (**28**) exhibited modest rat and human β_3-ARs agonistic activity. Although **28** agonistic activity for rat and human β_3-ARs was lower than that of SR 58611A, its agonistic activity for human β_3-AR was higher than that of CL 316243. A significant loss in rat and human β_3-ARs agonistic activity was observed with the regioisomer of **28**, *i.e.* the 2-benzo[*b*]thiophene derivative **29**. The 3-benzo[*b*]furan analogue **30** did not show improved rat β_3-AR agonistic activity, but its human β_3-AR agonistic activity was more potent than that of **28**. To our delight, the 1-(3-chlorophenyl)-2-[(1*H*-indol-3-yl)propyl]aminoethanol (**31**) was found to have the most potent human β_3-AR agonistic activity (Table 1), with 53% cAMP accumulation at 10^{-7} M. The regioisomer of **31**, *i.e.* the 2-(1*H*-indole) analogue **32**, like the benzo[*b*]thiophene series, had significantly low rat and human β_3-ARs agonistic activity. In terms of tether-length modification, a comparison of the data obtained for **33** and **34** with that obtained for **31** indicated that both longer and shorter RHS indole nucleus chains lead to diminished rat and human β_3-ARs agonistic activity. In addition, replacement of the 1*H*-indole nucleus of **31** by an indoline, benzoisoxazole, 1*H*-indazole, or benzothiazole ring (giving **32**—**38**, respectively) considerably reduced the agonistic activity for human β_3-AR. From the findings above, compound **31** was selected as lead compound and its *in vitro* ability to inhibit rat uterus spontaneous motility (β_2-AR agonistic activity) and to induce chronotropic effects on guinea-pig right atria (β_1-AR agonistic activity) were assayed. Our results show that compound **31** slightly relaxed both rat uterus (β_2-AR; $EC_{50}=557.3\pm149.4$ nM) and guinea-pig atria (β_1-AR, $EC_{50}=>10000$ nM) and was therefore characterized as a potent rat β_3-AR agonist even thought its agonistic activity was weaker than that of SR 58611A or CL 316243. Compound **31** also showed weak agonistic activity at both β_1- and β_2-ARs and moderate agonistic activity at human β_3-AR. Compound **31** was thus classified as a full agonist ($ED_{50}=62$ nM as shown in Table 2) at human β_3-AR with 99% activation relative to (+)-isoproterenol [defined as Intrinsic Activity (IA)>90%].

Inspection of the structure of the β_3-AR agonists BRL 37344 and CL 316243 revealed some similarities including the presence of a methyl group at the α-position of the phenethylamine moiety. As expected, introduction of a methyl group (yielding **39**, Table 2) into the tryptamine sidechain of **31** resulted in an enhancement of β_3-AR agonistic activity ($EC_{50}=12$ nM, IA=114%), when compared with that of **31**. However, modification of the alkyl substituent from a methyl to an ethyl group in **39** (yielding **40**) significantly reduced the agonistic activity for human β_3-AR. Eli Lilly scientists have studied a series of amides as anti-obesity/anti-diabetic agents and discovered the potent and selective β_3-AR agonist LY-377604 having a dimethyl group at the α-position of the phenethylamine moiety (see Chart 1).[40] In our study, further introduction of a methyl group into **39** (yielding **41**) resulted in *ca.* 6-fold decrease in β_3-AR agonistic activity ($EC_{50}=70$ nM). Compound **42** having a tetrahydrocarbazole ring like SR 58611A exhibited significantly poor agonistic activity at the β_3-AR. Furthermore, introduction of other functionalities such as methoxycarbonyl, carbamoyl, hydroxymethyl, and carboxylic acid into **31** (yielding **43**—**49**) remarkably decreased β_3-AR agonistic activity. As a result of the SAR studies described above, **39** bearing 1*H*-indole nucleus was selected as a potential human β_3-AR agonist and the useful RHS was concluded to be 1-(1*H*-indol-3-yl)-2-propylamine moiety.

From studies on β-ARs agonistic activity of the four optical isomers of BRL 37344, the (*R*,*R*)-configuration was proved to be important for enhancing rat β_3-AR agonistic activity while keeping that for β_1- and β_2-ARs low.[42] To study the relationship between the stereoconfiguration of the methyl and hydroxy groups in the selected tryptamine derivative **39** and β-ARs agonistic activity, the four stereoisomers **50**—**53** were prepared and were assessed by measuring cAMP accumulation in CHO cells expressing cloned human β_1-, β_2-, and β_3-ARs and rat β_3-AR (Table 3). As expected, the optical isomer **50**, having *R*-configuration in both the hy-

Table 1. Physical Data and Rat and Human β_3-AR Agonistic Activity of 1-(3-Chlorophenyl)-2-aminoethanol Derivatives

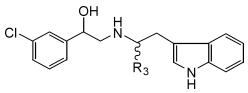


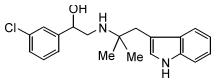
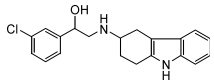
Compd[a]	Het.	*n*	mp, °C (Recryst. solvent[b])	Formula[c]	Rat spontaneous colonic contraction[d] ED_{50} (nM)	cAMP accumulation[e] (% at 10^{-7} M)
24		2	195—197 (E)	$C_{15}H_{19}ClN_2O \cdot C_2H_2O_4$[f]	46.1±2.1	4
25		2	88—89 (T–H)	$C_{14}H_{16}ClNOS$	NT[g]	0
26		2	161—164 (E)	$C_{15}H_{17}ClN_2O \cdot 2C_2H_2O_4 \cdot 1/2H_2O$[f]	154±25.7	9
27		2	131—133 (E)	$C_{15}H_{17}ClN_2O \cdot C_2H_2O_4 \cdot 1/4H_2O$[f]	639±36.6	1
28		2	106—107 (E)	$C_{18}H_{18}ClNOS$	20.3±3.1	17
29		2	198—200 (E–DE)	$C_{18}H_{18}ClNOS \cdot C_2H_2O_4$[f]	618.3±142.6	0
30		2	174—177 (E)	$C_{18}H_{18}ClNO_2 \cdot C_2H_2O_4$[f]	92.7±26.2	28
31		2	173—176 (E–DE)	$C_{18}H_{19}ClN_2O \cdot HCl \cdot 1/4H_2O$	22.9±3.1	53
32		2	187—188.5 (E)	$C_{18}H_{19}ClN_2O \cdot C_2H_2O_4$[f]	135.4±13.9	0
33[d]		1	140—142 (E)	$C_{17}H_{17}ClN_2O$	>1000	1
34		3	124—126 (E–DE)	$C_{19}H_{21}ClN_2O$	90.6±1.0	1
35		2	171—173 (E)	$C_{18}H_{21}ClN_2O \cdot 2C_2H_2O_4 \cdot 1/4H_2O$[f]	45.4±8.5	9
36		2	101—102 (T)	$C_{17}H_{17}ClN_2O_2$	504.9±131.7	0
37		2	85—88 (E–DE)	$C_{17}H_{18}ClN_3O \cdot C_2H_2O_4$[f]	NT[g]	0
38		2	98—99 (T)	$C_{17}H_{17}ClN_2OS$	NT[g]	9
			SR 58611A		2.7±0.5	34
			CL 316243		0.6±0.1	6

a) All compounds except for **33** were prepared by method X (See Experimental Section). All compounds were racemic mixture. *b*) Abbreviation for the solvents used are as follows: E=ethanol, DE=diethyl ether, T=toluene, H=hexane. *c*) All compounds were analyzed for C, H, N, S, and halogen; analytical results were within±0.4% for the theoretical values. *d*) See Experimental Section. *e*) Activity relative to (−)-isoproterenol. *f*) Oxalic acid. *g*) NT; not tested.

droxy and α-methyl centers, exhibited a potent agonistic activity at both the human and rat β_3-ARs compared with that of other stereoisomers **51** and **52** and the corresponding enantiomer **53**. On the other hand, BRL 37344 was found to be a potent rat β_3-AR and moderate human β_3-AR agonist. The human β_3-AR agonistic activity of **50** was *ca.* 2-fold and *ca.* 7-fold more potent than that of **39** and BRL 37344, respectively. Although **50** exhibited a more potent agonistic activity than the original **39** at the human and rat β_3-ARs, it had basically no selectivity against the β_1-AR. Other enantiomers; **51**—**53** showed low agonistic activity at the β_3- and β_2-ARs.

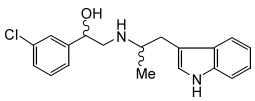
Table 2. Physical Data and Human β_3-AR Agonistic Activity of 3-[2-[2-(3-Chlorophenyl)-2-hydroxyethyl]aminoalkyl]-1*H*-indoles



Compd	R_3	Configuration of R_3 substituent center	Synthetic method[a]	mp, °C (Recryst. solvent[b])	Formula[c]	Human β_3-AR agonistic activity	
						EC_{50} (nM)[d] (IA %)[e]	cAMP accumulation[f] (% at 10^{-7} M)
31[g]	H	—				62 (99)	53
39[h]	Me	*R*+*S*	X	80—86 (E–DE)	$C_{19}H_{21}ClN_2O \cdot 1/4H_2O$	12 (114)	
40[h]	Et	*R*+*S*	X	oil	$C_{20}H_{23}ClN_2O$		20
41[g]			X	210—212 (E)	$C_{20}H_{23}ClN_2O \cdot C_4H_4O_4$[j]	70 (118)	
42[h]			X	109—113 (E–DE)	$C_{20}H_{21}ClN_2O \cdot C_2H_2O_4$[k]		0
43[i]	CO_2Me	*R*	X	124—128 (E–DE)	$C_{20}H_{21}ClN_2O_3 \cdot HCl \cdot 1/4H_2O$		0
44[i]	CO_2Me	*S*	X	123—127 (E–DE)	$C_{20}H_{21}ClN_2O_3 \cdot HCl$		0
45[i]	$CONH_2$	*R*	Y	129—133 (E–DE)	$C_{19}H_{20}ClN_3O_2 \cdot C_2H_2O_4$[k] $\cdot 1/2H_2O$		0
46[i]	$CONH_2$	*S*	Y	131—135 (E–DE)	$C_{19}H_{20}ClN_3O_2 \cdot C_2H_2O_4$[k] $\cdot 1/4H_2O$		12
47[i]	CH_2OH	*R*	Y	171—173 (E–DE)	$C_{19}H_{21}ClN_2O_2 \cdot C_2H_2O_4$[k]		0
48[i]	CH_2OH	*S*	Y	169—171 (E–DE)	$C_{19}H_{21}ClN_2O_2 \cdot C_2H_2O_4$[k]		24
49[i]	CO_2H	*R*	Y	216—219 (aq. M)	$C_{19}H_{19}ClN_2O_3 \cdot 1/4H_2O$		0

a) See Experimental Section. *b*) Abbreviaion for the solvents used are as follows: E=ethanol, DE=diethyl ether, M=Methanol. *c*) See footnote *c* in Table 1. *d*) β_3-AR agonistic activity was assessed by measurement of cAMP accumulation level in CHO cells expressing human β_3-AR (150000 receptors/cell). *e*) The maximal amount of cAMP obtained by (−)-isoproterenol and the amonunt of cAMP in the absence of agonists were defined as 100 and 0%, respectively, and the relative maximal response of each compound is expressed as intrinsic activity (IA). EC_{50} value is a concentration of the test compound to be required to achieve 50% of cAMP accumulation. *f*) Activity relative to (−)-isoproterenol. *g*) Racemic mixture. *h*) Mixture of four diastereomers. *i*) Mixture of two diastereomers. *j*) Fumaric acid. *k*) Oxalic acid.

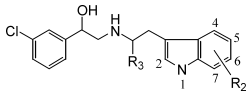
Table 3. Physical Data and β_3-AR Agonistic Activity of Compound **39**, Its Individual Diastereomers, and Reference Compound at Cloned Human β_1-, β_2-, and β_3-ARs and at the Cloned Rat β_3-AR[a]



Compd[b]	Configuration of hydroxy center	Configuration of methyl center	mp, °C (Recryst. solvent[c])	$[\alpha]^t_D$ (MeOH, *c*)	Formula[d]	Agonistic activity; EC_{50} (nM)[e] (IA %)[f]			
						Human β_3-AR	Human β_2-AR	Human β_1-AR	Rat β_3-AR
39	*R*+*S*	*R*+*S*				12 (114)	23 (46)	NT[g]	0.97 (107)
50[h]	*R*	*R*	113—115 (aq. IP)	−46.4° 28 °C (0.50)	$C_{19}H_{21}ClN_2O$	5.4 (110)	25 (50)	1.9 (65)	0.36 (98)
51[i]	*R*	*S*	166—168 (E–AC)	−13.5° 26 °C (0.10)	$C_{19}H_{21}ClN_2O \cdot C_2H_2O_4$[k] $\cdot 1/4H_2O$	240 (97)	—[j]	9.4 (50)	13 (96)
52[l]	*S*	*R*	169—171 (E–EA)	+14.6° 26 °C (0.10)	$C_{19}H_{21}ClN_2O \cdot C_2H_2O_4$[k]	220 (119)	330 (23)	47 (70)	11 (108)
53[m]	*S*	*S*	115—116 (aq. IP)	+48.5° 26 °C (0.50)	$C_{19}H_{21}ClN_2O$	3300 (62)	—[j]	140 (47)	33 (108)
BRL 37344						17 (89)	380 (20)	1100 (23)	0.40 (130)

a) β-ARs agonistic acitivities were assessed by measurement of cAMP accumulation levels in CHO cells expressing various β-ARs. Expressing levels of human β_1-, β_2-, and β_3-ARs and rat β_3-AR were 12000 receptors/cell, 30000 receptors/cell, 150000 receptors/cell, and 880000 receptors/cell, respectively.[62] *b*) All compounds were prepared by method X (See Experimental Section). *c*) Abbreviaion for the solvents used are as follows: E=ethanol, AC=acetone, EA=ethyl acetate, IP=isopropanol. *d*—*f*) See footnotes *c*—*e* in Table 2. *g*) NT: not tested. *h*) Enantiomeric excess; >99.5%, diastereomeric excess; >99.5%. *i*) Enantiomeric excess; >99.5%, diastereomeric excess; >99.5%. *j*) Could not be calculated because of Low IA. *k*) Oxalic acid. *l*) Enantiomeric excess; >99.5%, diastereomeric excess; 99.5%. *m*) Enantiomeric excess; >99.5%, diastereomeric excess; 98%.

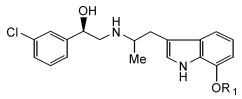
Table 4. Physical Data and Rat and Human β_3-AR Agonistic Activity of Substituted 3-[2-[2-(3-Chlorophenyl)-2-hydroxyethyl]aminoalkyl]-1*H*-indoles



Compd[a]	R_2	R_3	Synthetic method of tryptamines[b] (starting material)	mp, °C (Recryst. solvent[c])	Formula[d]	Human β_3-AR agonistic activity	
						EC_{50} (nM)[e] (IA %)[f]	cAMP accumulation[g] (% at 10^{-7} M)
54[h]	1-Me	H	*i*	101—102.5 (CH–DE)	$C_{19}H_{21}ClN_2O$		6
55[h]	2-Me	H	*i*	202—205 (E)	$C_{19}H_{21}ClN_2O \cdot C_2H_2O_4$[j]		5
56[h]	4-Me	H	*i*	99—101 (E–DE)	$C_{19}H_{21}ClN_2O$		20
57[k]	4-Me	Me	B-1 (P)	165—169 (EA)	$C_{20}H_{23}ClN_2O \cdot 1/10H_2O$	96 (165)	
58[h]	5-Me	H	*i*	172—174 (E–DE)	$C_{19}H_{21}ClN_2O \cdot HCl$		7
59[h]	6-Me	H	*i*	139—141 (E)	$C_{19}H_{21}ClN_2O \cdot 1/4H_2O$	31 (194)	
60[k]	6-Me	Me	A (Q)	124—128 (EA–H)	$C_{20}H_{23}ClN_2O \cdot 1/4H_2O$	12 (95)	
61[h]	7-Me	H	*i*	91—93 (E–DE)	$C_{19}H_{21}ClN_2 \cdot 1/2C_4H_4O_4$[l] $\cdot 1/4H_2O$		0
62[k]	7-Me	Me	A (Q)	97—104 (DE–H)	$C_{20}H_{23}ClN_2O$	35 (91)	
63[h]	4-OMe	H	B-1 (P)	126—128 (CH–DE)	$C_{19}H_{21}ClN_2O_2 \cdot 1/4H_2O$		0
64[h]	5-OMe	H	*i*	166—169 (E)	$C_{19}H_{21}ClN_2O_2 \cdot C_2H_2O_4$[j]		22
65[h]	6-OMe	H	*i*	100—101 (CH–DE)	$C_{19}H_{21}ClN_2O_2$	29 (140)	
66[k]	6-OMe	Me	B-1 (P)	oil	$C_{20}H_{23}ClN_2O_2$	22 (102)	
67[h]	7-OMe	H	*i*	157—158 (E–DE)	$C_{19}H_{21}ClN_2O_2 \cdot HCl$		36
68[k]	7-OMe	Me	A (Q)	143—147 (E–DE)	$C_{20}H_{23}ClN_2O_2 \cdot C_4H_4O_4$[l]	1.7 (113)	
69[k]	6,7-diOMe	Me	A (Q)	203—207 (E–DE)	$C_{21}H_{25}ClN_2O_3 \cdot 1/2C_4H_4O_4$[l]	4.0 (98)	
70[h]	6-OCH_2Ph	H	B-2 (P)	133—134 (E)	$C_{25}H_{25}ClN_2O_2$		5
71[k]	6-OCH_2CO_2Me	Me	B-1 (*b*)	55—65 (CH–DE)	$C_{22}H_{25}ClN_2O_4 \cdot 3/4HCl \cdot 1/2H_2O$	25 (76)	
72[h]	4-Cl	H	B-2 (P)	142—143 (E–DE)	$C_{18}H_{18}Cl_2N_2O$		42
73[h]	5-Cl	H	*i*	135—137 (E–DE)	$C_{18}H_{18}Cl_2N_2O$		8
74[h]	6-Cl	H	*i*	147—148 (E)	$C_{18}H_{18}Cl_2N_2O$		69
75[k]	6-Cl	Me	B-2 (P)	132—135 (DE–H)	$C_{19}H_{20}Cl_2N_2O$	28 (131)	
76[h]	6-Br	H	B-2 (P)	125—127 (IP)	$C_{18}H_{18}BrClN_2O$	540 (103)	
77[k]	6-Br	Me	B-2 (P)	146—149 (EA–H)	$C_{19}H_{20}BrClN_2O$	38 (98)	
78[h]	6-F	H	A (Q)	137—139 (E)	$C_{18}H_{18}ClFN_2O$	53 (127)	
79[k]	6-F	Me	A (Q)	136—140 (EA)	$C_{19}H_{20}ClFN_2O \cdot 1/4H_2O$	5.7 (90)	
80[k]	6-CF_3	Me	A (Q)	oil	$C_{20}H_{20}ClF_3N_2O$	17 (111)	
81[k]	6-CO_2Me	Me	B-1 (P)	118—123 (EA–H)	$C_{21}H_{23}ClN_2O_3$	31 (92)	

a) All compounds were prepared by method X (See Experimental Section). *b*) See Experimental Section. P; substituted 1*H*-indoles, Q; substituted 3-formyl-1*H*-indoles. *c*) Abbreviation for the solvents used are as follows: E=ethanol, DE=diethyl ether, CH=chloroform, EA=ethyl acetate, H=hexane, IP=isopropanol. *d*—*h*) See footnotes *c*—*g* in Table 2. *i*) The commercially available substituted tryptamines as a starting material were used. *j*) Oxalic acid. *k*) Mixture of four diastereoisomers. *l*) Fumaric acid.

Table 5. Physical Data and Rat and Human β_3-AR Agonistic Activity of 7-Substituted 3-[2-[2-(3-Chlorophenyl)-2-hydroxyethyl]aminopropyl]-1*H*-indoles

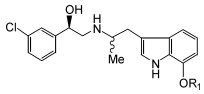


Compd[a)]	R_1	Synthetic method of tryptamines from substituted indoles[b)]	mp, °C (Recryst. solvent[c)])	Formula[d)]	Human β_3-AR agonistic activity EC_{50} (nM)[e)] (IA %)[f)]
82	Me	A	oil	$C_{20}H_{23}ClN_2O_2$	0.67 (114)
83	CH_2Ph	A	115—120 (E)	$C_{26}H_{27}ClN_2O_2$	11 (114)
84[b)]	H		oil	$C_{19}H_{21}ClN_2O_2$	1.7 (128)
85	Et	A	oil	$C_{21}H_{25}ClN_2O_2$	0.96 (96)
86	Pr	A	oil	$C_{22}H_{27}ClN_2O_2$	14 (103)
87	2-Pr	A	oil	$C_{22}H_{27}ClN_2O_2$	2.8 (87)
88	CH_2CO_2Me		oil	$C_{22}H_{25}ClN_2O_4$	0.92 (108)
89	$(CH_2)_2CN$	A	oil	$C_{22}H_{24}ClN_3O_2$	1.2 (113)
90[b)]	CH_2CO_2H		222—224 (dec.) (aq. E)	$C_{21}H_{23}ClN_2O_4 \cdot 3/4H_2O$	0.11 (124)
91	$(CH_2)_2OEt$	A	114—129 (E–DE)	$C_{23}H_{29}ClN_2O_3 \cdot C_2H_2O_4$[g)]	2.3 (87)

a) All compounds except for **84** and **90** were prepared by method X (See Experimental Section). All compounds were mixture of two diastereomers. *b*) See Experimental Section. *c*, *d*) See footnotes *b* and *c* in Table 1. *e*, *f*) See footnotes *d* and *e* in Table 2. *g*) Oxalic acid.

Next, to find selective and more potent human β_3-AR agonists, we introduced various substituents into the 1*H*-indole nucleus. Introduction of an electron-donating group such as methyl and methoxy groups at 1-, 2-, 4-, 5-, 6-, or 7-position of the indole nucleus of **31** and **39** gave a mixture of two diastereomers and four diastereomers **54**—**69** (Table 4). The 1-, 2-, 4-, 5-, and 7-methyltryptamine derivatives **54**—**58**, **61**, **62** displayed lower human β_3-AR agonistic activity than the parent compound **31** or **39**. On the other hand, the 6-methyltryptamines **59** and **60** displayed *ca.* 2-fold more potent, or comparable, β_3-AR agonistic activity than their parents **31** and **39**. In the methoxytryptamines **63**—**68**, on the other hand, the 7-methoxytryptamine **68** exhibited a dramatic improvement in β_3-AR agonistic activity with a much more potent human β_3-AR agonistic activity (EC_{50}=1.7 nM, IA=113%) than that of **39** and the des-methyl counterpart **67**. Other methoxytryptamines (**63**—**67**) had low or moderate agonistic activity at the human β_3-AR. Introduction of two methoxy groups at the 6- and 7-positions (yielding **69**) resulted in a well tolerated β_3-AR agonistic activity. Compound **70** with a more bulky 6-benzyloxy group was a weak human β_3-AR agonist compared with **66**. On the contrary, human β_3-AR agonistic activity of the 6-methoxycarbonylmethoxytryptamine **71** was comparable to that of **66**. Encouraged by these results, we prepared and tested tryptamine derivatives with an electron-withdrawing group. In the tryptamine derivatives **72**—**79** with a halogeno group at the 4-, 5-, and 6-positions, the 6-substituted tryptamines exhibited moderate or potent human β_3-AR agonistic activity. In particular, the 6-fluorotryptamine **79** showed potent β_3-AR agonistic activity (EC_{50}=5.7 nM, IA=90%). Compounds **80** and **81** with CF_3 and ethoxycarbonyl groups had an agonistic activity nearly equipotent to that of the corresponding 6-chlorotryptamine **75**. On the basis of human β_3-AR agonistic activity, compound **68** bearing a 7-methoxy group in the indole nucleus showed the most potent activity and was selected for further modifications of the *O*-substituent at the 7-position. From the above SAR studies on the configuration of the hydroxy center, the *R*-configuration was proved to be important for potent human β_3-AR agonistic activity. Thus, 7-*O*-substituted indole analogues with a (*R*)-hydroxy group were prepared and tested for their agonistic activity at the human β_3-AR. As shown in Table 5, the 7-methoxyindole derivative **82** showed an agonistic activity *ca.* 2.5-fold more potent than that of the mixture of the four diastereomers of **68**. Replacement of the methoxy group of **82** by a bulky benzyloxy group (yielding **83**) caused a profound decrease in agonistic activity. Removal of the benzyl group of **83** gave the 7-hydroxytryptamine **84**, which exhibited a slightly decreased agonistic activity at the human β_3-AR as compared to the 7-methoxytryptamine **82**. When the methoxy group of **82** was replaced with an alkoxy group such as ethoxy (**85**), propoxy (**86**), or isopropoxy (**87**) groups, no favorable influence on the agonistic activity was detected. The agonistic activity of the 7-ethoxytryptamine **85** was practically comparable or somewhat inferior to that of the 7-methoxytryptamine **82**. However, other compounds **86** and **87** showed *ca.* 20-fold and 4-fold, respectively, lower agonistic activity than that of **82**. Next, introduction of an electron-withdrawing group such as methoxycarbonyl, cyano, or free carboxylic acid into the 7-alkoxy group of **82** and **85** was carried out. Compounds **88** and **89** with methoxycarbonyl and cyano groups were essentially equipotent to the corresponding 7-methoxy and 7-ethoxytryptamines **82** and **85**. Interestingly, the acetic acid derivative **90** showed much more potent agonistic activity than the 7-methoxy and the 7-methoxycarbonylmethoxytryptamines **82** and **88** and conferred the highest human β_3-AR agonistic activity (EC_{50}=0.11 nM, IA=124%). It is unclear whether the superiority of the acetic acid **90** relative to the methoxy analogue **82**, the methyl ester **88**, and the cyano analogue **89** is a result of the presence of an acidic proton or subtle conformational difference that permits a more favorable interaction with the human β_3-AR (relative to β_1- and β_2-ARs) in the acetic acid analogue. On the other hand, introduction of an electron-donating group such as ethoxy group (yielding **91**) into the 7-ethoxy group of **85** caused a decrease

Table 6. Physical Data and β-AR Agonistic Activity of the Optical Isomers at Cloned Human β_1-, β_2-, and β_3-ARs and at the Cloned Rat β_3-AR[a]



Compd	R_1	Configuration of methyl center	mp, °C (Recryst. solvent[b])	$[\alpha]^t_D$ (solvent, *c*)	Formula[c]	Agonistic activity; EC_{50} (nM)[d] (IA %)[e]			
						Human β_3-AR	Human β_2-AR	Human β_1-AR	Rat β_3-AR
92	Me	*R*	87—87 (EA–H)	−41.1° 26 °C (MeOH, 1.00)	$C_{20}H_{23}ClN_2O_2$	0.36 (89)	0.13 (118)	5.2 (46)	0.15 (147)
93	Me	*S*	129—130 (EA–H)	+19.6° 26 °C (MeOH, 1.03)	$C_{20}H_{23}ClN_2O_2$	120 (51)	14 (107)	—[f]	6.5 (121)
94	CH_2CO_2Me	*R*	72—74 (M–DE)	−18.4° 24 °C (DMF, 0.10)	$C_{22}H_{25}ClN_2O_4 \cdot C_2H_2O_4$[g]	0.69 (117)	0.94 (24)	2.6 (32)	0.019 (117)
95	CH_2CO_2Me	*S*	98—101 (E)	−66.7° 24 °C (DMF, 0.12)	$C_{22}H_{25}ClN_2O_4$ $C_4H_4O_4$[h]$\cdot$ EtOH	8.6 (113)	NT[i]	22 (89)	NT[i]
96 (AJ-9677)	CH_2CO_2H	*R*	230—231 (dec.) (aq. E)	−23.3° 29 °C (1 N aq. NaOH, 1.00)	$C_{21}H_{23}ClN_2O_4$	0.062 (116)	13 (26)	6.4 (26)	0.016 (110)
97	CH_2CO_2H	*S*	216—218 (dec.) (aq. E)	+31.1° 25 °C (1 N aq. NaOH, 1.00)	$C_{21}H_{23}ClN_2O_4$	10 (84)	130 (83)	—[f]	1.2 (106)

a) See footnote *a* in Table 3. *b*—*e*) See footnotes *c*—*f* in Table 3. *f*) Could not be calculated because of low IA. *g*) Oxalic acid. *h*) Fumaric acid. *i*) NT: not tested.

in agonistic activity. As a result of the SAR studies described above, the optimum 7-substituent for the indole nucleus was concluded to a carboxylmethoxy group.

Finally, the 7-methoxy, 7-methoxycarbonylmethoxy, and 7-carboxylmethoxyindole derivatives (**82**, **88**, and **90**, respectively) with potent agonistic activity at the human β_3-AR were selected for diastereomers separation and agonistic activity examination at the human β_1-, β_2-, and β_3-ARs and rat β_3-AR (Table 6). The optical isomers **92**, **94**, and **96** with an (*R*)-α-methyl group showed much more potent agonistic activity than the corresponding diastereomers **93**, **95**, and **97**, respectively. However, **92** and **94** exhibited poor selectivity for human β_3-AR as they potently stimulated both human the β_1- and β_2-ARs. On the other hand, **96** had an outstanding profile with respect to its agonistic activity for both human and rat β_3-ARs (EC_{50}=0.062 and 0.016 nM, IA=116 and 110%, respectively) with a selectivity for human β_3-AR over 100-fold that for the β_1-AR (EC_{50}=6.4 nM, IA=26%) and 200-fold that for the β_2-AR (EC_{50}=13 nM, IA=26%). A comparison between human β_3-AR agonistic activity of the carboxylic acid derivative **96** and that of the corresponding ester **94** indicated that acidity of the carboxylic acid substituent in the RHS indole nucleus led to a remarkably increased (*ca.* 10-fold) activity at the human β_3-AR and enhanced selectivity at both the β_1- and β_2-ARs. This result is consistent with published data from earlier SARs[62—65] studies indicating that carboxylic acids are usually more selective than the corresponding esters. On the other hand, **50**, which lacks substituent on the RHS of the indole nucleus, displayed the most potent β_1-AR agonistic activity with a modest human β_3-AR agonistic activity. In addition, compound **92** having a 7-methoxy group in the indole nucleus showed the most potent β_2-AR agonistic activity as compared to **96**. Thus, the role of the carboxylic acid substituent in the RHS indole nucleus of **96** can be revealed by comparing its β-AR profile to that of the analogues **50**, **92**, and **94**. These findings indicate that the presence of a 7-carboxylmethoxy group and an *R*-configuration for the α-methyl group are necessary for potent β_3-AR agonistic activity and selectivity against human β_1- and β_2-ARs.

Since compound **96** showed the best *in vitro* profile with respect to humans β_3-AR agonistic activity and selectivity, we decided to investigate its ability to elicit *in vivo* β_3-AR mediated response (anti-obesity and anti-diabetic effects). Compound **96** (0.1 mg/kg) was orally administrated into genetically diabetic obese KK-A^y/Ta mice for 14 d. Control KK-A^y/Ta diabetic obese mice exhibited marked hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and high free fatty acid (FFA) levels compared with normal C57BL/6J mice (Fig. 2). In the mice treated with **96**, plasma glucose concentration and plasma insulin concentration decreased. Furthermore, **96** significantly reduced FFA and triglyceride (TG) concentrations in plasma. These results suggest that **96** stimulates lipid metabolism and improves insulin resistance in diabetic obese mouse model. As shown in Fig. 3, although treatment with **96** (0.1 mg/kg, *p.o.*) did not alter total food intake (control mice; 87.0±4.0 g *vs.* **96**-treated mice; 87.0±2.9 g), no increase in body weight was observed in diabetic obese mice after 14 d (control; 38.1±0.6 g to 40.2±0.7 g *vs.* 37.8±0.8 g to 37.8±1.1 g). It is therefore believed that compound **96** would be useful for the treatment of type II diabetes and obesity.

Conclusion

In this study, replacement of RHS moiety of BRL 37344 and CL 316243 with a heterocycle and further introduction of a variety of substituents on the RHS indole nucleus of the lead compound **31** resulted in the identification of a number of potent and full agonists at the human β_3-AR. In particular, introduction of a polar carboxylic acid functionality at the 7-

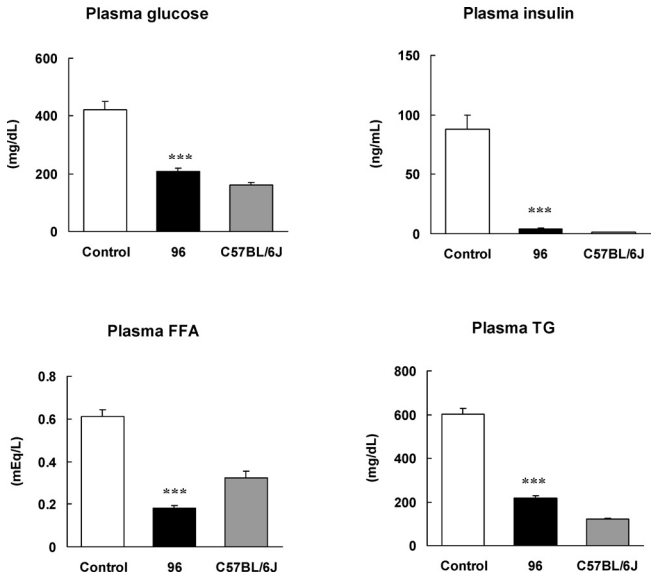


Fig. 2. Effect of **96** (0.1 mg/kg/d, *p.o.*) on Treatment for 14 d on Plasma Parameters in KK-A^y/Ta Diabetic Obese Mice

Data are means±S.E.; $n=6$—8 in KK-A^y/Ta groups and 5 in the C57BL/6J group. Blood was collected under well-fed conditions. ***$p<0.001$ *versus* the control group.

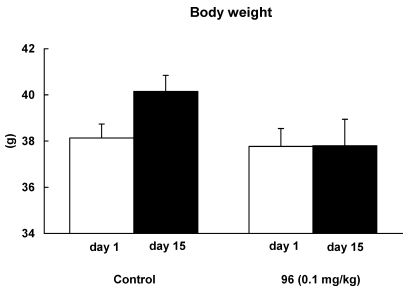


Fig. 3. Effect of **96** (0.1 mg/kg/d, *p.o.*) on Treatment for 14 d on the Body Weight in KK-A^y/Ta Diabetic Obese Mice

The data are expressed as mean±S.E., $n=6$.

position of the indole nucleus diminished both β_1- and β_2-ARs agonistic activity and increased the that for human β_3-AR. Optical resolution of **90** having a carboxylic acid functionality gave **96** (AJ-9677) with an (*R*,*R*)-configuration at the benzyl position, and a hydroxy group and adjacent methyl group at the amino group. Compound **96** exhibited potent human β_3-AR full agonistic activity and good selectivity for human β_3-AR over human β_1- and β_2-ARs. More importantly, we were pleased to discover that the most promising compound **96** we have found exhibits not only strong human β_3-AR agonistic activity with high β_1/β_3 and β_2/β_3 selectivity ratios but also strong rat β_3-AR potency ($EC_{50}=0.016$ nM, IA=110%).

Finally, repeated oral administration of **96** inhibited body weight gain and significantly decreased glucose, insulin, free fatty acid, and triglyceride concentrations in plasma in KK-A^y/Ta mice. On the basis of this pharmacological profile, **96** entered clinical development as a drug for the treatment of type II diabetes and obesity.

Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus without correction. IR spectra were recorded on a Shimadzu FTIR-8200 spectrometer with KBr disks. Atmospheric pressure chemical ionization mass spectra were obtained on a Hitachi M-1000 spectrometer. ^{1}H-NMR spectra were recorded on a Varian Gemini-200 (200 MHz) or a JEOL JNM-LA300 (300 MHz) using dilute solution in $CDCl_3$ unless otherwise stated and coupling constants (*J*) are given in Hz. Chemical shifts are expressed as δ (ppm) values from tetramethylsilane as an internal standard. Optical rotations were measured at 589 nm with a Jasco P-1020 digital polarimeter. Analytical HPLC was performed with a Shimadzu LC-6A, SPD-6A instruments. Organic extracts were dried over anhydrous $MgSO_4$ or anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure. Merck silica gel 60 (70—230 mesh), Fuji Silysia FL60D silica gel (60 μm), or Fuji Silysia BW-200 silica gel (150—350 mesh) was used for column chromatography. The following known compounds were prepared according to the cited literature: 3-(2-aminoethyl)thiophene,[67] 2-(2-aminoethyl)benzothiazole,[68,69] 3-(2-aminoethyl)-1*H*-indazole,[43,44] 3-(2-aminopropyl)-1*H*-indole,[70,71] 3-(2-aminobutyl)-1*H*-indole,[70,71] 3-(2-amino-2-methylpropyl)-1*H*-indole,[72] 1,2,3,4-tetrahydro-3-aminocarbazole,[73] 3-(2-aminoethyl)benzo[*b*]furan,[74] 3-(2-aminoethyl)benzoisoxazole,[75] 2-(2-aminoethyl)benzo[*b*]thiophene,[76,77] 3-(2-aminoethyl)benzo[*b*]thiophene,[78] 6,7-dimethoxy-1*H*-indole,[79] 3-(2-aminoethyl)-2,3-dihydro-1*H*-indole,[80] 2-(2-aminomethyl)-1-methylpyrrole,[81] 2-(2-aminoethyl)-1*H*-indole,[82] 3-(3-aminopropyl)-1*H*-indole,[83] 3-(3-aminopropyl)-7-methoxy-1*H*-indole.[84]

Methyl (1*H*-Indol-6-yloxy)acetate A mixture of the commercially available 6-hydroxy-1*H*-indole (2.9 g, 22 mmol), methyl chloroacetate (2.7 g, 25 mmol), K_2CO_3 (5.5 g, 40 mmol), KI (0.2 g), and acetone (100 ml) was heated to reflux for 8 h and cooled to room temperature. The insoluble materials were removed by filtration, and the filtrate was concentrated to dryness. The residue was chromatographed on silica gel with $CHCl_3$ to give 2.8 g (63%) of the title compound as an oil. ^{1}H-NMR δ: 3.78 (s, 3H), 4.67 (s, 2H), 6.49 (m, 1H), 6.83 (m, 1H), 6.88 (s, 1H), 7.11 (m, 1H), 7.53 (m, 1H), 8.2 (br s, 1H). MS *m*/*z*: 206 (MH^+). In a manner similar to that described above, 7-ethoxy-, 7-propoxy-, 7-isopropoxy-, and 7-(2-ethoxyethoxy)-1*H*-indoles were prepared from 7-hydroxy-1*H*-indole and the corresponding halides (iodoethane, 1-bromopropane, 2-iodopropane, 2-bromoethyl ethyl ether) in *N*,*N*-dimethylformamide (DMF) instead of acetone at 90 °C.

7-(2-Cyanoethoxy)-1*H*-indole A mixture of 7-hydroxy-1*H*-indole (5.0 g, 38 mmol), triton B (0.5 ml), and acrylonitrile (9 ml, 0.14 mol) was heated to reflux for 20 h and cooled to room temperature. The reaction mixture was concentrated to dryness, and the residue was purified by silica gel column chromatography with AcOEt/hexane=2/1 to give 6.2 g (89%) of the title compound as an yellow oil. ^{1}H-NMR δ: 2.8 (t, 2H, *J*=6), 4.30 (t, 2H, *J*=6), 6.4—7.5 (m, 5H), 8.6 (br s, 1H). MS *m*/*z*: 187 (MH^+).

Method A. General Procedure (1) Phosphorus oxychloride (5 ml) was added dropwise to DMF (16 ml) under ice-cooling. The mixture was stirred at the same temperature for 10 min. A solution of substituted 1*H*-indole (44 mmol) in DMF (16 ml) was added dropwise to the mixture at *ca.* 5 °C. After the temperature was raised to room temperature, the mixture was stirred for 2 h. Then, 30% aqueous NaOH (30 ml) was added under ice-cooling, and the mixture was heated at 80 °C for 5 min and cooled to room temperature. The resulting precipitate was collected by filtration, washed with water, and dried to give of substituted 3-formyl-1*H*-indole.

(2) A mixture of ammonium acetate (3.6 g, 47 mmol), Ac_2O (1 ml), and AcOH (3.2 ml) was heated at 50 °C for 20 min. After addition of substituted 3-formyl-1*H*-indole (26 mmol), nitromethane or nitroethane (16 ml), and AcOH (19.2 ml), the mixture was raised to 100 °C and AcONa (2.25 g, 27 mmol) was added. The whole was heated to reflux for 2 h while Ac_2O (3.2 ml) was gradually added dropwise. After being cooled to room temperature, water was added to the resulting mixture. The precipitate was collected by filtration, washed with water, and dried to afford of 1-(substituted 1*H*-indol-3-yl)-2-nitroethylene or -2-nitropropene.

(3) A solution of 1-(substituted 1*H*-indol-3-yl)-2-nitropropene or -2-nitroethylene (14 mmol) in anhydrous THF (60 ml) was added dropwise to a suspension of $LiAlH_4$ (3.2 g, 84 mM) in anhydrous Et_2O (60 ml) under ice-cooling. The mixture was heated to reflux for 5.5 h and cooled to *ca.* 5 °C. A saturated aqueous sodium potassium tartarate was carefully added to the mixture at the same temperature, and the insoluble materials were removed by filtration. The filtrate was dried over anhydrous $MgSO_4$. The solvent was evaporated to give substituted 3-(2-aminoethyl or propyl)-1*H*-indole, which was used in the next step without further purification.

Method B-1. Typical Procedure; Methyl [3-(2-Aminopropyl)-1*H*-indol-6-yloxy]acetate (1) A mixture of methyl (1*H*-indol-6-yloxy)acetate (2.8 g, 14 mmol), 2-nitro-1-propene (3.6 g, 41 mmol), and benzene (68 ml) was heated to reflux for 15 h and cooled to room temperature. The solvent was evaporated, and the residue was chromatographed on silica gel with $CHCl_3$ to give 2.15 g (54%) of methyl [3-(2-nitropropyl)-1*H*-indol-6-

yloxy]acetate as an oil. ^{1}H-NMR δ: 1.56 (d, 3H, J=7), 3.10—3.48 (m, 2H), 3.77 (s, 3H), 4.64 (s, 2H), 4.92 (m, 1H), 6.76—7.05 (m, 3H), 7.48 (m, 1H), 8.08 (s, 1H). MS m/z: 293 (MH^+).

(2) A solution of methyl [3-(2-nitropropyl)-1*H*-indol-6-yloxy]acetate (2.1 g, 7.2 mmol) in EtOH (50 ml) was hydrogenated over Raney-nickel (*ca.* 100 mg) at 50 °C under atmospheric pressure. After the theoretical amount of hydrogen was consumed, the catalyst was filtered off. The filtrate was concentrated to dryness to give *ca.* 1.9 g (quantitative yield) of the title compound as an oil, which was used in the next step without further purification. MS m/z: 263 (MH^+).

Method B-2. Typical Procedure; 3-(Aminopropyl)-7-(2-cyanoethoxy)-1*H*-indole (1) A mixture of 7-(2-cyanoethoxy)-1*H*-indole (6.1 g, 33 mmol), a solution of 2-nitro-1-propene (8.6 g, 99 mmol) in toluene (62 ml), molecular sieves 13X (1.2 g), and toluene (60 ml) was heated at 100 °C for 16 h and cooled to room temperature. After evaporation of the solvent, the residue was chromatographed on silica gel with AcOEt/hexane=1/2 to give 6.3 g of crude 7-(2-cyanoethoxy)-3-(2-nitropropyl)-1*H*-indole. MS m/z: 274 (MH^+).

(2) A solution of crude 7-(2-cyanoethoxy)-3-(2-nitropropyl)-1*H*-indole (6.0 g) in EtOH (50 ml) was added to a mixture of Fe (12 g, 0.21 mol), NH_4Cl (3.0 g, 56 mmol), EtOH (100 ml), and water (100 ml) at room temperature. The mixture was heated to reflux for 2.5 h and cooled to room temperature. The insoluble materials were filtered off, and the volatiles were evaporated. After addition of aqueous K_2CO_3 to the resulting aqueous solution, the mixture was extracted with $CHCl_3$. The extract was washed with brine and concentrated to dryness to give 2.3 g (30% yield for 2 steps) of the title compound as a brown oil, which was used in the next step without further purification. MS m/z: 244 (MH^+).

Methyl [3-(2-Aminopropyl)-1*H*-indol-7-yloxy]acetate (8) (1) A solution of 3-(2-aminopropyl)-7-benzyloxy-1*H*-indole (**4**, 3.7 g, 13 mmol), di-*tert*-butyl dicarbonate (2.9 g, 13 mmol), and Et_3N (1.8 ml, 13 mmol) in $CHCl_3$ (50 ml) was stirred at room temperature for 16 h. The reaction mixture was washed successively with water, 10% aqueous citric acid, water, and brine and dried over anhydrous $MgSO_4$. The solvent was evaporated and the residue was chromatographed on silica gel with $CHCl_3$ to 4.0 g (80%) of 7-benzyloxy-3-[2-(*tert*-butoxycarbonyl)aminopropyl]-1*H*-indole (**5**) as a white solid, which was used in the next step without further purification.

(2) A solution of **5** (8.0 g, 21 mmol) in EtOH (80 ml) was hydrogenated at atmospheric pressure over 10% Pd on carbon (0.8 g) at *ca.* 50 °C. After the theoretical amount of hydrogen was absorbed, the catalyst was filtered off. The filtrate was concentrated to dryness to give *ca.* 6 g of 3-[2-(*tert*-butoxycarbonyl)aminopropyl]-7-hydroxy-1*H*-indole (**6**) as an oil.

(3) A mixture of crude **6** (*ca.* 6 g, *ca.* 21 mmol), methyl chloroacetate (2.3 g, 21 mmol), K_2CO_3 (2.9 g, 21 mmol), KI (0.2 g), MeOH (40 ml), and acetone (80 ml) was heated to reflux for 4 h and cooled to room temperature. After the insoluble materials were filtered off, the filtrate was concentrated to dryness. The residue was chromatographed on silica gel with AcOEt/hexane=2/3 to give 4.5 g (59% yield from **5**) of methyl [3-[2-(*tert*-butoxycarbonyl)aminopropyl]-1*H*-indol-7-yloxy]acetate (**7**) as a brown oil. ^{1}H-NMR δ: 1.10 (d, 3H, J=6), 1.44 (s, 9H), 2.82—3.02 (m, 2H), 3.81 (s, 3H), 4.00 (m, 1H), 4.42 (br s, 1H), 4.86 (s, 2H), 6.57 (d, 1H, J=8), 7.00 (t, 1H, J=8), 7.02 (d, 1H, J=2), 7.31 (d, 1H, J=8), 8.65 (br s, 1H). MS m/z: 363 (MH^+), 307, 263, 231.

(4) A solution of **7** (4.2 g, 12 mmol) in 10% HCl in MeOH (21 ml) was stirred at room temperature for 1 h. After addition of aqueous K_2CO_3, the mixture was extracted with $CHCl_3$. The extract was washed with brine and concentrated to dryness to give 3.0 g (quantitative yield) of **8** as a gray solid, which was used in the next step without further purification. ^{1}H-NMR δ: 1.16 (d, 3H, J=6), 1.45 (br s, 2H), 2.63 (dd, 1H, J=8, 15), 2.86 (ddd, 1H, J=1, 6, 15), 3.28 (m, 1H), 3.81 (s, 3H), 4.76 (s, 2H), 6.58 (d, 1H, J=8), 6.99 (t, 1H, J=8), 7.04 (s, 1H), 7.28 (d, 1H, J=8), 8.74 (br s, 1H). MS m/z: 263 (MH^+).

Method X. Typical Procedure; 1-(3-Chlorophenyl)-2-[2-(7-methoxy-1*H*-indol-3-yl)propyl]aminoethanol Fumarate (68) A solution of 3-chlorostyrene oxide (**9**, 0.77 g, 5.0 mmol) and 3-(2-aminopropyl)-7-methoxy-1*H*-indole (2.16 g, 10.6 mmol) in MeOH (20 ml) was stirred at room temperature for 64 h. After evaporation of the solvent, the residue was chromatographed on silica gel with $CHCl_3$/MeOH=12/1 to give 1.1 g (62% from **9**) of the free base of **68** as an oil. ^{1}H-NMR δ: 1.12 (d, 3H, J=7), 2.61 (m, 1H), 2.78—2.86 (m, 2H), 2.88—3.12 (m, 2H), 3.95 (s, 3H), 4.56 (m, 1H), 6.65 (m, 1H), 6.93—7.09 (m, 2H), 7.11—7.29 (m, 4H), 7.33 (m, 1H), 8.25 (br s, 1H). MS m/z: 359 (MH^+). The oil was converted to the fumarate in the usual manner.

1-(3-Chlorophenyl)-2-[2-(1*H*-indol-3-yl)butyl]aminoethanol (40) ^{1}H-NMR δ: 1.00 (dt, 3H, J=1, 6), 1.42—1.62 (m, 2H), 2.59 (ddd, 1H, J=9, 7, 12.5), 2.65—3.05 (m, 6H), 4.48 (dd, 0.5H, J=4, 7), 4.61 (dd, 0.5H, J=4, 7), 7.00 (dd, 1H, J=2, 8), 7.02—7.4 (m, 7H), 7.58 (m, 1H), 8.01 (br s, 1H). MS m/z: 343 (MH^+), 212, 172, 130.

1-(3-Chlorophenyl)-2-[2-(6-methoxy-1*H*-indol-3-yl)propyl]aminoethanol (66) ^{1}H-NMR δ: 1.15 (dd, 3H, J=3, 6), 2.4—3.15 (m, 7H), 3.81 (s, 3H), 4.52 (dd, 0.5H, J=4, 6), 4.64 (dd, 0.5H, J=4, 6), 6.7—6.94 (m, 3H), 7.05—7.47 (m, 4H), 7.42 (dd, 1H, J=6, 10), 7.96 (br s, 1H). MS m/z: 359 (MH^+).

1-(3-Chlorophenyl)-2-[2-(6-trifluoromethyl-1*H*-indol-3-yl)propyl]-aminoethanol (80) ^{1}H-NMR δ: 1.13 (d, 3H, J=6), 2.5—3.12 (m, 6H), 4.52 (dd, 0.5H, J=2, 6), 4.64 (dd, 0.5H, J=2, 6), 6.93 (m, 1H), 7.05—7.43 (m, 5H), 7.58—7.65 (m, 2H), 8.35 (br s, 1H). MS m/z: 397 (MH^+).

(1*R*)-(3-Chlorophenyl)-2-[2-(7-methoxy-1*H*-indol-3-yl)propyl]aminoethanol (82) ^{1}H-NMR δ: 1.13 (d, 3H, J=6), 1.8 (br, 2H), 2.61 (dt, 1H, J=12, 8), 2.77—3.12 (m, 4H), 3.96 (s, 3H), 4.50 (dd, 0.5H, J=4, 7), 4.61 (dd, 0.5H, J=4, 7), 6.65 (d like, 1H, J=8), 6.98 (dd, 1H, J=2, 6), 7.04 (dt, 1H, J=2, 8), 7.12—7.25 (m, 4H), 7.32 (m, 1H), 8.20 (br s, 1H). MS m/z: 359 (MH^+).

(1*R*)-(3-Chlorophenyl)-2-[2-(7-ethoxy-1*H*-indol-3-yl)propyl]aminoethanol (85) ^{1}H-NMR δ: 1.12 (d, 3H, J=6), 1.48 (t, 3H, J=7), 2.4 (br, 2H), 2.60 (dt, 1H, J=12, 8), 2.77—2.96 (m, 3H), 3.04 (m, 1H), 4.20 (q, 2H, J=7), 4.49 (dd, 0.5H, J=4, 9), 4.60 (dd, 0.5H, J=4, 9), 6.63 (d, 1H, J=7), 6.91—7.06 (m, 2H), 7.10—7.25 (m, 4H), 7.32 (m, 1H), 8.27 (br s, 1H). MS m/z: 373 (MH^+).

(1*R*)-(3-Chlorophenyl)-2-[2-(7-propoxy-1*H*-indol-3-yl)propyl]aminoethanol (86) ^{1}H-NMR δ: 1.05 (d, 3H, J=6), 1.11 (t, 3H, J=7), 1.90 (sex, 2H, J=7), 2.1 (br, 2H), 2.61 (dt, 1H, J=12, 12), 2.81 (d, 2H, J=6), 2.87—3.12 (m, 2H), 4.10 (t, 2H, J=7), 4.49 (dd, 0.5H, J=3, 8), 4.60 (dd, 0.5H, J=3, 8), 6.62 (d, 1H, J=7), 6.95 (m, 1H), 7.03 (dd, 1H, J=2, 7), 7.1—7.25 (m, 4H), 7.31 (d, 1H, J=2), 8.25 (br s, 1H). MS m/z: 387 (MH^+), 188.

(1*R*)-(3-Chlorophenyl)-2-[2-(7-isopropoxy-1*H*-indol-3-yl)propyl]-aminoethanol (87) ^{1}H-NMR δ: 1.12 (d, 3H, J=6), 1.40 (d, 6H, J=7), 2.0 (br, 2H), 2.61 (m, 1H), 2.81 (d, 2H, J=7), 2.88—3.15 (m, 2H), 4.50 (dd, 0.5H, J=4, 9), 4.60 (dd, 0.5H, J=4, 9), 4.72 (quint, 1H, J=7), 6.64 (d, 1H, J=7), 6.9—7.1 (m, 2H), 7.1—7.3 (m, 4H), 7.32 (d, 1H, J=3), 8.22 (br s, 1H). MS m/z: 387 (MH^+), 188.

Methyl [3-[2-[[(2*R*)-(3-Chlorophenyl)-2-hydroxyethyl]amino]propyl]-1*H*-indol-7-yloxy]acetate (88) ^{1}H-NMR δ: 1.13 (d, 3H, J=6), 2.38 (br, 2H), 2.62 (dt, 1H, J=12, 8), 2.77—3.13 (m, 4H), 2.81 (s, 3H), 4.52 (dd, 0.5H, J=4, 7), 4.62 (dd, 0.5H, J=4, 7), 4.75 (s, 2H), 6.57 (d, 1H, J=8), 6.92—7.06 (m, 2H), 7.11—7.36 (m, 5H), 8.77 (br s, 1H). MS m/z: 417 (MH^+), 385.

(1*R*)-(3-Chlorophenyl)-2-[2-[7-(2-cyanoethoxy)-1*H*-indol-3-yl]propyl]-aminoethanol (89) ^{1}H-NMR δ: 1.14 (d, 3H, J=6), 2.22 (br, 2H), 2.63 (dt, 2H, J=10, 14), 2.90 (t, 2H, J=6), 3.05 (m, 1H), 2.7—3.0 (m, 2H), 4.36 (t, 2H, J=6), 4.54 (dd, 0.5H, J=4, 9), 4.63 (dd, 0.5H, J=4, 9), 6.62 (d, 1H, J=8), 6.95—7.10 (m, 2H), 7.10—7.40 (m, 5H), 8.35 (br s, 1H). MS m/z: 398 (MH^+), 345.

Method Y. Typical Procedure; (2*R*)-[2-(3-Chlorophenyl)-2-hydroxyethyl]amino-3-(1*H*-indol-3-yl)propionamide Oxalate (45) A mixture of the free base of **43** (1.0 g, 2.7 mmol), 28% aqueous NH_3 (10 ml), and saturated NH_3 in EtOH (10 ml) was stirred at room temperature for 20 h and concentrated. The residue was dissolved in $CHCl_3$ and the solution was washed successively with water and brine and dried over anhydrous $MgSO_4$. The solvent was evaporated to give *ca.* 1 g (quantitative yield) of the free base of **45** as an oil. The oil was converted to the oxalate in usual manner. ^{1}H-NMR [dimethylsulfoxide (DMSO)-d_6] δ: 2.73—3.0 (br, 2H), 3.1—3.35 (br, 2H), 3.89 (br, 1H), 4.86 (br, 1H), 6.85—7.6 (m, 10H), 7.64 (d, 1H, J=7), 7.75 (s, 2H), 10.99 (s, 1H). MS m/z: 358 (MH^+).

1-(3-Chlorophenyl)-2-[3-hydroxy-(2*R*)-(1*H*-indol-3-yl)propyl]aminoethanol Oxalate (47) To a mixture of the free base of **43** (1.12 g, 3.0 mmol), anhydrous LiCl (0.25 g, 5.9 mmol), $NaBH_4$ (0.22 g, 5.8 mmol), and anhydrous THF (10 ml) was added dropwise anhydrous EtOH (20 ml) under ice-cooling. The mixture was stirred at *ca.* 20 °C for 16 h and concentrated to dryness. The resultant residue was diluted with water and extracted with $CHCl_3$. The extract was washed with brine, and the solvent was evaporated. The residue was chromatographed on silica gel with $CHCl_3$/MeOH=10/1 to give 0.42 g (41%) of the free base of **47** as an oil. The oil was converted to the oxalate in usual manner. ^{1}H-NMR (DMSO-d_6) δ: 2.88—3.73 (m, 7H), 4.98 (m, 1H), 6.94—7.16 (m, 3H), 7.24 (s, 1H), 7.26—7.55 (m, 4H), 7.64 (d, 1H, J=7), 11.00 (s, 1H). MS m/z: 345 (MH^+).

(2*R*)-[2-(3-Chlorophenyl)-2-hydroxyethyl]amino-3-(1*H*-indol-3-yl)propionic Acid (49) A mixture of the free of **43** (1.0 g, 2.7 mmol), 1 N aqueous NaOH (5 ml, 5.0 mmol), and MeOH (5 ml) was stirred at room temperature for 3 h. After being cooled to *ca.* 5 °C, 1 N aqueous HCl (5 ml) was added to the reaction mixture. The resulting precipitate was collected by filtration, washed successively with water and 20% aqueous MeOH, and dried to give 0.62 g (64%) of **49** as a white crystal. ^{1}H-NMR (DMSO-d_6) δ: 2.5—2.8 (m, 2H), 2.8—3.5 (m, 2H), 3.51 (td, 1H, J=7, 14), 4.65 (dd, 1H, J=4, 6), 6.9—7.5 (m, 8H), 7.53 (d, 1H, J=7), 10.86 (br s, 1H). MS *m/z*: 359 (MH^+).

Method Z. (1*R*)-(3-Chlorophenyl)-2-[(2*S*)-(1*H*-indol-3-yl)propyl]-aminoethanol Oxalate (51) (1) Triethylamine (1.1 ml, 7.9 mmol) was added to a mixture of (*R*)-(−)-3-chloromandelic acid [(*R*)-**10**, 840 mg, 4.5 mmol], (*S*)-3-(2-aminopropyl)-1*H*-indole [(*S*)-**11**, 780 mg, 4.5 mmol], benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (1.99 g, 4.5 mmol), and DMF (10 ml) at *ca.* 20 °C. The mixture was stirred at the same temperature for 2 h. After addition of AcOEt, the solution was washed successively with water, 10% aqueous HCl, water, 1 N aqueous NaOH, water, and brine. The organic solution was dried over anhydrous Na_2SO_4 and concentrated to give *ca.* 1.5 g of *N*-[(2*S*)-(1*H*-indol-3-yl)propyl]-(*R*)-3-chloromandelamide (**13**) as an yellow oil. ^{1}H-NMR δ: 1.16 (d, 3H, J=7), 2.91 (d, 2H, J=7), 2.95 (br, 1H), 4.34 (m, 1H), 4.89 (s, 1H), 6.12 (br d, 1H, J=10), 6.91 (s, 1H), 6.96—7.31 (m, 6H), 7.39 (dd, 1H, J=2, 8), 7.60 (dd, 1H, J=2, 8), 8.08 (br s, 1H). MS *m/z*: 343 (MH^+). In a manner similar to that described above, *N*-[(2*R*)-(1*H*-indol-3-yl)propyl]-(*R*)-3-chloromandelamide (**12**) was prepared from (*R*)-(−)-3-chloromandelic acid and (*S*)-3-(2-aminopropyl)-1*H*-indole, mp 126—129 °C (AcOEt/hexane). ^{1}H-NMR δ: 1.18 (d, 3H, J=6.5), 2.87 (d, 2H, J=6.0), 2.88 (br, 1H), 4.34 (m, 1H), 4.85 (s, 1H), 5.99 (br d, 1H, J=8), 6.70 (d, 1H, J=2.5), 7.04—7.38 (m, 7H), 7.53 (dd, 1H, J=2, 9.5), 8.03 (br s, 1H). MS *m/z*: 343 (MH^+). $[\alpha]_D^{28}$ −26.3° (c=0.51, MeOH).

(2) 1 M Borane–THF complex (20 ml, 20 mmol) was added dropwise to the solution of **13** (*ca.* 1.5 g) in anhydrous THF (30 ml) at *ca.* 20 °C. The mixture was heated to reflux for 4 h and cooled to *ca.* 5 °C. After careful addition of MeOH (10 ml), the mixture was heated to reflux for 1.5 h and cooled to room temperature. The solvent was evaporated and the residue was dissolved in AcOEt. The solution was washed successively with water and brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was chromatographed on silica gel with $CHCl_3$/MeOH=15/1 to 10/1 to give 730 mg [49% from (*R*)-**10**] of the free base of **51** as a reddish oil. ^{1}H-NMR δ: 1.21 (d, 3H, J=6), 2.63 (dd, 1H, J=9, 12), 2.93 (dd, 2H, J=6.5, 3.5), 3.0—3.2 (m, 2H), 3.4 (br, 2H), 4.75 (dd, 1H, J=9, 3.5), 7.05 (d, 1H, J=2), 7.0—7.42 (m, 7H), 7.57 (dd, 1H, J=7, 2), 8.14 (br s, 1H). MS *m/z*: 329 (MH^+). The oil was converted to the oxalate in usual manner. In a manner similar to that described above, **50** was obtained from **12** in 58% yield. ^{1}H-NMR δ: 1.15 (d, 3H, J=6), 2.5 (2H, br), 2.64 (dd, 1H, J=9, 12), 2.85 (d, 1H, J=6), 2.88 (dd, 2H, J=4, 16), 3.05 (m, 1H), 4.51 (dd, 1H, J=8.5, 3.5), 7.03 (d, 1H, J=2), 7.07—7.42 (m, 7H), 7.61 (dd, 1H, J=9.5, 2), 8.04 (br s, 1H). MS *m/z*: 329 (MH^+). $[\alpha]_D^{28}$ −46.4° (c=0.50, MeOH). In a manner similar to that described above, the corresponding enantiomers **53** and **52** of **51** and **50**, respectively, were prepared from (*S*)-**10** and (*R*)-**11** or (*S*)-**11**.

By chiral HPLC analysis under the conditions described below, the optical purity of **50**—**53** was determined. Retention time of HPLC: **50**; 14.7 min, **51**; 27.7 min, **52**; 16.4 min, **53**; 18.3 min. Separation conditions for HPLC: Column; Ultron ES-OVM (4.6 mmϕ×150 mm; Shinwa Chemical Industries, Ltd., Japan), Eluent; 20 mM aqueous KH_2PO_4 (pH 3.6)/(MeOH/MeCN=3/1)=92/8, Flow rate; 1.0 ml/min, Temperature; 25 °C, Detection; 220 nm.

1-(3-Chlorophenyl)-2-[(1*H*-indol-3-yl)methyl]aminoethanol (33) A mixture of 3-formy-1*H*-indole (730 mg, 5.0 mmol), 2-amino-1-(3-chlorophenyl)ethanol (940 mg, 5.5 mmol), $NaHCO_3$ (630 mg, 7.5 mmol), and MeOH (10 ml) was heated to reflux for 3 h and cooled to *ca.* 5 °C. After addition of $NaBH_4$ (280 mg, 7.4 mmol), the reaction mixture was stirred at room temperature for 2 h and concentrated to dryness. The residue was dissolved in a mixture of water and $CHCl_3$, and the organic layer was separated and dried over anhydrous $MgSO_4$. The solvent was evaporated, and the residue was chromatographed on silica gel with $CHCl_3$/MeOH=9/1 to give 230 mg (15% from 3-formyl-1*H*-indole) of **33** as a solid. ^{1}H-NMR (DMSO-d_6) δ: 2.6—2.85 (m, 2H), 3.90 (d, 1H, J=13), 3.98 (d, 1H, J=13), 4.7 (m, 1H), 6.85—7.16 (m, 2H), 7.16—7.46 (m, 6H), 7.59 (d, 1H, J=7). MS *m/z*: 301 (MH^+).

3-[(2*R*)- and 3-[(2*S*)-(7-Benzyloxy-1*H*-indol-3-yl)propyl]-(5*R*)-(3-chlorophenyl)-1,3-oxazolidin-2-ones (16, 17) (1) A solution of 3-(3-aminopropyl)-7-benzyloxy-1*H*-indole (**4**, 9.0 g, 32 mmol) and (*R*)-**9** (3.1 g, 20 mmol) in MeOH (100 ml) was stirred at room temperature for 28 h and concentrated to dryness. The residue was chromatographed on silica gel with $CHCl_3$/MeOH=20/1 to $CHCl_3$/MeOH/saturated aqueous NH_3=10/1/0.1 to give 4.1 g [47% from (*R*)-**9**] of **83** as a pale brown solid and to recover 3.1 g (34%) of **4** as a pale yellow solid in that order.

(2) A mixture of **83** (3.9 g, 9.0 mmol), *N*,*N*′-carbonyldiimidazole (CDI, 3.1 g, 19 mmol), Et_3N (7.9 ml, 57 mmol), and THF (40 ml) was stirred at room temperature for 16 h and concentrated to dryness. The residue was dissolved in AcOEt, and the solution was washed successively with water, 10% aqueous citric acid, water, and brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue containing a mixture of **16** and **17** was chromatographed on silica gel with AcOEt/hexane=1/3 to 1/1 to give 2.0 g (48%) of **16** as a pale brown oil and 1.5 g (36%) of **17** as a pale brown oil in that order. **16**; ^{1}H-NMR δ: 1.30 (d, 3H, J=6), 2.94 (d, 2H, J=6), 3.22 (dd, 1H, J=5, 7), 3.80 (t, 1H, J=5), 4.40 (m, 1H), 5.20 (s, 2H), 5.31 (dd, 1H, J=6, 8), 6.71 (d, 1H, J=7), 6.85 (d like, 1H, J=7), 6.99 (dd, 2H, J=7, 7), 7.05—7.12 (m, 2H), 7.12—7.28 (m, 2H), 7.34—7.52 (m, 5H), 8.26 (br s, 1H). MS *m/z*: 461 (MH^+), 460. **17**; ^{1}H-NMR δ: 1.27 (d, 3H, J=6), 3.00 (t, 2H, J=6), 3.22 (dd, 1H, J=6, 6), 3.69 (t, 1H, J=5), 4.30 (m, 1H), 5.20 (s, 2H), 5.21 (dd, 1H, J=6, 8), 6.74 (d, 1H, J=7), 7.02—7.14 (m, 3H), 7.19—7.32 (m, 5H), 7.32—7.52 (m, 4H), 8.29 (br s, 1H). MS *m/z*: 461 (MH^+), 460.

[3-[(2*R*)- and [3-[(2*S*)-[[(2*R*)-(3-Chlorophenyl)-2-hydroxyethyl]amino]-propyl]-1*H*-indol-7-yloxy]acetic Acids (96, 97) (1) A solution of **16** (0.8 g, 1.8 mmol) in EtOH (15 ml) was hydrogenated at atmospheric pressure over 5% Rh on carbon (0.1 g) at 50—55 °C. After *ca.* 36 h, the catalyst was filtered off, and the filtrate was concentrated to dryness. The residue was chromatographed on silica gel with AcOEt/hexane=1/1 to give 0.18 g (23% recovery) of **16** and 0.49 g (77%) of (5*R*)-(3-chlorophenyl)-3-[(2*R*)-(7-hydroxy-1*H*-indol-3-yl)propyl]-1,3-oxazolidin-2-one (**18**) as a pale brown amorphous solid in that order. Crude **18** was used in the next step without further purification.

(2) A mixture of **18** (0.45 g, 1.3 mmol), methyl chloroacetate (0.16 g, 1.5 mmol), K_2CO_3 (0.18 g, 1.3 mmol), KI (20 mg), MeOH (1 ml), and acetone (10 ml) was heated to reflux for 3 h and cooled to room temperature. The reaction mixture was concentrated to dryness, and residue was chromatographed on silica gel with AcOEt/hexane=3/2 to give 0.36 g (67%) of methyl [3-[(2*R*)-[(5*R*)-(3-chlorophenyl)-1,3-oxazolidin-2-one-3-yl]propyl]-1*H*-indol-7-yloxy]acetate (**20**) as a pale yellow oil, which was used in the next step without further purification. ^{1}H-NMR δ: 1.30 (d, 3H, J=7), 2.94 (d, 2H, J=8), 3.23 (dd, 1H, J=8, 9), 3.80 (t, 1H, J=9), 3.81 (s, 3H), 4.41 (m, 1H), 4.76 (2H, s), 5.33 (dd, 1H, J=7, 8), 6.57 (d, 1H, J=8), 6.88 (d, 1H, J=8), 6.9—7.03 (m, 2H), 7.05—7.2 (m, 3H), 7.23 (d, 1H, J=8), 8.61 (br s, 1H). MS *m/z*: 443 (MH^+), 247, 220.

(3) A solution of **20** (0.36 g, 0.81 mmol), NaOH (0.9 g, 22.5 mmol), water (4.5 ml), and EtOH (9 ml) was heated to reflux for 18 h and cooled to room temperature. After evaporation of the volatiles, the residue was dissolved in 2 N aqueous HCl (15 ml). The solution was purified by CHP 20P (75—150 μ, Mitsubishi Chemical Co., Ltd., Japan) with water to 70% aqueous MeCN, and the fractions containing **96** were collected. The solvent was evaporated, and the residue was dissolved in a mixture of EtOH and Et_2O. After reevaporation of the volatiles, the residual solid was triturated with aqueous EtOH to give 0.24 g (73%) of **96** as a gray solid. IR ν_{max}; 3134, 1609, 1406, 1250, 1057, 783 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.93 (d, 3H, J=6.0), 2.5—3.3 (m, 8H), 4.55 (s, 2H), 4.85 (dd, 1H, J=9.8, 2.6), 6.42 (d, 1H, J=7.8), 6.78 (t, 1H, J=7.8), 6.94 (d, 1H, J=2.0), 6.99 (d, 1H, J=7.8), 7.27—7.33 (m, 3H), 7.46 (s, 1H), 11.02 (br s, 1H). MS *m/z*: 403 (MH^+).

In a manner similar to that described above, **97** was prepared from **17** *via* **19** and **21**. **21**; ^{1}H-NMR δ: 1.26 (d, 3H, J=7), 2.89—3.12 (m, 2H), 3.24 (dd, 1H, J=8, 9), 3.69 (t, 1H, J=9), 3.82 (s, 3H), 4.37 (sex, 1H, J=7), 4.77 (2H, s), 5.24 (t, 1H, J=8), 6.58 (d, 1H, J=8), 7.03 (t, 1H, J=8), 7.07—7.16 (m, 2H), 7.23—7.35 (m, 4H), 8.61 (br s, 1H). MS *m/z*: 443 (MH^+), 411, 198. **97**; IR ν_{max}; 1603, 1421, 1261, 1057, 787 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.91 (d, 3H, J=6.0), 2.61 (m, 1H), 2.78—3.19 (m, 8H), 4.55 (s, 2H), 4.88 (m, 1H), 6.48 (d, 1H, J=7.8), 6.78 (t, 1H, J=7.8), 6.92—7.05 (m, 2H), 7.28—7.39 (m, 3H), 7.46 (s, 1H), 11.02 (br s, 1H). MS *m/z*: 403 (MH^+).

Retention time of HPLC: **96**; 29.7 min, **97**; 15.0 min. Separation conditions for HPLC: Column; Chiral AGP (4.0 mmϕ×100 mm; Shinwa Chemical Industries Ltd., Japan), Eluent; 20 mM aqueous K_2HPO_4+2 M aqueous $Bu_4N^+HSO_4^-$ (pH=7.0)/2-PrOH=92/8, Flow rate; 0.7 ml/min, Temperature; 30 °C, Detection; 220 nm.

Methyl [3-[(2*R*)-[[(2*R*)-(3-Chlorophenyl)-2-hydroxyethyl]amino]propyl]-1*H*-indol-7-yloxy]acetate Oxalate (94) and Methyl [3-[(2*S*)-[[(2*R*)-(3-Chlorophenyl)-2-hydroxyethyl]amino]propyl]-1*H*-indol-7-yloxy]ac-

etate Fumarate (95) To a solution of **96** (1.0 g, 2.5 mmol) in MeOH (50 ml) was added $SOCl_2$ (0.55 ml, 7.5 mmol) under ice-cooling. The mixture was raised to room temperature and stirred at room temperature overnight. The volatiles were evaporated and the residue was dissolved in $CHCl_3$. The solution was washed successively with water, 10% aqueous $NaHCO_3$, water, and brine and dried over anhydrous $MgSO_4$. The solvent was evaporated and the residue was chromatographed on silica gel with $CHCl_3$/MeOH=9/1 to give 980 mg (95%) of **94** as a pale yellow oil, which was converted to the oxalate in usual manner. ^{1}H-NMR (DMSO-d_6) δ: 1.14 (d, 3H, J=6), 2.85 (dd, 1H, J=10, 14), 2.95—3.65 (m, 4H), 3.70 (s, 3H), 4.93 (s, 2H), 5.02 (br d, 1H, J=9), 6.55 (d, 1H, J=7), 6.90 (t, 1H, J=7), 7.16 (d, 1H, J=2), 7.24 (d, 1H, J=7), 7.3—7.5 (m, 3H), 7.50 (s, 1H), 8.5 (br, 2H), 11.11 (br s, 1H). MS m/z: 457 (MH^+).

In a manner similar to that described above, **95** was prepared from **97**.

(1*R*)-(3-Chlorophenyl)-2-[(2*R*)-(7-methoxy-1*H*-indol-3-yl)propyl]-aminoethanol (92) and (1*R*)-(3-Chlorophenyl)-2-[(2*S*)-(7-methoxy-1*H*-indol-3-yl)propyl]aminoethanol (93) In a manner similar to that described above, **92** and **93** were prepared by the reaction of **18** and **19** with MeI in the presence of K_2CO_3 and KI, followed by alkaline hydrolysis of the resultants **22** and **23**, respectively. **92**; ^{1}H-NMR δ: 1.12 (d, 3H, J=7), 1.75 (br, 2H), 2.57 (dd, 1H, J=7, 12), 2.81 (d, 2H, J=7), 2.91—3.09 (m, 2H), 3.95 (s, 3H), 4.59 (dd, 1H, J=4, 8), 6.64 (d, 1H, J=8), 6.95 (d, 1H, J=2), 7.03 (dd, 1H, J=8, 8), 7.10—7.24 (m, 4H), 7.32 (m, 1H), 8.25 (br s, 1H). MS m/z: 359 (MH^+). **93**; ^{1}H-NMR δ: 1.12 (d, 3H, J=7), 1.5 (br, 2H), 2.62 (td, 1H, J=12, 9), 2.82 (d, 2H, J=7), 2.89 (dd, 1H, J=4, 12), 3.05 (m, 1H), 3.96 (s, 3H), 4.49 (dd, 1H, J=4, 9), 6.65 (d, 1H, J=8), 6.99 (d, 1H, J=2), 7.04 (dd, 1H, J=8, 8), 7.12—7.25 (m, 4H), 7.33 (m, 1H), 8.26 (br s, 1H). MS m/z: 359 (MH^+). Retention time of HPLC: **92**; 20.3 min, **93**; 24.5 min. Separation conditions for HPLC: Column; CHIRALPAK AD (4.6 mmϕ×250 mm; Daicel Chemical Indastrial, Ltd., Japan), Eluent; hexane/EtOH/Et_2NH=85/15/0.1, Flow rate; 0.4 ml/min, Temperature; 40 °C, Detection; 254 nm.

(1*R*)-(3-Chlorophenyl)-2-[2-(7-hydroxy-1*H*-indol-3-yl)propyl]aminoethanol (84) A solution of **83** (0.9 g, 2.1 mmol) in EtOH (20 ml) was hydrogenated at atmospheric pressure over 5% Rh on carbon (0.12 g) at 45—50 °C. After 10 h, the catalyst was filtered off, and the filtrate was concentrated to dryness. The residue was chromatographed on silica gel with $CHCl_3$/MeOH=20/1 to 10/1 to give 0.5 g (70%) of **84** as a pale yellow oil. ^{1}H-NMR (DMSO-d_6) δ: 1.09 (d, 3H, J=6), 2.7 (m, 1H), 2.8—3.45 (m, 4H), 3.32 (s, 1H), 4.86 (br t, 1H, J=10), 6.05 (br s, 1H), 6.49 (d, 1H, J=7), 6.78 (dt, 1H, J=2, 7), 7.01 (dd, 1H, J=2, 7), 7.07 (dd, 1H, J=2, 2), 7.25—7.45 (m, 3H), 7.46 (d, 1H, J=7), 9.50 (br s, 1H), 10.76 (br s, 1H). MS m/z: 345 (MH^+), 146.

[3-[2-[[(2*R*)-(3-Chlorophenyl)-2-hydroxyethyl]amino]propyl]-1*H*-indol-7-yloxy]acetic Acid (90) A mixture of **88** (1.0 g, 2.4 mmol), NaOH (0.2 g, 5.0 mmol), water (10 ml), and MeOH (20 ml) was stirred at room temperature for 16 h. After evaporation of the volatiles, the residue was purified by CHP 20P with water and 85% aqueous MeCN to 75% aqueous MeCN, and the fractions containing **90** were collected. The solvent was evaporated, and toluene was added to the residue. After reevaporation of the solvent, the residual solid was triturated with aqueous EtOH to give 0.4 g (41%) of **90** as a pale brown solid.

β_3-AR Agonistic Activity: Inhibition of Spontaneous Motility (Rhythmic Phasic Contractions) in the Rat Isolated Colon Isolated proximal colon, from male Jcl:SD rats (350—400 g), were placed in Krebs–Henseleit solution (composition: 118 mmol NaCl, 4.75 mmol KCl, 2.5 mmol $CaCl_2$, 1.2 mmol KH_2PO_4, 1.2 mmol $MgSO_4$, 25 mmol $NaHCO_3$, and 10 mmol glucose) containing 10 μmol phentolamine, 0.5 μmol desmethylimipramine and 30 μmol hydrocortisone hemisuccinate. The first 2.5—3 cm segment of the proximal colon, starting from the ileo-caecal junction, was mounted longitudinally in Krebs–Henseleit solution in a 10 cm organ bath maintained at 37 °C and aerated with 95% O_2, 5% CO_2. Spontaneous motility (rhythmic phasic contractions) was recorded isometrically under a constant load of 1 g. After obtaining stable responses, compounds were added cumulatively. Responses were expressed as the percentage of the amplitude of contractions obtained before addition of the tested compounds. Concentrations of tested compounds reducing spontaneous contractions by 50% (IC_{50}) were obtained from log-concentration response curves built up by the individual responses in each preparation.

β_2-AR Agonistic Activity: Inhibition of Spontaneous Contractions in the Rat Isolated Uterus Uterine horns from unprimed oestrus female Wistar rats mounted in Locke solution (composition: 157 mmol NaCl, 5.6 mmol KCl, 2.2 mmol $CaCl_2$, 18 mmol $NaHCO_3$, and 5.6 mmol glucose) in a 20 ml organ bath maintained at 37 °C and aerated with 95% O_2, 5% CO_2. Spontaneous contractions were recorded isometrically under a constant load of 1 g in presence of 12 μmol phenoxybenzamine before addition of the tested compounds. After obtaining stable responses, tested compounds were added cumulatively. Responses were expressed as the percentage reduction of the amplitude of contractions. IC_{50} of tested compounds were calculated by the same methods.

β_1-AR Agonistic Activity: Enhancing Effect of Spontaneously Beating in the Guinea-pig Isolated Right Atria Spontaneously beating right atria, from male Hartley guinea-pig were set up in Krebs solution (composition: 122.2 mmol NaCl, 4.2 mmol KCl, 2.5 mmol $CaCl_2$, 1 mmol $MgCl_2$, 1.2 mmol $NaHPO_4$, 15.5 mmol $NaHCO_3$, and 11.5 mmol glucose containing 12 μmol phenoxybenzamine) in a 20 ml organ bath maintained at 37 °C and aerated with 95% O_2, 5% CO_2. The number of spontaneously beating was recorded isometrically under a constant load of 1 g. After 30 min, tested compounds were added cumulatively.

Cloning of Various β-AR cDNAs and Establishment of the Cell Lines Expressing Them Human β_3-AR cDNA was cloned from total RNA extracted from human neuroblastoma cell SK-N-MC (ATCC HTB 10). The sequence of the cDNA was identical with that reported by Lelias *et al.*[85] The cDNA was inserted to pKCN1, a plasmid vector containing SV40 promoter and G-418 resistant gene. This plasmid was named as pKREX10. The Chinese hamster ovary cell line CHO-K1 (ATCC CCL61) was transformed with the pKREX10 by calcium phosphate method and transformants were selected with antibiotics G-418. Sixty-nine G-418 resistant clones were harvested and disrupted by low osmotic pressure. This suspension was incubated with (−)-3-[^{125}I]iodocyanopindolol at 4 °C for 2 h and the reaction mixture was filtered and washed with glass filter and radioactivity on the filter was counted. One clone named CHO/pKREX10-36, which showed highest radioactivity, was used for human β_3-AR-expressing cell line. Rat β_3-AR, human β_1-AR, and human β_2-AR cDNAs were cloned from fat cell cDNA library RL1011b (Clontech), total RNA from human neuroblastoma cell SK-N-MC, and mRNA derived from human whole brain (Clontech 6516-1), respectively. Each cDNA was inserted to expression vector pKCN1 and introduced into CHO-K1 cell as in the case of human β_3-AR. Resulting cell lines CHO/pKREX6-47, CHO/pKREX23-46, and CHO/pKREX21-8 were used as rat β_3-AR-, human β_1-AR-, and human β_2-AR-expressing cells, respectively.

Measurement of Intracellular cAMP Accumulating Activity The cells were peeled off and suspended with Hanks' balanced salts containing 1 mmol L-ascorbic acid and 1 mM 3-isobutyl-1-methylxanthine. This suspension and each test compound were incubated at 37 °C for 30 min, followed by boiling for 5 min to terminate the reaction. After centrifugation, the amount of cAMP in the supernatant was measured by enzyme immunoassay. The maximal amount of cAMP obtained by (−)-isoproterenol or the amount of cAMP when not adding any agonists were defined as 100% and 0%, respectively, and the relative maximal response of each compound is expressed as intrinsic activity (IA). EC_{50} value which is a concentration of the test compound to be required to achieve 50% of cAMP accumulation was calculated by least squares regression analysis of concentration-response curve of each compound.

Animals and Drug Treatment Male 9-week-old KK-A^y/Ta mice and 11-week-old C57BL/6J mice were purchased from Clea Japan, Inc. (Tokyo, Japan) and allowed to adjust to the facilities and handling for 1 week before all experimental protocols. Under standardized conditions, mice were given solid chow (CE-2; Oriental Yeast, Tokyo, Japan) and water ad libitum. KK-A^y/Ta mice were divided into the control and treatment groups so that the mean body weight of the two groups was comparable one day before the starting day (day 1). The treatment group was given **96** by gavage at 0.1 mg/kg/d in the morning for 14 d. The control group was given only 0.5% tragacanth solution. Twenty-four hours after the last administration of **96**, blood samples were collected from the cut ends of the tails and centrifuged to separate plasma. Blood samples from 12-week-old C57BL/6J mice were also collected by the same method. Concentrations of glucose, insulin, free fatty acid (FFA), and triglyceride (TG) in plasma were measured by commercially available kits.

Single-Crystal X-Ray Analysis. X-Ray Structure Determination of 96 Suitable crystals of **96** were obtained from 28% ammonia solution–methanol (9 : 1 v/v) solution: $C_{21}H_{23}ClN_2O_4$; M=402.86; monoclinic; $P2_1$; a=7.447(4) Å; b=8.936(2) Å; c=15.166(3) Å; β=102.85(2)°; V=984.0(6) Å^3; Z=2; ρ(calc)=1.360 g cm^{-1}; F(000)=424; μ(Cu$K\alpha$)=19.72 cm^{-1}; T=298 K; crystal size, 0.2×0.08×0.02 mm. All measurements were made on a Rigaku AFC5R diffractometer with graphite monochromated Cu$K\alpha$(λ=1.54178 Å). The cell constants and orientation matrix for data collections in the range 6.57°>θ>12.52°. The data were collected

using the ω-2θ scan technique to a maximum 2θ value of 135.18. Scans of $(1.26+0.30\tan\theta)°$ were made at a speed of $8.0° \min^{-1}$. Stationary background counts were recorded in each side of the reflections. A total of 2772 reflections were collected. The intensities of three representative reflections were measured after every 150 reflections. No decay correction was applied. The data were corrected for Lorenz and polarization effects. A correction for secondary extinction was applied. The structure was solved by direct methods (SIR92) and expanded using Fourier technique (DIRDIF94). The non-hydrogen atoms were refined with anisotropic temperature factors. All hydrogen atoms were included at idealized positions but not refined. The final cycle of full matrix least-squares refinement (SHELXL-97) was based on 1879 observed reflections and 255 variable parameters. All calculations were performed using the teXsan (Molecular Structure Co.). A refinement of the Flack's χ parameter was carried out to determine the absolute configuration. The value of refined χ [0.00(6)] indicated that to be the correct absolute stereochemistry. The final R value was 0.065. The standard deviation of an observation of unit weight (goodness of fit) was 1.57. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.32 and $-0.42\,e^-/A^3$, respectively. Further details of the X-ray structure are available from the Cambridge Crystallographic Data Centre (deposition number CCDC 203815).

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