Relationship between Stereochemistry and the β_3-Adrenoceptor Agonistic Activity of 4′-Hydroxynorephedrine Derivative as an Agent for Treatment of Frequent Urination and Urinary Incontinence

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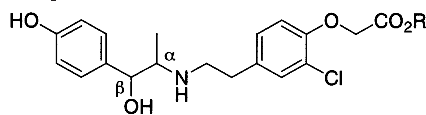
This report proposes a β_3-adrenoceptor (AR) selective agonist, 2-[2-chloro-4-(2-{[(1*S*,2*R*)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)phenoxy]acetic acid (**1a**), as a novel agent for treating urinary bladder dysfunction. This compound and its relatives have a unique feature among β_3-AR agonists: two chiral carbons are adjacently structured on the left side of the molecule. To study the relationship between the stereoconfiguration of the vicinal chiral carbons in **1a** and β-AR agonistic activity, the four stereoisomers were synthesized via oxazolidinone prepared by intracyclization involving inversion of the β-hydroxy group. The in vitro assays using rat atria for β_1-AR, rat uteri for β_2-AR, and ferret detrusor for β_3-AR showed that **1a** possessed potent β_3-AR agonistic activity (EC_{50} = 3.85 nM) and 3700- and 1700-fold selectivity for β_3-AR relative to β_1- and β_2-AR, respectively. Comparison of the four isomers revealed that the ($\alpha S,\beta R$)-compound (**1a**) was not only the most potent agonist but was also the most selective for β_3-AR. In the anesthetized rat, intravenous administration of **1a** brought about a sufficient decrement of the intrabladder pressure (ED_{50} = 12 μg/kg), and intraduodenal administration of **2a**, which is the ethyl ester of **1a**, led to same result (ED_{50} = 0.65 mg/kg). Moreover, no effects on the cardiovascular system were observed in either test.

Introduction

Human urinary bladder is controlled by the sympathetic and parasympathetic nervous systems.[1] Urinary bladder dysfunctions, such as frequent urination and urinary incontinence, arise from disturbance of this dual nervous mechanism, which can be caused by various factors such as neurologic disease. Anti-muscarinic drugs are the most commonly used to treat such diseases.[2,3] Many investigators have pursued alternative therapeutic approaches not only to avoid the side effects of anti-muscarinic agents but to eliminate the various causes of urinary dysfunction, which has led to the development of a new classes of drugs based on the other mechanisms,[4] including potassium channel openers[5] and 5-HT and NE reuptake inhibitors.[6] β_3-Adrenoceptor (AR) stimulation has attracted attention recently as a new therapeutic approach. Some reports on the distribution and function of β_3-ARs in human bladder have suggested that β_3-ARs play a significant role in urinary storage.[7−9] Moreover, β_3-AR agonists prolong the micturition interval in a rat bladder hyperactivity model.[10] We have been exploring a new type of selective β_3-AR agonist to treat such diseases and assessed them by novel assay using a ferret bladder strip, which is relaxed via β_3-AR subtype, as in the human bladder.[11] Previously, we presented *N*-phenylglycine derivatives as the new class of β_3-AR agonists in our previous report.[12] Here, we report on a 2-(2-chlorophenoxy)acetic acid derivative (**1a**) and its ester (**2a**) bearing 4′-hydroxynorephedrine as a potent and selective β_3-AR agonists (Table 1). Two vicinal asymmetric centers are characteristic of these compounds, which occurred as four stereoisomers. There are no reports on the β_3-AR agonist's stereochemistry of the two vicinal chiral carbons involved in the β_3-AR agonistic activity except for the study of ephedrine isomers, which are not selective β_3-AR agonists. Most of the β_3-AR agonists do not possess vicinal chiral carbons atoms but are constructed with separated asymmetrical carbon atoms that are connected by the aminomethylene group.[13−15] Some reports have examined the agonistic activity of the four isomers on β-ARs.[16,17] Our previous investigation of ($\alpha S,\beta R$)-4′-hydroxynorephedrine-structured β_3-AR agonists was based on the evidence that β_2-AR agonistic activity was enhanced on the ($\alpha S,\beta R$)-configuration.[18] First, it was necessary to define whether the ($\alpha S,\beta R$)-configuration was important in determining β_3-AR agonistic activity.

Table 1. Structure and Analytical Data of 4′-Hydroxynorephedrine Derivatives



compd	configuration	R	formula	MW	anal. data[a]
1a	$\alpha S,\beta R$	H	$C_{19}H_{22}ClNO_5$	379.83	C, H, N
1b	$\alpha R,\beta S$	H	$C_{19}H_{22}ClNO_5$	379.83	C, H, N
1c	$\alpha S,\beta S$	H	$C_{19}H_{22}ClNO_5 \cdot 0.5H_2O$	388.84	C, H, N
1d	$\alpha R,\beta R$	H	$C_{19}H_{22}ClNO_5 \cdot 0.5H_2O$	388.84	C, H, N
2a	$\alpha S,\beta R$	Et	$C_{21}H_{26}ClNO_5 \cdot HCl$	444.36	C, H, N

[a] Analytical data were within ±0.4% of the theoretical values.

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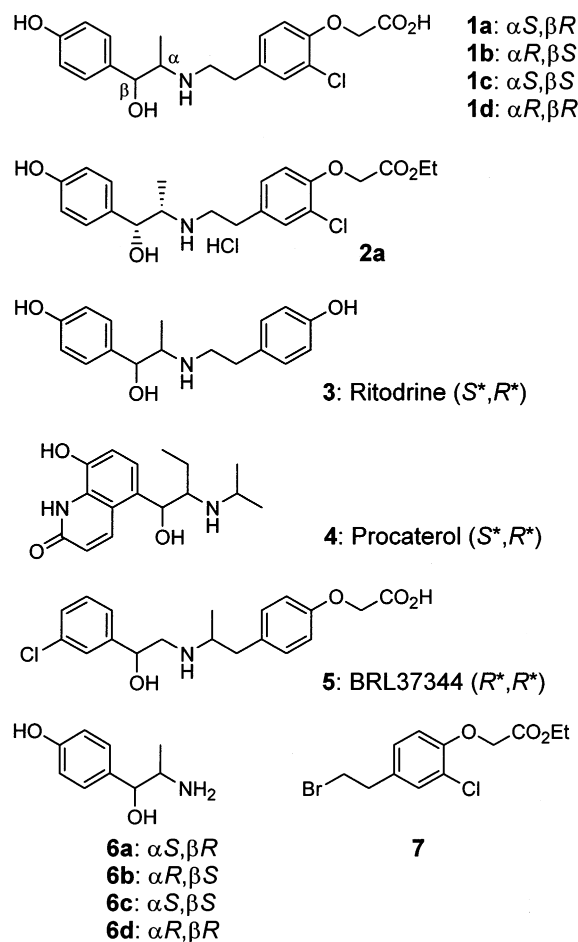


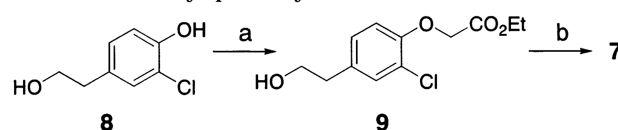
Figure 1. Structures of the novel β_3-adrenergic agonists (**1a**–**d**, **2a**) and their synthetic intermediates (**6a**–**d**, **7**), the launched β_2-adrenergic agonists (**3** is ritodrine; **4** is procaterol), and the classic β_3-adrenergic agonist (**5**: BRL37344). The *R* and *S* next to α or β indicate the absolute configuration of the hydroxy and methyl groups. The *R** and *S** in parentheses indicate the relative configuration of the hydroxy and methyl groups.

In this study, we synthesized the four stereoisomers of 2-(2-chlorophenoxy)acetic acid derivatives (**1a**–**d**) via oxazolidinone prepared by cyclization–inversion from 4′-hydroxynorephedrine (Scheme 2) and estimated their agonistic activity for β_1-, β_2-, and β_3-ARs. Stereochemistry vs activity relationship is discussed in the following section. Additionally, we report on the pharmacological tests that measured the antagonist activity of 3-(2-allylphenoxy)-1-[(1*S*)-1,2,3,4-tetrahydronaphth-1-ylamino]-(2*S*)-2-propranol (SR-58894A)[19] against compound **1a** and the in vivo measurements of the effects of intraduodenal administration of the ester of **1a**.

Chemistry

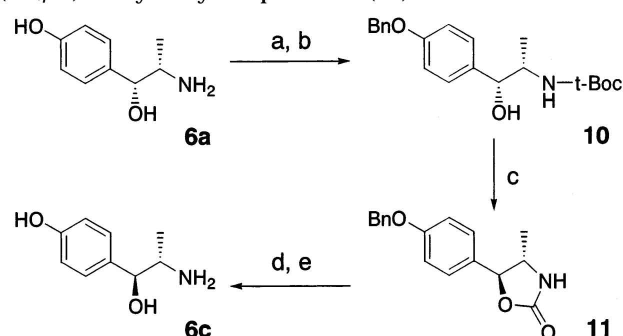
The isomers of 2-(2-chlorophenoxy)acetic acid derivatives (**1a**–**d**), as shown in Figure 1, were synthesized by N-alkylation of the corresponding 4′-hydroxynorephedrine isomers (**6a**–**d**) with ethyl 2-[4-(2-bromoethyl)-2-chlorophenoxy]acetate (**7**) under heating, followed by saponification via the corresponding esters. The phenethyl bromide derivative (**7**) was prepared as illustrated in Scheme 1. Reduction of 4′-hydroxy-2′-chlorophenylacetic acid with a borane–methyl sulfide complex afforded 2-chloro-4-(2-hydroxyethyl)phenol (**8**),

Scheme 1. Synthesis of Ethyl 2-[4-(2-Bromoethyl)phenoxy]acetate (**7**)[a]



[a] Reagents: (a) ethyl bromoacetate, K_2CO_3; (b) CBr_4, Ph_3P.

Scheme 2. Synthesis of (α*S*,β*S*)-4′-Hydroxynorephedrine (**6c**)[a]



[a] Reagents: (a) *t*-Boc_2O; (b) BnBr, Cs_2CO_3; (c) MsCl, Et_3N; (d) aqueous KOH; (e) H_2, Pd–C.

which was converted to the 2-phenoxyacetic acid ester (**9**) by alkylation using ethyl bromoacetate with K_2CO_3. The hydroxy group of **9** was converted into bromine with CBr_4 and Ph_3P to give the phenethyl bromide derivative (**7**). Optical resolution of the racemic *erythro*-**6** was performed using optically active tartaric acid according to a previous report by H. E. Smith et al.[20] to give the optically active **6a** and **6b**. The isolation of optically active *threo*-**6** by optical resolution with 10-camphorsulfonic acid was also described in that previous report. However, the racemic *threo*-**6** is not commercially available. We adopted inversion of configuration of the hydroxy groups of **6a** and **6b** via oxazolidinones by intramolecular cyclization of *N*-Boc-β-amino alcohols.[21,22] The synthesis of **6c** is outlined in Scheme 2. The *erythro*-isomer (**6a**) was protected with Boc and the benzyl group. Treatment of **10** with methanesulfonyl chloride led to ready intramolecular–cyclization to form oxazolidinone (**11**). Following hydrolysis of the urethane moiety of the oxazolidinone, the benzyl group was removed by hydrogenation to obtain the optically active *threo*-isomer (**6c**). The enantiomer **6d** was derived from **6b** with the exact same route as shown in Scheme 2.

Results

***β_1*-AR Agonistic Activity.** The β_1-AR agonistic activity of the compounds was estimated from their chronotropic effect on rat atria. The EC_{20} value is the mean concentration required to increase the basal rate of the rat atria by 20 beats per minute (Table 2). No isomer of the acid (**1a**–**d**) had remarkable effects at concentrations up to 10 μM. Even the most active (α*S*,β*R*)-isomer (**1a**) was 90000-fold and 100-fold weaker than isoproterenol and BRL37344, respectively. The ethyl esterification of **1a** resulted in an increase of β_1-AR agonistic activity by 1 order of magnitude. The rank order of potency for these compounds was the following: ester (**2a**) > (α*S*,β*R*)-compound (**1a**) > (α*R*,β*R*)-compound (**1d**) > (α*S*,β*S*)-compound (**1c**) ≫ (α*R*,β*S*)-compound (**1b**).

Table 2. β-AR Agonistic Activity and Selectivity of 4′-Hydroxynorephedrine Derivatives

compd	β_1-AR pEC$_{20}$ ± SE (EC$_{20}$, nM)[a]	β_2-AR pIC$_{50}$ ± SE (IC$_{50}$, nM)[b]	β_3-AR pEC$_{50}$ ± SE IA[c] (EC$_{50}$, nM)[d]	β_3-AR selectivity[e] β_1/β_3	β_2/β_3
1a	4.86 ± 0.04 (14000)	5.19 ± 0.20 (6400)	8.42 ± 0.23 0.88 (3.8)	3700	1700
1b	4> (>100000)	4> (>100000)	6.31 ± 0.15 0.77 (490)	>200	>200
1c	4.26 ± 0.18 (55000)	>4 (>100000)	6.21 ± 0.19 0.74 (610)	90	>160
1d	4.68 ± 0.07 (21000)	4.22 ± 0.25 (60500)	5.24 ± 0.13 0.66 (5800)	3.6	10
2a	5.80 ± 0.10 (1600)	6.28 ± 0.03 (520)	8.13 ± 0.38 0.81 (7.4)	220	70
BRL37344	6.92 ± 0.04 (120)	8.04 ± 0.10 (9.1)	8.66 ± 0.08 0.96 (2.2)	55	4.1
isoproterenol	9.82 ± 0.08 (0.15)	10.0 ± 0.03 (0.1)	7.06 ± 0.11 0.99 (87)	0.002	0.001

[a] The value in parentheses is the EC$_{20}$ (nM), which is the mean concentration required to increase the heart rate of rat atrium by 20 beats per minute ($n \geq 3$). [b] The value in parentheses is the IC$_{50}$ (nM), which is the mean concentration required to produce 50% inhibition of uterine contractions in the rat uterus ($n \geq 3$). [c] The intrinsic activity (IA) is given as the ratio of the maximum stimulation with forskolin (10^{-5} M). [d] The value in parentheses is the EC$_{50}$ (nM), which is the mean concentration required to produce 50% relaxation of the detrusor ($n \geq 3$). [e] The selectivity is the concentration ratio of β_3 (EC$_{50}$) to β_1 (EC$_{20}$) or β_2 (IC$_{50}$) for each drug.

β_2-AR Agonistic Activity. The β_2-AR agonistic activity of the compounds was assessed by their inhibitory effect on spontaneous contractions in isolated rat uterus. The IC$_{50}$ value is expressed as the concentration required to effect a 50% inhibition of the spontaneous contraction of the rat uterus (Table 2). The two compounds with the *S*-configuration at the benzyl carbon (**1b**,**c**) had no β_2-AR agonistic activity up to 100 μM. The ($\alpha S,\beta R$)-compound (**1a**) was a full agonist with modest potency (IC$_{50}$ = 6.40 μM, IA = 0.94) while the ($\alpha R,\beta R$)-compound (**1d**) was a partial agonist (IA = 0.56) relative to the maximal response to isoproterenol. The rank order of potency for these compounds was the following: ester (**2a**) > ($\alpha S,\beta R$)-compound (**1a**) > ($\alpha R,\beta R$)-compound (**1d**) ≫ ($\alpha R,\beta S$)-compound (**1b**), ($\alpha S,\beta S$)-compound (**1c**).

β_3-AR Agonistic Activity. The β_3-AR agonistic activity of the compounds was evaluated by the relaxing ability of the isolated ferret detrusor basal tone. The EC$_{50}$ value is expressed as the concentration required to effect 50% relaxation of the ferret detrusor strip (Table 2). The ($\alpha S,\beta R$)-compound (**1a**) was more potent for β_3-AR agonistic activity (EC$_{50}$ = 3.80 nM; IA = 0.88) with almost full agonism relative to isoproterenol. Its potency was comparable to that of BRL37344. By contrast, the other isomers (**1b**–**d**) showed modest potency with partial agonism (EC$_{50}$ = 0.49–5.80 μM; IA = 0.66–0.77). The rank order of the potency of these compounds was the following: BRL37344 = ($\alpha S,\beta R$)-compound (**1a**) > ester (**2a**) > isoproterenol > ($\alpha R,\beta S$)-compound (**1b**) = ($\alpha S,\beta S$)-compound (**1c**) > ($\alpha R,\beta R$)-compound (**1d**).

Effect of a β_3-AR Antagonist, SR58894A, on Compound 1a Induced Inhibition of Spontaneous Contraction in the Rat Proximal Colon. The interaction between ($\alpha S,\beta R$)-compound (**1a**) and 3-(2-allylphenoxy)-1-[(1*S*)-1,2,3,4-tetrahydronaphth-1-ylamino]-(2*S*)-2-propranol (SR-58894A), a specific β_3-AR antagonist developed by Sanofi Co., Ltd.,[19] was set out in isolated rat proximal colon. The ($\alpha S,\beta R$)-compound (**1a**) suppressed spontaneous contraction of the colon. SR58894A caused a rightward shift of the concentration–response curve for **1a** in a concentration-dependent manner, as

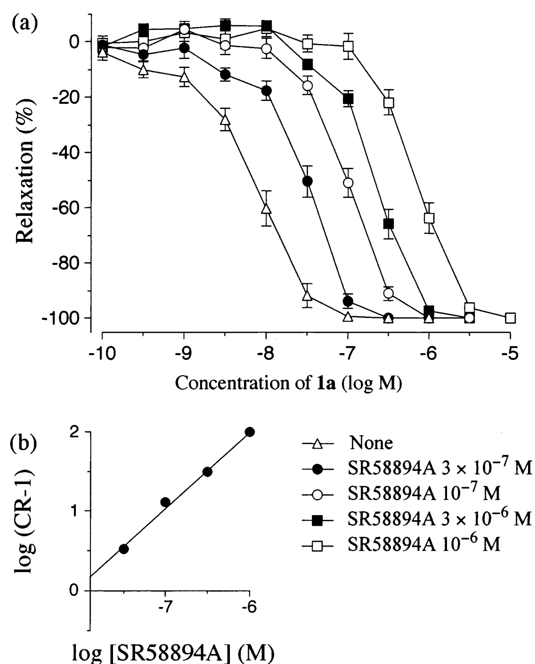


Figure 2. Effect of SR58894A on the **1a**-induced inhibition of spontaneous contraction in isolated rat proximal colon preparations. All experiments were carried out in the presence of CGP-20712A (10^{-7} M), ICI-118,551 (10^{-7} M), and phentolamine (10^{-6} M). (a) Concentration–response relationships for **1a**, either alone or in the presence of SR58894A (3×10^{-7}, 10^{-7}, 3×10^{-6}, and 10^{-6} M). Each point represents the mean ± SEM (n = 7–12). The data are expressed as a percentage of the maximal relaxation induced by 10^{-5} M forskolin. (b) Schild plot for the inhibition of the **1a**-induced relaxation produced by SR58894A.

shown in Figure 2. A Schild plot analysis yielded a pA$_2$ value of 8.1 and a slope of 0.96.

In Vivo Studies of iv or id Administration in the Anesthetized Rat. The results of the in vivo examinations of the compound are shown in Figures 3 and 4. In the test of **1a**, the intravenous administration of isoproterenol decreased the intrabladder pressure from 6.5 ± 0.6 to 3.8 ± 0.5 cmH$_2$O. This change was defined as the maximum (100%) response to which the effect of **1a** was compared (Figure 3a). The blood pressure is expressed as the percentage change from the initial blood

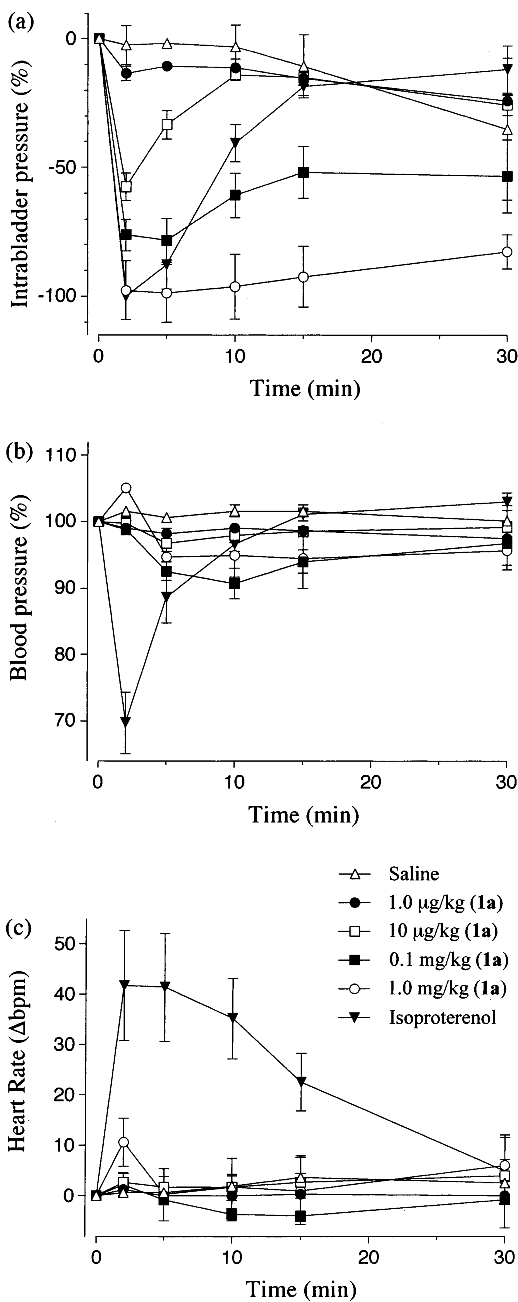


Figure 3. Time course of intrabladder pressure (a), heart rate (b), and blood pressure (c) in anesthetized rats after intravenous injection of saline (1 mL/kg), isoproterenol (10 μg/kg), or **1a** (1.0 μg/kg, 10 μg/kg, 0.1 mg/kg, 1.0 mg/kg) (n = 3). Intrabladder pressure (a) is expressed as a percentage of the maximal relaxation with isoproterenol. Heart rate (b) is expressed as the difference from the value before drug administration. Blood pressure (c) is expressed as a percentage of the value before drug administration.

pressure (92.9 ± 2.9 mmHg), and the heart rate (Δbpm, change in beats per minute) is expressed as the change from the initial rate (371.3 ± 5.8 beats/min). In the test of **2a**, the intrabladder pressure was initially 4.6 ± 0.4 cmH_2O and changed to 3.1 ± 0.3 cmH_2O after administering isoproterenol iv. The vertical axis in Figure 4a is the same as in Figure 3a. The basal heart rate was 363.3 ± 6.7 beats/min. Intravenous administration of the (α*S*,β*R*)-compound (**1a**) decreased the bladder pressure in the anesthetized rat in a dose-dependent manner without increasing the heart rate (ED_{50} = 12 μg/kg).

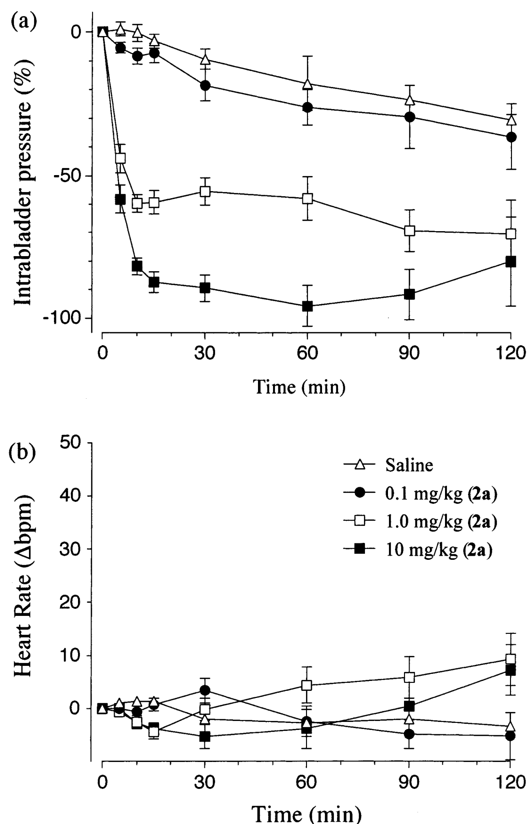


Figure 4. Time course of the changes in intrabladder pressure (a) and heart rate (b) in anesthetized rats after intraduodenal administration of saline (1 mL/kg) and **2a** (0.1, 1.0, 10 mg/kg) (n = 3). Intrabladder pressure (a) is expressed as a percentage of the maximal relaxation with isoproterenol (iv) at 2 min. Heart rate (b) is expressed as the difference from the value before drug administration.

Isoproterenol was 20-fold more potent (ED_{50} = 0.6 μg/kg) than **1a** but with a shorter duration of action and producing a significant increase in heart rate.

Intraduodenal administration of the ester compound (**2a**) produced sufficient decrement of intrabladder pressure in the anesthetized rats in a dose-dependent manner. Moreover, it did not increase the heart rate. The ED_{50} value of **2a** was 0.65 mg/kg when the maximal response of the iv isoproterenol-induced decrement of intrabladder pressure was 100%.

Discussion

It is well-known that β-AR agonists as phenylethanolamines essentially require *R*-configuration at the benzyl position with the hydroxy group to enhance their affinities and agonist activity for β-ARs.[23] However, there are few studies of phenylpropanolamines, such as ephedrine, ritodrine **3**, and procaterol **4** (Figure 1), with respect to the correlation of β-AR agonist activity with the relative stereochemistry between the benzyl carbon with its hydroxy group (β-carbon) and the vicinal carbon with an amino group (α-carbon). Meiji Seika Company and Otsuka Pharmaceutical Company researchers reported on two ritodrine enantiomers[18] and four procaterol stereoisomers,[24] respectively. (−)-(α*S*,β*R*)-*erythro*-Ritodrine and procaterol are more potent β_2-AR agonists than the corresponding (+)-(α*R*,β*S*)-*erythro*-enantiomers, and *threo*-procaterols (*RR*- and *SS*-compounds) showed mid β_2-AR agonistic activity between the (−)-

erythro-isomer and the (+)-*erythro*-isomer. When comparing the β_2-AR agonistic activity of the stereoisomers of our compounds (**1**) even with their low potency, the (−)-($\alpha S,\beta R$)-*erythro*-isomer (**1a**) was the most potent. The rank order for β_2-AR agonistic activity was the following: (−)-($\alpha S,\beta R$)-*erythro*-isomer > (−)-($\alpha R,\beta R$)-*threo*-isomer ≫ (+)-($\alpha S,\beta S$)-*threo*-isomer = (+)-($\alpha R,\beta S$)-*erythro*-isomer. It was apparent that β_2-AR was extremely sensitive to the β-hydroxy group in the *R*-configuration. These results are in agreement with the results reported on ritodrine and procaterol. Besides, the rank order for β_1-AR agonistic activity was almost the same as for β_2-AR. Thus, the *R*-configuration at the β-carbon bearing the hydroxy group was required for the best enhancement of β_1- or β_2-AR agonistic activity, and the *S*-configuration at the α-carbon bearing the methyl and amino group complementarily enhanced the activity. In 1999, Feller et al. reported the stereochemistry studies of ephedrine for β_3-AR agonistic activity.[25] ($\alpha S,\beta R$)-*Erythro*-ephedrine was shown to have the most potent β_3-AR agonistic activity of four isomers on human β_3-AR expressed cells, whereas ephedrine shows significantly lower β_3-AR agonistic activity than β_1- and β_2-AR with low intrinsic activity (IA = 0.07−0.31). We postulated that a more potent β_3-AR agonist with vicinal chiral carbons was necessary to decide the activity order of its isomers. Our selective β_3-AR agonists (**1a**−**d**) had EC_{50} values below 5.80 μM and intrinsic activities (IA) greater than 0.6. The ($\alpha S,\beta R$)-isomer (**1a**) was the most potent of the four; however, the rank order for β_3-AR agonistic activity was inconsistent with the orders for β_1- and β_2-AR activity. The two *erythro*-isomers (**1a**,**b**) had greater β_3-AR agonistic activity than the *threo*-isomers (**1c**,**d**) regardless of the configuration at the benzyl position (β-carbon), although there was a slight difference in activities of **1b** and **1c**. **1a**−**d** were selective compounds for β_3-AR agonistic activity significantly because of their low potency for β_1- and β_2-AR. The rank order for the β_3-AR selectivity depended largely on the β_3-AR agonistic activity. It is noteworthy that the *erythro*-compounds (**1a** and **1b**) were over 200-fold selective for β_3-AR agonistic activity relative to their β_1- and β_2-AR activity.

The *erythro*-compound (**1a**) with the best β_3-AR agonistic activity and selectivity of its isomers was 22-fold more potent for β_3-AR agonistic activity than isoproterenol and almost the same as BRL37344. Next, we studied the interaction between **1a** and the β_3-AR selective antagonist, SR58894A, on β_3-AR. The respective pA_2 and slope of the Schild plot of SR58894A are 6.24 ± 0.20 and 0.68 ± 0.31 in human detrusor,[7b] 7.64 ± 0.36 and 0.43 ± 0.19 in ferret detrusor,[11] and 8.06 ± 0.43 and 1.06 ± 0.40 in rat proximal colon.[19b] Therefore, the rat proximal colon is better for estimating the antagonism of SR58894A on β_3-AR. SR58894A produced a parallel rightward shift of the concentration−response curve for **1a** (Figure 2). The slope 0.96 from Schild plots indicates a competitive form of antagonism.

In the in vivo study using the anesthetized rat, the intravenous injection of **1a** significantly reduced the intrabladder pressure, as shown in Figure 3, implying that **1a** increases the urine storage in the bladder. The effect of **1a** (ED_{50} = 12 μg/kg) was 20-fold weaker than that of isoproterenol (ED_{50} = 0.6 μg/kg), although **1a** had more potent β_3-AR agonistic activity than isoproterenol, as shown in Table 2. This reversal in their potency order was largely due to the potent β_2-AR agonistic activity of isoproterenol. A previous report confirmed that the rat detrusor is relaxed through both β_2- and β_3-AR stimulations.[26] In fact, the in vitro study on the isolated rat detrusor showed that the relaxing effect of **1a** (EC_{50} = 140 nM) was weaker than that of isoproterenol (EC_{50} = 10 nM). We predict that a selective β_3-AR agonist (**1a**) would produce a sufficient effect on the human bladder, which is predominately mediated via the β_3-AR subtype over the other β-ARs.

Esterification of **1a** brought about retainment of β_3-AR agonistic activity and a slight increase of β_1- and β_2-AR agonistic activity by 8- and 12-fold, respectively. The ester compound (**2a**) is hydrolyzed into **1a** during intestinal absorption. Intraduodenal administration of **2a** significantly decreased the intrabladder pressure in a dose-dependent manner. Moreover, no cardiac effect was observed, even with complete relaxation by administration of 10 mg of **2a** (Figure 4). On the basis of these findings, the ester compound (**2a**) appears to be an oral prodrug of **1a** without the cardiac effects.

Summary

We synthesized a novel β_3-AR selective agonist (**1a**) with two vicinal chiral carbons and asymmetric isomers by inversion of the hydroxy group via oxazolidinone. The correlation between the stereochemistry of **1a**−**d** and the β-AR agonist activity of each was investigated by in vitro assay using rat atria for β_1-AR, rat uteri for β_2-AR, and ferret detrusor for β_3-AR. All four isomers were selective for β_3-AR over β_1- and β_2-AR. The ($\alpha S,\beta R$)-configuration proved to be essential for enhancing β-AR agonism, although no stereoisomer had the same effect on each β-AR. The ($\alpha S,\beta R$)-isomer (**1a**) was the most potent and selective β_3-AR agonist of the four. Intravenous administration of **1a** in the anesthetized rat showed a decrement of intrabladder pressure without influence on heart rate and blood pressure. The β_3-AR agonist activity of the ester (**2a**) was equipotent to that of **1a**, although it was slightly less selective. Intraduodenal administration of **2a** in the rat produced a sufficient decrement of intrabladder pressure without an increase of heart rate. In preliminary pharmacokinetic tests, the ester (**2a**) had an oral bioavailability of 26% in dogs with a half-life of 90 min. One of the problems of conventional β_3-AR agonists, which were selected using rat adipose tissue, was their low potency, low efficacy, or lack of adequate selectivity against human β_3-AR. Therefore, we investigated the potency of our compounds, which were selected using ferret detrusor, on human β_3-AR expressed by CHO cells.[27] Compound **1a** produced a concentration-dependent increase in cAMP accumulation with full agonistic activity, as did isoproterenol. The EC_{50} of **1a** and isoproterenol was 1.50 μM (IA = 1.03) and 0.13 μM (IA = 1.00), respectively. We are now working on the preclinical profiles of **1a** and **2a** as drug candidates. The results of further pharmacokinetic and toxicologic studies and the pharmacodynamics of these compounds in several species will be reported in due course.

Experimental Section

General Methods. Melting points were taken on a Yanaco MP-3S Micro melting point apparatus and are uncorrected. Infrared spectra were measured on a Nicolet 510 FT-IR spectrophotometer and are reported in reciprocal centimeters. Proton NMR spectra were recorded at 400 or 500 MHz with a Bruker AMX 400 or DRX 500 instrument, and chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane as the internal standard. The peak patterns are shown as the following abbreviations: br = broad, d = doublet, m = multiplet, s = singlet, t = triplet, q = quartet. The mass spectra (MS) were carried out with a Thermo Quest FINNIGAN AQA electrospray ionization mass spectrometer. Elemental analyses were performed by the Yanaco CHN MT-5 analyzer. The analytical results obtained were within ±0.4% of the theoretical values unless otherwise stated. Silica gel 60 F_{254} precoated plates on glass from Merck KGaA or aminopropyl silica gel (APS) precoated NH plates from Fuji Silysia Chemical Ltd. were used for thin-layer chromatography (TLC). Medium-pressure liquid column chromatography (MPLC) was performed on silica gel 60 N (particle size 40−50 μm) from Kanto Chemical Co., Inc. or APS Daisogel IR-60 (particle size 25−40 μm) from Daiso Co., Ltd. Analytical HPLC was run on a Shimadzu LC-VP instrument equipped with an CHIRALPAK AD-H, 4.6 mm × 250 mm column (Daicel Chemical Industries, Ltd.) under two elution conditions: isocratic conditions in hexane/EtOH/diethylamine/trifluoroacetic acid = 940/60/1/1 (method a) and in hexane/2-propanol = 88/12 (method b); flow rate = 1.0 mL/min, λ = 225 nm. The column temperature was maintained at 25 °C. All reagents and solvents were commercially available unless otherwise indicated. Yields were not optimized.

2-Chloro-4-(2-hydroxyethyl)phenol (8). To a solution of 3-chloro-4-hydroxyphenylacetic acid (30.0 g, 160 mmol) in THF (300 mL) was added $BH_3 \cdot Me_2S$ complex (40.0 mL, 400 mmol) dropwise at 10 °C. The mixture was stirred for 1 h at room temperature and then heated under reflux for 1 h. After the mixture was cooled by an ice bath, MeOH (50 mL) was carefully added and the mixture was concentrated in vacuo. The residue was partitioned between 1 M HCl (100 mL) and EtOAc (200 mL). The EtOAc layer was washed successively with water (100 mL), saturated aqueous $NaHCO_3$ (100 mL), and brine (50 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was triturated with hexane, and the resulting precipitates were collected by filtration to give 25.6 g (92%) of **8** as a colorless solid: mp 75−76 °C; ^{1}H NMR ($CDCl_3$) δ 1.55 (1H, br), 2.78 (2H, t, J = 6.5 Hz), 3.80−3.85 (2H, m), 5.64 (1H, br s), 6.94 (1H, d, J = 8.2 Hz), 7.03 (1H, dd, J = 8.2, 2.0 Hz), 7.19 (1H, d, J = 2.0 Hz).

Ethyl 2-[2-Chloro-4-(2-hydroxyethyl)phenoxy]acetate (9). To a solution of **8** (5.00 g, 29.0 mmol) in DMF (80 mL) were added K_2CO_3 (4.80 g, 34.8 mmol) and ethyl bromoacetate (3.86 mL, 34.8 mmol) at 5 °C, and the resulting suspension was stirred for 4 h at room temperature. Diethylamine (3.00 mL, 29.0 mmol) was added, and the mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with Et_2O (100 mL) and poured into ice−water (200 g). The aqueous layer was extracted with Et_2O (100 mL). The combined Et_2O layer was washed with water (50 mL) twice, 1 M HCl (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL) successively. After being dried over anhydrous $MgSO_4$, the organic layer was filtrated through a short column of APS (eluent, Et_2O). The filtrate was concentrated in vacuo to give 6.0 g (80%) of **9** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.29 (3H, t, J = 7.2 Hz), 1.44 (1H, t, J = 6.2 Hz), 2.79 (2H, t, J = 6.2 Hz), 3.82 (2H, q, J = 6.2 Hz), 4.27 (2H, q, J = 7.2 Hz), 4.67 (2H, s), 6.80 (1H, d, J = 8.4 Hz), 7.05 (1H, dd, J = 8.4, 2.2 Hz), 7.77 (1H, d, J = 2.2 Hz).

Ethyl 2-[4-(2-Bromoethyl)-2-chlorophenoxy]acetate (7). To a stirred solution of **9** (5.5 g, 21.3 mmol) and Ph_3P (5.86 g, 22.3 mmol) in CH_2Cl_2 (55 mL) was added CBr_4 (7.40 g, 22.3 mmol) at 5 °C. After the reaction mixture was stirred for 2 h at room temperature, EtOH (12 mL) was added, and the mixture was the stirred for 1 h. The solvent was removed in vacuo, and the residue was triturated with a mixture of Et_2O (60 mL) and hexane (20 mL), followed by filtration through a pad of Celite. The filtrate was concentrated in vacuo, and the residue was purified by MPLC on silica gel (eluent, hexane/Et_2O = 3/1) to give 6.5 g (95%) of **7** as a colorless oil: IR (KBr) 2977, 1756, 1605 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.29 (3H, t, J = 7.1 Hz), 3.08 (2H, t, J = 7.5 Hz), 3.54 (2H, t, J = 7.5 Hz), 4.27 (2H, q, J = 7.1 Hz), 4.69 (2H, s), 6.80 (1H, d, J = 8.4 Hz), 7.04 (1H, dd, J = 8.4, 2.2 Hz), 7.25 (1H, d, J = 2.2 Hz).

2-[2-Chloro-4-(2-{[(1*S*,2*R*)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)phenoxy]acetic Acid (1a). To a solution of **6a** (502 mg, 3.00 mmol) and **7** (984 mg, 3.06 mmol) in DMF (3 mL) was added diisopropylamine (0.46 mL, 3.30 mmol), and the mixture was stirred for 2 h at 80 °C. The reaction mixture was concentrated in vacuo, and the residue was partitioned between EtOAc (70 mL) and water (70 mL). The organic layer was washed with brine (50 mL), dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residual oil was purified by MPLC on APS (eluent, CH_2Cl_2/EtOH = 20/1) to give 690 mg (56%) of ethyl 2-[2-chloro-4-(2-{[(1*S*,2*R*)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)phenoxy]acetate as a glassy oil: ^{1}H NMR ($CDCl_3$) δ 0.98 (3H, d, J = 6.4 Hz), 1.33 (3H, t, J = 7.1 Hz), 2.60−2.85 (4H, m), 2.90−3.05 (1H, m), 4.31 (2H, q, J = 7.1 Hz), 4.47 (1H, d, J = 5.6 Hz), 4.69 (2H, s), 6.64−6.75 (3H, m), 6.91 (1H, dd, J = 8.4, 2.1 Hz), 7.06 (2H, d, J = 8.6 Hz), 7.13 (1H, d, J = 2.1 Hz).

Ethyl 2-[2-chloro-4-(2-{[(1*S*,2*R*)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)phenoxy]acetate (690 mg, 1.69 mmol) was dissolved in 1 M NaOH (8.5 mL), and the solution was stirred for 1 h at room temperature. To the reaction mixture was added 1 M HCl (8.5 mL) under ice-cooling with stirring, and collection of the resulting precipitates by filtration gave 464 mg (99%) of **1a** as a solid: mp 229−230 °C dec; $[\alpha]^{30}_D$ −5.7° (c 1.01, HOAc); IR (KBr) 3366, 3297, 3034, 2817, 1608, 1571 cm^{-1}; ^{1}H NMR (DMSO-d_6 + D_2O) δ 0.93 (3H, d, J = 6.7 Hz), 2.68−2.82 (2H, m), 3.00−3.17 (2H, m), 3.26−3.35 (1H, m), 4.47 (2H, s), 5.06 (1H, d, J = 2.2 Hz), 6.75 (2H, d, J = 8.5 Hz), 6.83 (1H, d, J = 8.6 Hz), 6.91 (1H, dd, J = 8.6, 2.1 Hz), 7.17 (2H, d, J = 8.5 Hz), 7.26 (1H, d, J = 2.1 Hz); MS m/z (relative intensity) 380 $(M + H)^+$, 382 (0.35). Anal. ($C_{19}H_{22}ClNO_5$, 379.83) C, H, N.

The following compounds were prepared from the corresponding 4′-hydroxynorephedrine isomers (**6b**−**d**) by a method similar to that described here.

2-[2-Chloro-4-(2-{[(1*R*,2*S*)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)phenoxy]acetic acid (1b): mp 230−234 °C dec; $[\alpha]^{26}_D$ +8.3° (c 1.10, 1 M HCl); MS m/z (relative intensity) 380 $(M + H)^+$, 382 (0.34). Anal. ($C_{19}H_{22}ClNO_5$, 379.83) C, H, N.

2-[2-Chloro-4-(2-{[(1*S*,2*S*)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)phenoxy]acetic acid (1c): mp 232−236 °C dec; $[\alpha]^{26}_D$ +43.7° (c 1.00, 1 M HCl); IR (KBr) 3326, 3246, 1608, 1565 cm^{-1}; ^{1}H NMR (DMSO-d_6 + CF_3CO_2D) δ 0.97 (3H, d, J = 6.7 Hz), 2.80−3.40 (5H, m), 4.42 (1H, d, J = 9.3 Hz), 4.81 (2H, s), 6.80 (2H, d, J = 8.6 Hz), 7.01 (1H, d, J = 8.5 Hz), 7.15−7.23 (3H, m), 7.41 (1H, d, J = 2.1 Hz), 14.98 (4H, br); MS m/z (relative intensity) 380 $(M + H)^+$, 382 (0.35). Anal. ($C_{19}H_{22}ClNO_5 \cdot 0.5H_2O$, 388.84) C, H, N.

2-[2-Chloro-4-(2-{[(1*R*,2*R*)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)phenoxy]acetic acid (1d): mp 242−246 °C dec; $[\alpha]^{26}_D$ −44.9° (c 1.10, 1 M HCl); MS m/z (relative intensity) 380 $(M + H)^+$, 382 (0.37). Anal. ($C_{19}H_{22}ClNO_5 \cdot 0.5H_2O$, 388.84) C, H, N.

Ethyl 2-[2-Chloro-4-(2-{[(1*S*,2*R*)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)phenoxy]acetate Hydrochloride (2a). To a stirred solution of ethyl 2-[2-chloro-4-(2-{[(1*S*,2*R*)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)phenoxy]acetate (1.50 g, 3.68 mmol) in EtOAc (40 mL) was added 4 M HCl in EtOAc (1.84 mL, 7.36 mmol) dropwise at 5 °C. The mixture was concentrated in vacuo, and the residue was triturated with Et_2O, followed by filtration to give 1.6 g (98%) of **2a** as a white solid: mp 196−198 °C; $[\alpha]^{30}_D$ −10.3° (c 1.00, EtOH); IR (KBr) 3297, 3160, 1737 cm^{-1}; ^{1}H

NMR (DMSO-d_6) δ 0.96 (3H, d, J = 6.7 Hz), 1.21 (3H, t, J = 7.1 Hz), 2.90−3.05 (2H, m), 3.15−3.40 (3H, m), 4.17 (2H, q, J = 7.1 Hz), 4.90 (2H, s), 5.08 (1H, br s), 5.90−6.00 (1H, m), 6.76 (2H, d, J = 8.6 Hz), 7.02 (1H, d, J = 8.6 Hz), 7.10−7.20 (3H, m), 7.40 (1H, d, J = 2.1 Hz), 8.85 (2H, br), 9.41 (1H, s); MS m/z (relative intensity) 408 $(M + H)^+$, 410 (0.35). Anal. ($C_{21}H_{27}Cl_2NO_5$, 444.36) C, H, N.

***tert*-Butyl *N*-[(1*S*,2*R*)-2-(4-Benzyloxyphenyl)-2-hydroxy-1-methylethyl]carbamate (10).** To a solution of t-Boc_2O (6.20 g, 28.4 mmol) in THF (60 mL) was added **6a** (5.00 g, 29.9 mmol) at room temperature. The mixture was stirred overnight and concentrated in vacuo. The residue was dissolved in EtOAc and washed with 10% aqueous citric acid, saturated aqueous $NaHCO_3$, and brine successively. The organic layer was dried over anhydrous $MgSO_4$ and evaporated in vacuo. The residue (7.57 g) was dissolved in DMF (140 mL). Cesium carbonate (10.0 g, 30.7 mmol) and benzyl bromide (3.57 mL, 30.0 mmol) were added, and the mixture was stirred overnight at room temperature. The reaction mixture was diluted with Et_2O and washed with water, 1 M NaOH, and brine successively. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give 9.50 g (93%) of **10** as a white solid: mp 135−138 °C dec; IR (KBr) 3360, 1682 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.99 (3H, d, J = 7.0 Hz), 1.46 (9H, s), 3.17 (1H, br), 3.90−4.05 (1H, m), 4.55−4.65 (1H, m), 4.75−4.85 (1H, m), 5.06 (2H, s), 6.96 (2H, d, J = 8.6 Hz), 7.25 (2H, d, J = 8.6 Hz), 7.30−7.50 (5H, m).

(4*S*,5*S*)-5-(4-Benzyloxyphenyl)-4-methyloxazolidin-2-one (11). To a stirred solution of **10** (9.00 g, 25.2 mmol) and triethylamine (6.97 mL, 50.0 mmol) in CH_2Cl_2 (100 mL) was added MsCl (2.16 mL, 27.9 mmol) dropwise at 5 °C, and the mixture was stirred overnight at room temperature. The reaction mixture was washed with 1 M HCl, saturated aqueous $NaHCO_3$, and brine successively. The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The residual solid was recrystallized with EtOAc to give 4.50 g (63%) of **11** as a white crystal: mp 180−181 °C; IR (KBr) 3263, 1751, 1708 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (3H, d, J = 6.1 Hz), 3.80−3.86 (1H, m), 4.98 (1H, d, J = 7.2 Hz), 5.08 (2H, s), 5.63 (1H, br), 7.00 (2H, d, J = 8.7 Hz), 7.30 (2H, d, J = 8.7 Hz), 7.31−7.45 (5H, m).

(α*S*,β*S*)-4′-Hydroxynorephedrine (6c). The mixture of **11** (4.50 g, 15.9 mmol) and KOH (1.06 g, 16.0 mmol) in water (16 mL) and dioxane (16 mL) was heated under reflux overnight. Additionally, KOH (1.06 g), water (16 mL), and dioxane (16 mL) were added and the mixture was heated under reflux for 5 h. The reaction mixture was concentrated in vacuo, and the residue was triturated with water (ca. 20 mL). The suspension was allowed to stand at 5 °C overnight. The precipitates were collected by filtration. After the mixture was dried under reduced pressure, the resulting solid (3.65 g) was dissolved in MeOH (70 mL), and 10% Pd on activated carbon (700 mg, wet) was added. The mixture was stirred overnight at room temperature under hydrogen and filtrated to remove the catalyst. The filtrate was concentrated in vacuo, and the residue was triturated with EtOAc. Filtration gave 2.36 g (89%) of **6c** as a white solid: mp 149−152 °C dec; $[\alpha]^{26}_D$ +34.2° (c 1.06, MeOH); IR (KBr) 3389, 2577 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.73 (3H, d, J = 6.4 Hz), 2.72 (1H, quint, J = 6.7 Hz), 3.96 (1H, d, J = 6.7 Hz), 6.69 (2H, d, J = 8.5 Hz), 7.06 (2H, d, J = 8.5 Hz). The enantiomeric excess and diastereomeric excess of **6c** were determined by HPLC loading of N-t-Boc-**6c**, prepared by treatment of **6c** with excess t-Boc_2O in EtOH, giving t_R = 25.0 min (method a), while nontreated **6c** gave t_R =16.4 min (method b): 100% ee, 99.4% de.

(α*R*,β*R*)-4′-Hydroxynorephedrine (6d). The title compound was prepared from **6b** by same method described in **6c**: mp 153−155 °C; $[\alpha]^{26}_D$ −34.7° (c 1.08, MeOH); t_R = 29.1 min, 100% ee, 99.7% de (method a).

In Vitro Experiments. The in vitro functional experiments, using pharmacologically characterized organs (rat atrium, rat uterus, and ferret detrusor), were performed as previously described in detail.[12] The rat colon preparation was used to examine the interaction of a β_3-AR antagonist (SR-58894A) with **1a**. SR-58894A was added to a Magnus bath 30 min before the addition of **1a**. Concentration−response curves for **1a** were obtained for each concentration of SR-58894A. All experiments were conducted in the presence of 10^{-6} M phentolamine. The pA_2 value for SR-58894A, as defined by Arunlakshana and Shild,[28] was obtained from linear regression analysis of the plot of the mean value of log(CR − 1) vs the negative log of the antagonist concentration (Figure 2).

In Vivo Experiments.[12,29] Male rats (300−350 g in body weight) were anesthetized with urethane (1.5 g kg^{-1}, sc.), and the midline abdomen was incised. The ureter on each side was ligated and cut proximal to the ligature to allow urine to drain into cotton wads. After ligation of the urethra, a polyethylene catheter (PE-50, Nihon Becton Dickinson, Tokyo, Japan) was inserted into the urinary bladder via the top of the bladder dome and connected through a three-way connector to a pressure transducer (SPB-108, NEC San-ei) and a syringe filled with warmed saline. The initial bladder pressure was adjusted to ca. 6 cmH_2O by instillation of warmed saline (37 °C) in 0.05 mL increments. An arterial catheter was inserted into the left carotid artery (PE-90, Nihon Becton Dickinson) and connected to a pressure transducer (SPB-108, NEC San-ei) to measure blood pressure. The heart rate was measured via a tachometer (132l, NEC San-ei) connected to a transducer amplifier (1829, NEC San-ei). Bladder pressure, blood pressure, and heart rate were recorded continuously on a rectigraph (Recti-Horiz-8K, NEC San-ei). The drug was injected through a venous catheter (PE-50, Nihon Becton Dickinson) inserted into the left femoral vein or through a catheter (PE-50, Nihon Becton Dickinson) inserted into the duodenum. The relaxing effect of each drug was expressed as a percentage of the maximal relaxation with isoproterenol. No animal was exposed to more than one of the test drugs.

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