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β_3-Adrenoceptor Agonists for the Treatment of Frequent Urination and Urinary Incontinence: 2-[4-(2-{[(1*S*,2*R*)-2-Hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)phenoxy]-2-methylpropionic Acid

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Abstract—In a search for novel analogues of β_3-adrenoceptor (AR) agonists relaxing the bladder for treatment of urinary dysfunction, 2-[4-(2-{[(1*S*,2*R*)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)phenoxy]-2-methylpropionic acids (**1a**–**e**), into which a fibrate-like structure had been incorporated, were synthesised. Compound **1a** was found to be a selective β_3-AR agonist in functional assays using the ferret detrusor (β_3-AR), rat uterus (β_2-AR), and rat atrium (β_1-AR); β_3: $EC_{50} = 7.8$ nM, β_2: $IC_{50} = 7{,}300$ nM, β_1: $EC_{20} = 23{,}000$ nM. The introduction of a chlorine atom or methyl substituent at the *ortho*-position on the phenyl ring of **1a** further improved β_3-AR selectivity. In an in vivo study, **1a** lowered intrabladder pressure ($ED_{50} = 31$ μg/kg) in rats, without increasing heart rate, in keeping with the in vitro results. Consequently, it is proposed that **1a** and its analogues (**1b**–**e**), possess β_3-AR agonistic activity in the absence of undesirable β_1- or β_2-AR mediated actions, and may be useful for clinical treatment and pharmacological studies.

Introduction

Recent reports suggest that the β_3-AR mRNA is expressed in human bladder and that β_3-AR in human bladder could play a significant role in urinary storage.[1–4] Moreover, exogenous β_3-AR agonists prolong the micturition interval in a rat bladder hyperactivity model.[5,6] These findings offer the opportunity for a new approach to the treatment of urinary disorders using β_3-AR agonists.

The β_3-AR belongs to the Gs protein-coupled receptor family that shares a common structure, consisting of seven transmembrane-spanning helices connected by interhelical loops. The endogenous catecholamines, adrenaline and noradrenaline, stimulate this receptor, which mediates metabolic functions such as lipolysis, thermogenesis and motility process control in the gastrointestinal tract.[7–9] The characterisation and expression of the gene encoding the β_3-AR was established ten years ago.[10,11] Since that time, a number of β_3-AR agonists have been developed for the treatment of obesity and type II diabetes.[12,13] Unfortunately, some drugs developed for such metabolic diseases failed to produce similar effects in humans, due to differences between humans and animals with regard to amino acid sequences of receptors, adverse reactions and/or pharmacokinetic actions of the drugs.[14] New generations of β_3-AR agonists have been found using the human β_3-AR expressed in Chinese hamster ovary (CHO) cells.[13]

It has not yet been determined whether the recombinant human β_3-AR assay is applicable for evaluation of relaxation of smooth muscle in the human bladder. We have reported that various β_3-AR agonists mediate relaxation of the ferret detrusor, as seen in humans.[15] To discover a new class of potent and selective β_3-AR agonists as therapeutic drugs for urological dysfunction, we have evaluated our synthetic compounds by a novel functional assay using the ferret detrusor preparation for analysis of β_3-AR agonistic activity. We have also employed conventional assays using rat atrium and rat uterus for estimation of agonistic activity at β_1-AR and β_2-AR respectively.[16] A previous report showed that

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2-(phenylamino)isobutyric acid derivatives had high β_3-AR selectivity against β_1- and β_2-AR.[17] Optimising these compounds, we found that the 2-phenoxyisobutyric acid derivative (**1a**) possessed potent β_3-AR agonistic activity and significant selectivity against β_1- and β_2-AR. Replacement of the nitrogen with oxygen preserved the β_3-AR activity and selectivity, in spite of the presence of a fibrate-like (anti-hyperlipidemic[18]) component in the structure. The effect of substituents on the phenyl ring of **1a** on the β-ARs agonistic activity, and the in vivo effects of **1a** in rats are reported below.

Chemistry

The 2-phenoxyisobutyric acid derivatives **1a–e**, as shown in Figure 1, were synthesized by *N*-alkylation of (−)-4′-hydroxynorephedrine (**2**)[19] with phenethyl bromide derivatives **3a–e** under heating, followed by saponification via the corresponding esters. The phenethyl bromides (**3a–e**) were prepared as shown in Scheme 1. Phenols (**4a–e**) were converted to 2-phenoxyisobutyric acid esters (**5a–e**) using 1,1,1-trichloro-2-methyl-2-propanol hemihydrate (Chloretone).[20] Friedel–Crafts reaction of **5a–e** with bromoacetyl bromide provided phenacyl bromides (**6a–e**). The phenethyl bromides (**3a–e**) were obtained by reduction of **6a–e** with triethylsilane under acidic conditions.

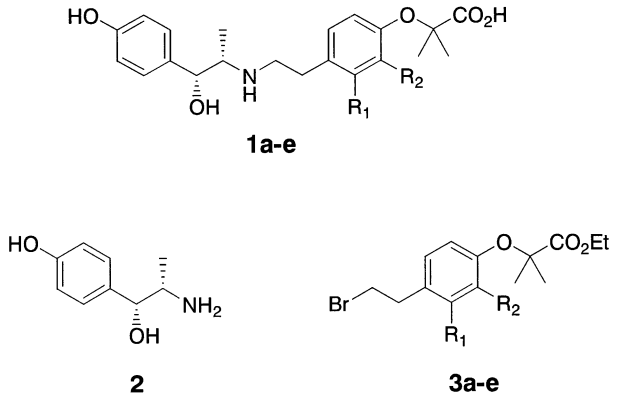
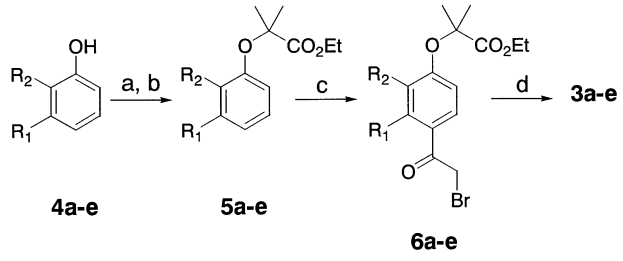


Figure 1. General formula of 2-phenoxyisobutyric acids (**1**) as a novel β_3-adrenoceptor agonist and synthetic intermediates.

Results and Discussion

All compounds in the in vitro study were evaluated for ability to increase heart rate, using isolated rat atrium (β_1-AR agonistic activity), inhibition of spontaneous motility on rat uterus (β_2-AR agonistic activity), and relaxation of ferret detrusor (β_3-AR agonistic activity). Selectivity was calculated by dividing β_1- or β_2-AR activity by β_3-AR activity. The results of the in vitro study are shown in Table 1.

The key compound **1a** consists of phenylethanolamine, a structure known to be required for β-agonistic activity, and 2-phenoxyisobutyrate, a structure characteristic of conventional fibrates. Interestingly, this simple structure led to 10-fold more potent β_3-AR agonistic activity and significantly higher selectivity for β_3-AR against β_1- and β_2-AR than isoproterenol (**1a**: $EC_{50} = 7.8$ nM, $\beta_1/\beta_3 = 2{,}940$-fold, $\beta_2/\beta_3 = 930$-fold). It has previously been convincingly shown that improvement of β_3-AR agonistic activity or selectivity was dependent on the position of halogens on the phenyl ring bearing the



Scheme 1. General synthesis of intermediates **3**. Reagents: (a) Chloretone, NaOH; (b) $SOCl_2$, EtOH; (c) bromoacetylbromide, $AlCl_3$; (d) Et_3SiH, TFA.

Table 1. Structure and β-AR Agonistic Activity of 2-Phenoxyisobutyric acids (**1**)

Compounds	R_1	R_2	Anal. (CHN)[a]	β_1-AR	β_2-AR	β_3-AR	Selectivity[f]	
				$pEC_{20} \pm$ S.E. (EC_{20}:nM)[b]	$pIC_{50} \pm$ S.E. (IC_{50}:nM)[c]	$pEC_{50} \pm$ S.E. IA.[d] (EC_{50}:nM)[e]	β_1/β_3	β_2/β_3
1a	H	H	$C_{21}H_{27}NO_5 \cdot 0.8H_2O$	4.64±0.03 (23,000)	5.14±0.58 (7,300)	8.11±0.08 0.87 (7.8)	2,949	936
1b	Me	H	$C_{22}H_{29}NO_5 \cdot 1.2H_2O$	4.96±0.02 (11,000)	5.68±0.77 (2,100)	8.32±0.10 0.86 (4.8)	2,292	438
1c	H	Me	$C_{22}H_{29}NO_5$	< 4 (100,000<)	< 4 (100,000<)	7.85±0.09 0.97 (14)	7,143<	7,143<
1d	Cl	H	$C_{21}H_{26}ClNO_5 \cdot 0.8H_2O$	5.19±0.08 (6,500)	5.51±0.03 (3,100)	7.72±0.17 0.85 (19)	342	163
1e	H	Cl	$C_{21}H_{26}ClNO_5 \cdot H_2O$	< 4 (100,000<)	4.42±0.09 (38,000)	8.62±0.10 0.96 (2.4)	41,667<	15,833
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]Elemental analyses were within ±0.4% of the theoretical values.
[b]In parentheses is the EC_{20} value (nM), the mean concentration required to increase heart rate by 20 beats per min in rat atrium ($n \geq 3$).
[c]In parentheses is the IC_{50} value (nM), the mean concentration required to produce 50% inhibition of uterine contraction in the rat uterus ($n \geq 3$).
[d]The intrinsic activity (IA) given as a ratio of the maximum stimulation with forskolin (10^{-5} M).
[e]In parentheses is the EC_{50} value (nM), the mean concentration required to produce 50% relaxation of the detrusor ($n \geq 3$).
[f]The selectivity is the concentration-ratio of β_3 (EC_{50}) to β_1 (EC_{20}) or β_2 (IC_{50}) for each drug.

carboxyalkylamino group.[17] Thus, further optimisation of the β_3-AR agonistic activity and selectivity of **1a** was carried out by chlorine (Cl) and methyl group (Me) substitutions. Introduction of a Me into the phenyl ring at the *meta*-position (**1b**: $EC_{50}=4.8$ nM) resulted in a 1.6-fold increase of β_3-AR agonistic activity, while *ortho*-substitution led to a 1.8-fold decrease in activity (**1c**: $EC_{50}=14$ nM). Replacement of Me with Cl at the *meta*-position (**1d**: $EC_{50}=19$ nM) resulted in a 2.4-fold decrease of β_3-AR agonistic activity, while *ortho*-substitution led to a 3.2-fold increase in activity (**1e**: $EC_{50}=2.4$ nM), when compared with **1a**. We conclude that enhancement of β_3-AR agonistic activity requires an electron-donating group (Me) at the *meta*-position or an electron-withdrawing group (Cl) at the *ortho*-position. On the other hand, substitution of either Cl or Me at the *meta*-position led to a 2–3.5-fold increase of β_1- and β_2-AR agonistic activity. In the case of *ortho*-substitution, a more than 5-fold decrease of β_1- and β_2-AR agonistic activity occurred. It was apparent that β_3-AR selectivity was improved by *ortho*-substitution but lowered by *meta*-substitution, regardless of their electric characterisations.

In an attempt to assess structure–activity relationships (SAR) by molecular modelling, the geometry optimisations of carboxylic acid derivatives of compounds **3a**–**e**, as simple models for **1a**–**e**, were calculated via semi-empirical AM1 utilising WinMOPAC® (Fujitsu Limited[21]). The most stable conformer of acid-**3c**, as a representative, is illustrated in Figure 2. Both the 2-bromoethyl and alkoxy groups (ether of the isobutyric acid moiety) of acids-**3a**–**e** were not coplanar with the phenyl ring, but almost located perpendicular to it, in an antiperiplanar relationship. The dihedral angles between the phenyl and 2-bromoethyl groups (C_{P5}–C_{P4}–C_{E1}–C_{E2}) on acids-**3a**–**e** were same ($98.2\pm0.4°$). The dihedral angles between the phenyl and alkoxy groups (C_{A1}–C_{A2}–O_1–C_{P1}) on acids-**3a**,**b**,**d** were also same ($96.3\pm0.3°$). However, the dihedral angles on acid-**3c**, **e** were changed by *ortho*-substitution to constrain a little movement of the alkoxy group (to $103.2\pm0.1°$ from $96.3\pm0.3°$). In addition, the isobutyric acid moiety was slightly rotated around the C_{A1}–O_1 bond (by $5.4\pm0.1°$). Simultaneously, the conformational flexibility of the alkoxy group on acids-**3c**, **e** was more severely limited than that of the unsubstituted or the *meta*-substituted analogues, since the population of conformers at the local minimum was decreased. The consequent difference in the conformational disposition of each carboxyl group at the global minimum would lead to the low affinity of *ortho*-substituted compounds for β_1- and β_2-AR, so that the β_1- and β_2-AR agonistic activities of **1c** and **1e** would be significantly decreased. Despite the conformational distinction, the β_3-AR agonistic activity of all compounds was retained, i.e., it resulted in an alteration of between half to twice the potency of the unsubstituted compound (**1a**). It can be assumed that there is a relatively extensive domain to which the isobutyric acid moiety binds for enhancement of β_3-AR agonistic activity. When comparing the substituents at the same position on the phenyl ring, Cl and Me had almost the same conformational effect, due to their similar size, though their inductive effects differed. However, the electrostatic potential charge on the phenyl ring or ether oxygen in acids-**3a**–**e** did not correlate with β_3-AR activity (data not shown). The

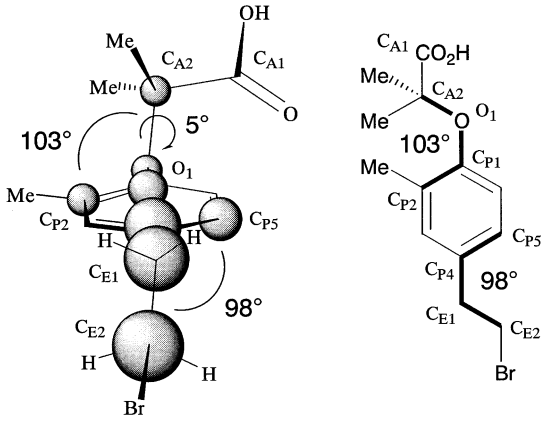


Figure 2. Structure of 2-[4-(2-bromoethyl)-2-methylphenoxy]-2-methylpropionic acid (right), the hydrolysed derivative of **3c**, and its conformer at the global minimum (left).

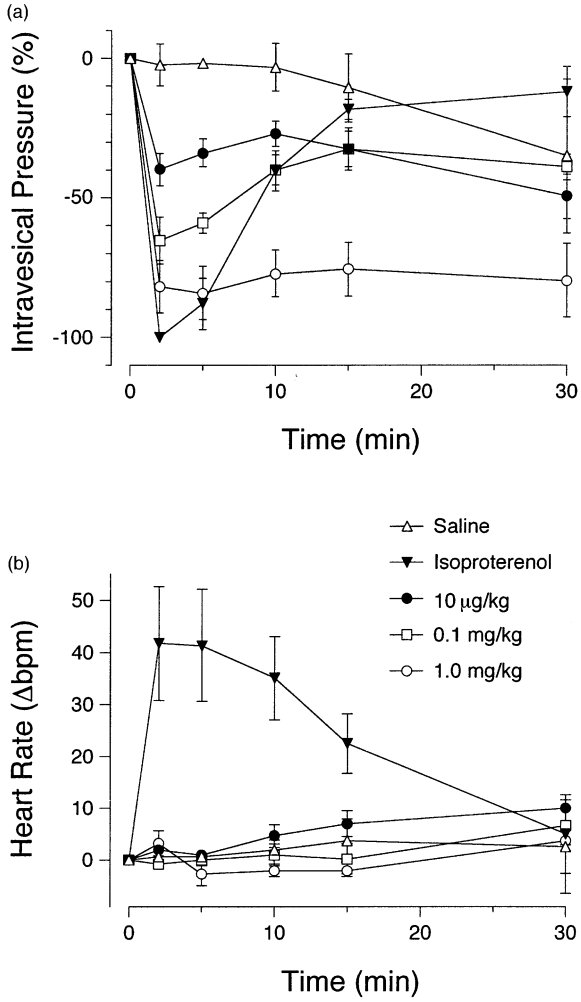


Figure 3. Time course of changes in intravesical pressure (a) and heart rate (b) in anaesthetized rats after administration of iv saline (1 mL/kg), isoproterenol (10 μg/kg), and **1a** (10 μg, 0.1 mg, 1.0 mg/kg) ($n=3$). Intravesical pressure (a) is expressed as a percentage of the maximal relaxation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

hypothesis as described above, which proposes contributions of electron distribution to β_3-AR agonistic activity, based on the profiles and positions of Cl and Me, was not supported by the calculated molecular charge. To analyse the SAR for β_3-AR agonistic activity using small molecular modelling would have needed a wider range of potencies of compounds examined. We conclude that construction of receptor models based on the rhodopsin structure as a template, together with more information on various phenyl ring substituents might be necessary. We are still investigating the synthesis and SAR of these analogues bearing other substituents.

The unsubstituted compound **1a** appeared to have the advantages of easier synthetic process and lower cost compared with the substituted compounds. It was therefore the first of these fibrate analogues assessed in vivo, using intravenously administered **1a** in anaesthetized rats. Compound **1a** lowered the intrabladder pressure of anaesthetized rats ($ED_{50} = 31$ μg/kg) with a 50-fold lower potency than isoproterenol ($ED_{50} = 0.6$ μg/kg), as shown in Figure 3a. The greater potency of isoproterenol may have been due to β_2-AR mediated relaxation, since the rat detrusor is relaxed via both β_2-AR and β_3-AR.[22] The β_3-AR selective compound **1a** exhibited sufficient intrabladder pressure lowering, in spite of being able to relax the detrusor via β_3-AR. In addition, **1a** showed little effect on heart rate, whereas isoproterenol increased heart rate (42 beats as a maximum value at 2 min) as shown in Figure 3b. It appeared that the in vivo results were closely related to those derived from in vitro assays, where **1a** was a potent and selective β_3-AR agonist.

Conclusion

Compound **1a** and its analogues, bearing a portion of the fibrate-like structure, produced significant relaxation of the ferret detrusor (β_3) and had little effect on both discharge rate of rat atrium (β_1) and spontaneous motility of rat uterus (β_2). In fact, β_3-AR agonistic activity exceeded β_1- or β_2-AR agonistic activity by three orders of magnitude. This evidence indicated that compounds **1a**–**e** were potent and selective β_3-AR agonists. Investigations of the substitution pattern on the phenyl ring suggested that Cl at the *ortho*-position, or Me at the *meta*-position, led to a slight increase of β_3-AR agonistic activity. Furthermore, substitution of either Cl or Me at the *ortho*-position led to significant improvement of selectivity for the β_3-AR with respect to β_1- or β_2-AR. Intravenous administration of **1a** in anaesthetized rats produced a lowering of intrabladder pressure. In a preliminary study, a reporter gene assay using the peroxisome proliferator response element (PPRE) upstream of the luciferase gene,[23] involving formation of PPAR-α and retinoid X receptor (RXR) heterodimers, showed no effect up to 10^{-4} M of **1a**, using bezafibrate and WAY14,643 as positive controls. It was presumed that **1a** would lack fibrate-like functions such as modulation of lipid metabolism, even if it possessed a fibrate-like structure. Additionally, **1a** showed nearly full agonistic activity in CHO cells expressing the cloned human β_3-AR (data not shown). Thus, **1a** should be a potent and selective β_3-AR agonist, acting as a urine-storage agent. This action would be mediated via relaxation of detrusor, without undesirable effects via β_1- and β_2-ARs, and PPAR-α. Therefore, we expect **1a** and its analogues will have roles in the treatment of bladder dysfunction and as new tools for studies of human bladder function.

Experimental

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 tetramethysilane 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) and the high resolution mass spectra (HRMS) were carried out with a JEOL JMS-SX102A mass spectrometer with capabilities for fast atom bombardment. Elemental analyses were performed by the Yanaco CHN corder MT-5 analyzer. The analytical results obtained were within 0.4% of the theoretical values (Table 1). Silica gel 60 F_{254} pre-coated plates on glass from Merck KGaA or aminopropyl silica gel (APS) pre-coated NH TLC from Fuji Silysia Chemical Ltd. was used for Thin-layer chromatography (TLC). Flash or medium pressure liquid column chromatography (MPLC) was performed on silica gel BW350 (particle size 25–44 μm) from Fuji Silysia Chemical Ltd. or APS Daisogel IR60 (particle size 25–40 μm) from Daiso Co., Ltd. All reagents and solvents were commercially available unless otherwise indicated. Yields were not optimised.

Ethyl 2-phenoxy-2-methylpropionate (5a)

To a solution of phenol (10.0 g, 106 mmol) and 1,1,1-trichloro-2-methyl-2-propanol hemihydrate (39.6 g, 212 mmol) in acetone (500 mL) was added NaOH (34.0 g, 850 mmol), and the mixture was stirred overnight at room temperature. After the solvent was removed under reduced pressure, the residue was dissolved in water and washed with Et_2O. The aqueous layer was acidified with concentrated HCl and extracted with Et_2O. The extract was washed twice with brine, dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The residue was dissolved in EtOH (500 mL), and $SOCl_2$ (7.75 mL, 106 mmol) was carefully added dropwise to the stirred solution at room temperature. The mixture was heated under reflux for 6 h. After the reaction mixture was concentrated under reduced pressure, the residue was dissolved in Et_2O and washed with water, saturated aqueous $NaHCO_3$, and brine successively. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was dissolved in hexane (200 mL) and filtrated

through a short column of APS (eluent:hexane). The filtrate was concentrated under reduced pressure to give 17.0 g of **5a** as a colourless oil (77%): ^{1}H NMR($CDCl_3$) δ 1.25 (3H, t, J = 7.1 Hz), 1.60 (6H, s), 4.24 (2H, q, J = 7.1 Hz), 6.82–6.87 (2H, m), 6.95–7.01 (1H, m), 7.20–7.27 (2H, m).

The following compounds (**5b–e**) were prepared as described for the preparation of **5a**, using the corresponding phenol derivatives (**4b–e**).

Ethyl 2-(3-Methylphenoxy)-2-methylpropionate (5b). ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, J = 7.1 Hz), 1.59 (6H, s), 2.29 (3H, s), 4.24 (2H, q, J = 7.1 Hz), 6.60–6.65 (1H, m), 6.67–6.69 (1H, m), 6.78–6.82 (1H, m), 7.11 (1H, t, J = 7.8 Hz).

Ethyl 2-(2-Methylphenoxy)-2-methylpropionate (5c). ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, J = 7.1 Hz), 1.59 (6H, s), 2.24 (3H, s), 4.25 (2H, q, J = 7.1 Hz), 6.64–6.68 (1H, m), 6.88 (1H, dt, J = 7.5, 1.0 Hz), 7.01–7.07 (1H, m), 7.12–7.16 (1H, m).

Ethyl 2-(3-Chlorophenoxy)-2-methylpropionate (5d). ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, J = 7.1 Hz), 1.60 (6H, s), 4.24 (2H, q, J = 7.1 Hz), 6.72 (1H, ddd, J = 8.1, 2.1, 0.9 Hz), 6.86 (1H, t, J = 2.1 Hz), 6.97 (1H, ddd, J = 8.1, 2.1, 0.9 Hz), 7.15 (1H, t, J = 8.1 Hz).

Ethyl 2-(2-Chlorophenoxy)-2-methylpropionate (5e). ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, t, J = 7.1 Hz), 1.62 (6H, s), 4.26 (2H, q, J = 7.1 Hz), 6.89 (1H, dd, J = 8.2, 1.5 Hz), 6.95 (1H, ddd, J = 7.9, 7.5, 1.5 Hz), 7.12 (1H, ddd, J = 8.2, 7.5, 1.7 Hz), 7.37 (1H, dd, J = 7.9, 1.7 Hz).

Ethyl 2-[4-(Bromoacetyl)phenoxy]-2-methylpropionate (6a). To a stirred suspension of $AlCl_3$ (3.84 g, 28.8 mmol) in CH_2Cl_2 (30 mL) was added bromoacetyl bromide (2.51 mL, 28.8 mmol) at room temperature, and the mixture was stirred for 1 h. Compound **5a** (2.0 g, 9.60 mmol) was added dropwise to the stirred mixture in an ice bath. The mixture was stirred overnight at room temperature and heated under reflux for 6 h. The reaction mixture was poured into ice–water and extracted with EtOAc. The extract was washed with water and dried over anhydrous $MgSO_4$ followed by concentration under reduced pressure. The residue was purified by MPLC on silica gel (eluent: hexane/EtOAc = 10/1) to give 0.74 g of **6a** as a light yellow solid (23%): ^{1}H NMR ($CDCl_3$) δ 1.22 (3H, t, J = 7.2 Hz), 1.67 (6H, s), 4.23 (2H, q, J = 7.2 Hz), 4.39 (2H, s), 6.85 (2H, d, J = 9.2 Hz,), 7.91 (2H, d, J = 9.2 Hz).

The following compounds (**6b–e**) were prepared as described for the preparation of **6a**, using the corresponding esters (**5b–e**).

Ethyl 2-[4-(Bromoacetyl)-3-methylphenoxy]-2-methylpropionate (6b). ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, t, J = 7.2 Hz), 1.65 (6H, s), 2.52 (3H, s), 4.23 (2H, q, J = 7.2 Hz), 4.38 (2H, s), 6.65 (1H, dd, J = 8.6, 2.4 Hz), 6.71 (1H, d, J = 2.4 Hz,), 7.67 (1H, d, J = 8.6 Hz).

Ethyl 2-[4-(Bromoacetyl)-2-methylphenoxy]-2-methylpropionate (6c). ^{1}H NMR ($CDCl_3$) δ 1.26 (3H, t, J = 7.1 Hz), 1.67 (6H, s), 2.28 (3H, s), 4.23 (2H, q, J = 7.1 Hz), 4.39 (2H, s), 6.62 (1H, d, J = 8.6 Hz), 7.72 (1H, dd, J = 8.6, 2.3 Hz,), 7.81 (1H, d, J = 2.3 Hz).

Ethyl 2-[4-(Bromoacetyl)-3-chlorophenoxy]-2-methylpropionate (6d). ^{1}H NMR ($CDCl_3$) δ 1.24 (3H, t, J = 7.1 Hz), 1.65 (6H, s), 4.24 (2H, q, J = 7.1 Hz), 4.53 (2H, s), 6.74 (1H, dd, J = 8.7, 2.5 Hz), 6.89 (1H, d, J = 2.5 Hz), 7.62 (1H, d, J = 8.7 Hz).

Ethyl 2-[4-(Bromoacetyl)-2-chlorophenoxy]-2-methylpropionate (6e). ^{1}H NMR ($CDCl_3$) δ 1.24 (3H, t, J = 7.1 Hz), 1.70 (6H, s), 4.24 (2H, q, J = 7.1 Hz), 4.36 (2H, s), 6.81 (1H, d, J = 8.7 Hz), 7.78 (1H, dd, J = 8.7, 2.3 Hz), 8.04 (1H, d, J = 2.3 Hz).

Ethyl 2-[4-(2-Bromoethyl)phenoxy]-2-methylpropionate (3a). A solution of **6a** (700 mg, 2.13 mmol) and triethylsilane (747 μL, 4.68 mmol) in TFA (10 mL) was stirred for 1 h at 70 °C. After the reaction mixture was concentrated under reduced pressure, the residue was purified by MPLC on silica gel (eluent: hexane/CH_2Cl_2 = 1:1) to give 640 mg of **3a** as a colourless oil (96%): ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, J = 7.1 Hz), 1.59 (6H, s), 3.09 (2H, t, J = 7.8 Hz), 3.52 (2H, t, J = 7.8 Hz), 4.23 (2H, q, J = 7.1 Hz), 6.79 (2H, d, J = 8.7 Hz), 7.07 (2H, d, J = 8.7 Hz).

The following compounds (**3b–e**) were prepared according as described for the preparation of **3a**, using the corresponding phenacyl bromides (**6b–e**).

Ethyl 2-[4-(2-Bromoethyl)-3-methylphenoxy]-2-methylpropionate (3b). ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, J = 7.1 Hz), 1.58 (6H, s), 2.26 (3H, s), 3.09 (2H, t, J = 8.1 Hz), 3.46 (2H, t, J = 8.1 Hz), 4.23 (2H, q, J = 7.1 Hz), 6.61 (1H, dd, J = 8.4, 2.7 Hz), 6.68 (1H, d, J = 2.7 Hz), 6.99 (1H, d, J = 8.4 Hz).

Ethyl 2-[4-(2-Bromoethyl)-2-methylphenoxy]-2-methylpropionate (3c). ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, J = 7.1 Hz), 1.58 (6H, s), 2.22 (3H, s), 3.05 (2H, t, J = 7.8 Hz), 3.50 (2H, t, J = 7.8 Hz), 4.24 (2H, q, J = 7.1 Hz), 6.60 (1H, d, J = 8.2 Hz), 6.87 (1H, dd, J = 8.2, 1.9 Hz), 6.98 (1H, d, J = 1.9 Hz).

Ethyl 2-[4-(2-Bromoethyl)-3-chlorophenoxy]-2-methylpropionate (3d). ^{1}H NMR ($CDCl_3$) δ 1.26 (3H, t, J = 7.1 Hz), 1.59 (6H, s), 3.20 (2H, t, J = 7.7 Hz), 3.54 (2H, t, J = 7.7 Hz), 4.24 (2H, q, J = 7.1 Hz), 6.69 (1H, dd, J = 8.4, 2.6 Hz), 6.89 (1H, d, J = 2.6 Hz), 7.11 (1H, d, J = 8.4 Hz).

Ethyl 2-[4-(2-Bromoethyl)-2-chlorophenoxy]-2-methylpropionate (3e). ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, t, J = 7.1 Hz), 1.61 (6H, s), 3.07 (2H, t, J = 7.6 Hz), 3.51 (2H, t, J = 7.6 Hz), 4.25 (2H, q, J = 7.1 Hz), 6.84 (1H, d, J = 8.4 Hz), 6.96 (1H, dd, J = 8.4, 2.2 Hz), 7.22 (1H, d, J = 2.2 Hz).

2-[4-(2-{[(1*S*,2*R*)-2-Hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)phenoxy]-2-methylpropionic acid (1a). A stirred mixture of **2** (630 mg, 2.0 mmol) and **3a** (670

mg, 4.0 mmol) in DMF (2 mL) was heated at 70 °C for 4 h. After cooling, the reaction mixture was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The residue was purified by MPLC on APS (eluent: CH_2Cl_2/EtOH = 40:1) to give 540 mg of ethyl 2-[4-(2-{[(1*S*,2*R*)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)phenoxy]-2-methylpropionate (67%). A solution of ethyl 2-[4-(2-{[(1*S*,2*R*)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)phenoxy]-2-methylpropionate (530 mg) in 1 N NaOH (2.0 mL) was stirred for 1 h at room temperature. To the reaction mixture was added dropwise 2 N HCl (2.0 mL), and precipitates were collected by filtration and dried under reduced pressure to give 380 mg of **1a** (77%): mp 216–219 °C dec; $[\alpha]_D^{31} = -13.1°$ (c = 1.00, 1 N HCl); IR (KBr) 3429, 1611, 1568, 1511; ^{1}H NMR (DMSO-d_6) δ 0.91 (3H, d, J = 6.6 Hz), 1.46 (6H, s), 2.60–2.80 (2H, m), 2.90–3.05 (2H, m), 3.15–3.35 (1H, m), 5.05 (1H, br s), 6.70–6.75 (4H, m), 6.86 (2H, d, J = 8.6 Hz), 7.14 (2H, d, J = 8.6 Hz), 9.40 (1H, br); MS m/z 374 $(M+H)^+$. HRMS: $(M+H)^+$ calcd for $C_{21}H_{28}NO_5$, 374.1967; found, 374.1936.

The following compounds (**1b**–**e**) were prepared according as described for the preparation of **1a**, using the corresponding phenethyl bromides (**3b**–**e**).

2-[4-(2-{[(1*S*,2*R*)-2-Hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)-3-methylphenoxy]-3-methylpropionic Acid (1b). Mp 209–212 °C dec; $[\alpha]_D^{30} = -10.2°$ (c = 0.90, 1N HCl); IR (KBr) 3405, 1613, 1570, 1513, 1502; ^{1}H NMR (DMSO-d_6) δ 0.91 (3H, d, J = 6.6 Hz), 1.45 (3H, s), 1.47 (3H, s), 1.99 (3H, s), 2.45–2.75 (2H, m), 2.80–2.95 (2H, m), 3.20–3.35 (1H, m), 5.10 (1H, br s), 6.55 (1H, dd, J = 8.3, 2.6 Hz), 6.59 (1H, d, J = 2.6 Hz), 6.68 (1H, d, J = 8.3 Hz), 6.73 (2H, d, J = 8.7 Hz), 7.16 (2H, d, J = 8.7 Hz), 9.35 (1H, br); MS m/z 388 $(M+H)^+$. HRMS: $(M+H)^+$ calcd for $C_{22}H_{30}NO_5$, 388.2124, found: 388.2169.

2-[4-(2-{[(1*S*,2*R*)-2-Hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)-2-methylphenoxy]-2-methylpropionic acid (1c). Mp 209–211 °C dec; $[\alpha]_D^{31} = -10.0°$ (c = 0.36, AcOH); IR (KBr) 3280, 1616, 1594, 1565, 1516; ^{1}H NMR (DMSO-d_6) δ 0.92 (3H, d, J = 6.6 Hz), 1.47 (6H, s), 2.11 (3H, s), 2.60–2.80 (2H, m), 2.85–3.05 (2H, m), 3.10–3.35(1H, m), 5.02 (1H, br s), 6.50–6.60 (1H, m), 6.65–6.75 (3H, m), 6.90 (1H, s), 7.13 (2H, d, J = 8.5 Hz); MS m/z 388 $(M+H)^+$. HRMS: $(M+H)^+$ calcd for $C_{22}H_{30}NO_5$, 388.2124, found: 388.2117.

2-[3-Chloro-4-(2-{[(1*S*,2*R*)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)phenoxy]-2-methylpropionic acid (1d). Mp 208–210 °C dec; $[\alpha]_D^{30} = -5.3°$ (c = 0.15, MeOH); IR (KBr) 3406, 1605, 1562, 1511; ^{1}H NMR (DMSO-d_6) δ 0.92 (3H, d, J = 6.5 Hz), 1.48 (3H, s), 1.49 (3H, s), 2.70–3.10 (4H, m), 3.20–3.40 (1H, m), 5.05 (1H, br s), 6.65–6.80 (3H, m), 6.85 (1H, d, J = 2.4 Hz), 6.95 (1H, d, J = 8.5 Hz), 7.16 (2H, d, J = 8.5 Hz); MS m/z (relative intensity) 408 $(M+H)^+$, 410 (0.31). HRMS: $(M+H)^+$ calcd for $C_{21}H_{27}ClNO_5$, 408.1578, found: 408.1539.

2-[2-Chloro-4-(2-{[(1*S*,2*R*)-2-hydroxy-2-(4-hydroxyphenyl)-1-methylethyl]amino}ethyl)phenoxy]-2-methylpropionic Acid (1e). Mp 182–184 °C dec; $[\alpha]_D^{31} = -6.9°$ (c = 0.75, AcOH); IR (KBr) 3491, 1631, 1611, 1568, 1511; ^{1}H NMR (DMSO-d_6) δ 0.91 (3H, d, J = 6.6 Hz), 1.50 (6H, s), 2.60–2.85 (2H, m), 2.90–3.50 (3H, m), 5.09 (1H, br s), 6.67 (1H, d, J = 8.6 Hz), 6.72 (2H, d, J = 8.5 Hz), 6.88 (1H, d, J = 8.6 Hz), 7.15 (2H, d, J = 8.5 Hz), 7.23 (1H, s), 9.35 (2H, br); MS m/z (relative intensity) 408 $(M+H)^+$, 410 (0.36). HRMS: $(M+H)^+$ calcd for $C_{21}H_{27}ClNO_5$, 408.1578, found: 408.1539.

Pharmacological experiments. In vitro and in vivo experiments were performed as described previously.[15–17] Using isolated preparations (rat atrium, rat uterus, and ferret detrusor) in the in vitro experiments, the effect of cumulative addition of compound **1** and isoproterenol to a Magnus bath were measured and estimated values calculated as follows: the EC_{20} value for β_1-AR was the concentration of each compound required increase the heart rate by 20 beats per min. The IC_{50} value for β_2-AR was the concentration of each compound required to produce a 50% inhibition of spontaneous uterine contraction, calculated as the total uterine contraction during 5 min following addition of each compound, when the total before addition of the compound was expressed as 100%. The EC_{50} value for β_3-AR was the concentration of each compound required to produce a 50% relaxation of urinary bladder smooth muscle, taking the relaxation induced by 10^{-5} M forskolin to be 0%, while tension before addition of the compound was 100%. In the in vivo experiment with **1a**, a polyethylene catheter was inserted into the urinary bladder of urethane-anaesthetized male rats. The bladder pressure was measured via a pressure transducer connected to the catheter and heart rate was measured simultaneously. Drug effects on bladder pressure (ED_{50} value) were quantified by expressing post-administration value as a percentage of the value before administration of **1a** or isoproterenol.

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