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Synthesis, binding affinity and selectivity of new β_1- and β_2-adrenoceptor blockers

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Abstract

The synthesis of a new series of sesamol derivatives with β-adrenergic blocking activity is described. The affinity and selectivity of these compounds for β_1- and β_2-adrenoceptors were studied in comparison with those of ICI-118551 and propranolol. Some of the synthesized compounds show very good affinity for the β_2-receptors of rat lung membranes and two of them provide interesting selectivity. © 1999 Elsevier Science S.A. All rights reserved.

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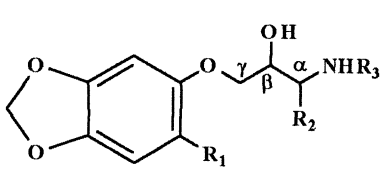
1. Introduction

Receptors should be regarded as the major targets of therapeutic agents and thus the bases for drug design. β-Adrenoceptors are known to play an important role in the regulation of the autonomic nervous system and β-blockers have been shown to be useful in the pharmacotherapy of serious and widespread cardiovascular diseases [1].

Lands et al. [2] proposed the existence of two β-adrenoceptor subtypes: the β_1-adrenoceptor, equally sensitive to norepinefrine and epinefrine, was dominant in heart muscle, whereas β_2-subtype, the dominant receptor in airway smooth muscle, was mainly sensitive to epinefrine. The cloning of β-adrenoceptor proteins have confirmed the presence of β_1- [3] and β_2-adrenoceptors [4], and finally that of a third gene coding for β_3-subtypes that are localized in adipose tissue [5]. The β_2-adrenoceptors also function as '*autoreceptors*' in the brain, because when activated they mediate a positive feedback on sympathetic neurotransmission and then enhance stimulus-evoked norepinefrine release.

It was recently reported that introducing extra copies of the gene coding for β_2-adrenoceptor into mice greatly increases the contractility of the heart, even in absence of epinefrine [6]. These considerations led to the 'epinephrine hypothesis' of essential hypertension, which suggests that an overstimulation of β_2-adrenoceptors leads to illness. With regard to the chemical aspect, it appears that aryloxypropanolamines are particularly efficient as β-blocking drugs and it has been demonstrated that the *ortho* substitution with a chloro atom increases the β-antagonistic potency whereas the α-methyl substitution brings about a shift towards β_2-selective activity [7].

In order to obtain compounds binding selectively to β_1- or β_2-adrenoceptors, we have synthesized a series of sesamol derivatives, both with and without a methyl group at the α carbon atom and a bromine atom in the *ortho* position of the aromatic ring:



R_1 = H or Br

R_2 = H or CH_3

The synthesis of all the derivatives is reported in Scheme 1.

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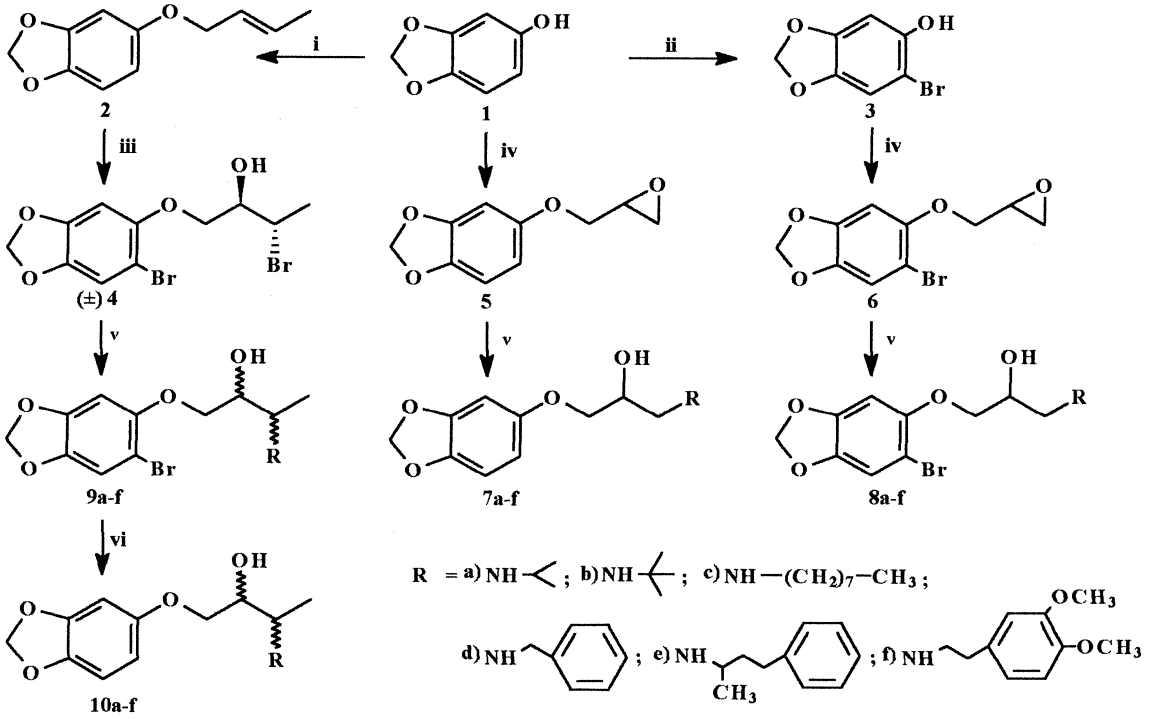


Scheme 1. (i) Crotyl chloride, CH_3COCH_3, K_2CO_3; (ii) Br_2, Ac, Ac; (iii) DMSO, H_2O, NBS. (iv) Method A: epichorohydrin, acetone, K_2CO_3. Method B; epichlorohydrin, Bu_4NHSO_4, NaOH/toluol; (v) a–f, EtOH; (vi) $LiAlH_4$, THF.

2. Chemistry

The starting material for the whole series of compounds was the 3,4-methylenedioxyphenol (sesamol). To obtain the α-methylamino derivatives **9a**–**f** and **10a**–**f**, sesamol **1** was reacted with *trans*-crotyl chloride to give the olefine **2**, which was treated with *N*-bromosuccinimide (NBS) to obtain the *trans*-bromidrine **4**. The reaction mechanism [8] is a *trans* addition of HOBr to olefin by means of moist dimethyl sulfoxide and NBS. The structure of bromohydrin **4** was confirmed by NMR spectroscopy.

A large excess of amine was refluxed in ethanol with the bromohydrin **4** for 2 days to give aminoalcohols **9a**–**f**, that were debrominated by $LiAlH_4$ to yield derivatives **10a**–**f**.

The other two series of compounds **7a**–**f** and **8a**–**f** were obtained starting from sesamol **1** and 6-bromosesamol **3**, respectively, which were refluxed in acetone with epichlorohydrin and K_2CO_3 for 3 days to give epoxy derivatives **5** and **6** (yield 64 and 66%, respectively). The same reaction was performed using a phase transfer catalysis. Compound **1** (or **3**) and epichlorohydrin were suspended in a mixture of toluene and aqueous sodium hydroxide (50% v/v) in the presence of tetrabutylammonium hydrogen sulfate as the catalyst. The reaction time was 6 h at room temperature (r.t.), but the yield decreased (35–40%). Epoxydes **5** and **6** were reacted in dry ethanol at r.t. for 2 days with three equivalents of amine to give the final products **7a**–**f** and **8a**–**f**.

3. Experimental

3.1. Chemistry

Melting points were determined using a Köfler block and are uncorrected. Elemental analyses for C, H, N were performed in a Perkin–Elmer elemental analyzer model 240 and the data are within ±0.4% of the theoretical values. ^1H NMR and ^{13}C NMR spectra were recorded at 25°C on a Brucker AC200F and chemical shifts are expressed as δ (ppm). FTIR spectra were recorded on a Mattson 3000 spectrometer. All the compounds were checked for purity by thin layer chromatography (TLC) on Merck 60 F_{254} silica plates. For column chromatography Merck 60 silica gel, 230–400 mesh, was used.

3.1.1. 3,4-Methylenedioxy-1-crotyloxybenzene (2)

To a solution of 1 g (7.25 mmol) of sesamol in 25 ml of acetone, 1 g (7.25 mmol) of K_2CO_3 and 1.5 ml (14.5 mmol) of *trans*-crotyl chloride were added. The mixture was refluxed and stirred for 48 h, water was added and the solution was extracted with ethyl acetate. The acetate extracts were collected, washed with water to neutrality, dried and evaporated. The residue was

loaded onto a column of silica gel 60 and eluted with a mixture of (9:1) petroleum ether–Et_2O, yielding 1.075 g (86%) of oily product.

^{1}H NMR. (200.13 MHz): ($CDCl_3$) δ 6.7 (d, 1H, Ar, J_o 7.8 Hz); 6.5 (d, 1H, Ar, J_m 3.1 Hz); 6.32 (dd, 1H, Ar, J_o 7.8 Hz, J_m 3.1 Hz); 5.9 (s, 2H, OCH_2O); 5.9–5.6 (m, 2H, CH=CH); 4.36 (d, 2H, $ArOCH_2$, J 5.9 Hz); 1.72 (d, 3H, CH_3, J 5.9 Hz). IR: ν_{max} 1503, 1487, 1182, 1042, 968 cm^{-1}.

*3.1.2. 2-Bromo-4,5-methylenedioxyphenol (**3**)*

To a solution of 2.07 g (15 mmol) of sesamol in 4.5 ml of glacial acetic acid, a solution of 6 ml (113 mmol) of bromine in 2.2 ml of glacial acetic acid was added dropwise, keeping the temperature below 10°C. The mixture was poured into ice-water and filtered. The solid was washed with cold water and dried to obtain 3.6 g (90%) of pure product. M.p. 84°C (dec.) [9].

*3.1.3. (±)6-Bromo-1-(3-bromo-2-hydroxy)butoxy-3,4-methylenedioxybenzene (**4**)*

To a solution of 985 mg (5.12 mmol) of **2** in 20 ml of DMSO, stirred under nitrogen at 10°C, 0.23 ml (12.8 mmol) of H_2O and 1.82 g (10.25 mmol) of NBS were added. After 30 min a solution of 0.2 N $NaHCO_3$ was added and the solution was extracted with diethyl ether. The ether extracts were collected, washed with water to neutrality, dried and evaporated. The residue was loaded onto a column of silica gel 60 and eluted with (8:2) hexane–Et_2O and then (4:6) hexane–Et_2O, yielding 908 mg (48%) of oily product.

^{1}H NMR. (200.13 MHz): ($CDCl_3$) δ 6.98 (s, 1H, Ar); 6.59 (s, 1H, Ar); 5.95 (s, 2H, OCH_2O); 4.34 (q, 1H, CH_3CHBr, J 6.9 Hz); 4.16 (d, 2H, $ArOCH_2$, J 5.6 Hz); 4.05 (m, 1H, CHOH); 2.74 (d, 1H, OH, J 5.3 Hz); 1.81 (d, 3H, CH_3, J 6.9 Hz). ^{13}C NMR. (50.33 MHz): ($CDCl_3$) δ 149 (C_q, Ar); 148 (C_q, Ar); 143 (C_q, Ar); 112.5 (CH, Ar); 103 (C_q, Ar); 102 (OCH_2O); 98 (CH, Ar); 72 ($ArOCH_2$); 68.5 (CHOH); 56.5 (CHBr); 20 (CH_3). DEPT. (50.33 MHz): ($CDCl_3$) δ 112.5 (CH, Ar); 102.0 (OCH_2O); 98.0 (CH, Ar); 72.0 ($ArOCH_2$); 68.5 (CHOH); 56.5 (CHBr); 20.0 (CH_3). IR: ν_{max} 1505, 1476, 1182, 1123, 1040, 936, 566 cm^{-1}.

*3.1.4. Synthesis of 3,4-methylenedioxy-1-oxyranylmethoxybenzene (**5**) and 6-bromo-3,4-methylenedioxy-1-oxyranylmethoxybenzene (**6**)*

Method A. To a solution of 3.62 mmol of **1** (or **3**) in 10 ml of acetone 500 mg (3.62 mmol) of K_2CO_3 and 0.9 ml (10.86 mmol) of epichlorohydrine were added. The mixture was refluxed and stirred for 60 h, water was then added and the solution was extracted with chloroform. The chloroform extracts were collected, washed with water to neutrality, dried and evaporated. The residue was loaded onto a column of silica gel 60 and eluted with a mixture of (98:2) $CHCl_3$–CH_3OH, yielding 450 mg (64%) of **5** (650 mg (66%) of **6**) as an oily product.

Method B. To a solution of 21.7 mmol of **1** or **3** in 30 ml of toluene and 50 ml of NaOH 50% (w/v), 5.3 ml (65.2 mmol) of epichlorohydrine and 600 mg (1.77 mmol) of tetrabutylammonium hydrogen sulfate were added. The mixture was stirred for 6 h, water was then added and the solution was extracted with chloroform. The chloroform extracts were collected, washed with water to neutrality, dried and evaporated. The residue was loaded onto a column of silica gel 60 and eluted with a mixture of (98:2) $CHCl_3$–CH_3OH, yielding 1.7 g (40%) of **5** and 2 g (35%) of **6** as oily products.

Compound **5**. ^{1}H NMR. (200.13 MHz): (DMSO) δ 6.80 (d, 1H, Ar, J 8.8 Hz); 6.62 (d, 1H, Ar, J 2.8 Hz); 6.38 (m, 1H, Ar, J); 5.96 (s, 2H, OCH_2O); 4,87 (m, 1H, OCH_2CHO); 4,61 (m, 1H, OCH_2CHO); 3.94–3.72 (m, 3H, CH-oxyranic).

Compound **6**. ^{1}H NMR (200.13 MHz): ($CDCl_3$) δ 6.95 (s, 1H, Ar); 6.58 (s, 1H, Ar); 5.92 (s, 2H, OCH_2O); 4.23–4.15 (m, 1H, OCH_2CHO); 3.97–3.89 (m, 1H, OCH_2CHO); 3.37–3.31 (m, 1H, CHO); 2.89–2.75 (m, 2H, CH_2O). IR: ν_{max} 1488.00, 1244.00, 1184.00, 1040.00, 908.00 cm^{-1}.

*3.1.5. Synthesis of 1-[2-hydroxy-3-(substituted-amino)]-propoxy-3,4-methylenedioxybenzene (**7a–f**) and 6-bromo-1-[2-hydroxy-3-(substituted-amino)]-propoxy-3,4-methylenedioxybenzene (**8a–f**) derivatives*

To a solution of 2.57 mmol of epoxy derivative (**5** or **6**) in 6 ml of dry ethanol was added 6.64 mmol of the appropriate amine and the mixture was stirred at r.t. After 48–60 h the solution was evaporated and the residue was dissolved in a solution of NaOH 25% (w/v) and extracted with chloroform. The chloroform extracts were collected, washed with water to neutrality, dried and evaporated. The residue was loaded onto a column of silica gel 60 and eluted. Yields and spectroscopic data are shown in Table 1.

*3.1.6. Synthesis of 6-bromo-1-[2-hydroxy-3-(substituted-amino)]-butoxy-3,4-methylenedioxybenzene derivatives (**9a–f**)*

A solution of 130 mg (0.35 mmol) of **4** and 1.06 mmol of appropriate amine in 3 ml of dry ethanol was stirred and refluxed. After 48 h the solution was evaporated and the residue was dissolved in a solution of NaOH 25% (w/v) and extracted with chloroform. The chloroform extracts were collected, washed with water to neutrality, dried and evaporated. The residue was loaded onto a column of silica gel 60 and eluted. Yields and spectroscopic data are shown in Table 1.

*3.1.7. Synthesis of 1-[2-hydroxy-3-(substituted-amino)]butoxy-3,4-methylenedioxybenzene derivatives (**10a–f**)*

To a solution of 0.43 mmol of derivative **9a–f** in 1.7 ml of dry THF was added 1.7 mmol of $LiAlH_4$ and the

Table 1
Chemical and physical data of the assayed compounds

Compound	Purification	NMR data
7a	Chromatography on SiO_2. $CHCl_3/CH_3OH$ gradient 95:5, 9:1, 8:2. M.p. 77–78°C. Yield 64%. M.p. of HCl salt: 131–132°C	^1H NMR (200.13 MHz) DMSO-d_6: δ 6.81 (d, 1H, Ar., J_o 8.7 Hz); 6.63 (d, 1H, Ar, J_m 2.4 Hz); 6.39 (dd, 1H, Ar., J_m 2.4, J_o 8.4 Hz); 5.97 (s, 2H, OCH_2O); 3.84 (m, 5H, $CHOH+OCH_2Oar+NH$); 2.85–2.50 (m, 3H, $CH_2N+CH(CH_3)_2$); 1.03 (d, 6H, $-CH(CH_3)_2$, J 6.1 Hz)
8a	Chromatography on SiO_2. $CHCl_3/CH_3OH$ gradient 42:3; 45:5. M.p. 70–72°C. Yield 45%	^1H NMR (200.13 MHz) DMSO-d_6: δ 7.13 (s, 1H, Ar); 6.92 (s, 1H, Ar); 5.99 (s, 2H, OCH_2O); 3.91–3.80 (m, 3H, OCH_2CHOH); 2.80–2.55 (m, 3H, CH_2N+CHN); 0.98 (d, 6H, $CH(CH_3)_2$)
9a	Chromatography on SiO_2. $CHCl_3/CH_3OH$ 8:2. P.f. 96–100°C. Yield 56%	^1H NMR (200.13 MHz) $CDCl_3$: δ 6.98 (s, 1H, Ar); 6.58 (s, 1H, Ar); 5.95 (s, 2H, OCH_2O); 4.02 (m, 2H, CH_2OAr); 3.86 (q, 1H, $CHOH$, J 4.2 Hz); 3.00 (m, 2H, $CHNH+CH(CH_3)_2$); 1.97 (s, 2H, $NH+OH$); 1.06 (m, 9H, $CH(CH_3)_2+CH_3$). IR ($CHCl_3$): ν_{max} 2969, 1505, 1474, 1182, 1119, 1040, 935 cm^{-1}
10a	Chromatography on SiO_2. AcOEt/CH_3OH 1:1. Yield 50%. M.p. 90–94°C	^1H NMR (200.13 MHz) $CDCl_3$: δ 6.69 (d, 1H, Ar, J_o 8.3 Hz); 6.51 (m, 1H, Ar); 6.34 (m, 1H, Ar); 5.90 (s, 2H, OCH_2O); 4.00–3.80 (m, 3H, OCH_2CHOH); 3.72–3.05 (m, 2H, $CH_3CHN+CH(CH_3)_2$); 2.00 (s broad, $NH+OH$, D_2O exchangeable); 1.10 (m, 9H, $CH(CH_3)_2+CH_3$)
7b	Chromatography on SiO_2. $CHCl_3/CH_3OH$ gradient: 95:5, 9:1, 8:2. Yield 67%. M.p. of HCl salt: 143–145°C	^1H NMR (200.13 MHz) $CDCl_3$: δ 6.67 (d, 1H, Ar., J_o 8.4 Hz); 6.51 (d, 1H, Ar., J_m 2.4 Hz); 6.33 (dd, 1H, Ar., J_m 2.4, J_o 8.4 Hz); 5.89 (s, 2H, OCH_2O); 3.90 (m, 3H, $CHOH+CH_2OAr$); 2.85 (s, 2H, $NH+OH$, D_2O exchangeable); 2.83–2.68 (m, 2H, CH_2N); 1.13 (s, 9H, $-C(CH_3)_3$)
8b	Crystallized from diethyl ether. Yield 73%. M.p. 93°C	^1H NMR (200.13 MHz) DMSO-d_6: δ 7.16 (s, 1H, Ar); 6.96 (s, 1H, Ar); 6.02 (s, 2H, OCH_2O); 4.85 (s br, 1H, OH, D_2O exchangeable); 3.96–3.72 (mm, 3H, OCH_2CHOH); 2.53–2.52 (m, 2H, CH_2N); 1.03 (s, 9H, $C(CH_3)_3$)
9b	Chromatography on SiO_2. AcOEt/CH_3OH/Et_3N 95:2:3. Yield 26%. Oily	^1H NMR (200.13 MHz) $CDCl_3$: δ 6.98 (s, 1H, Ar); 6.58 (s, 1H, Ar); 5.94 (s, 2H, OCH_2O); 3.99 (d, 2H, CH_2OAr, J 4.6 Hz); 3.73 (q, 1H, $CHOH$, J 6.2 Hz); .09 (qp, 1H, $CHCH_3$, J 6.2 Hz); 1.44 (s br, 2H, $NH+OH$, D_2O exchangeable); 1.13 (m, 12H, $-C(CH_3)_3+CH_3$). IR ($CHCl_3$): ν_{max} 2969, 1505, 1474, 1229, 1184, 1117, 1040, 935 cm^{-1}
10b	Chromatography on SiO_2. AcOEt/CH_3OH 7:3. Yield 61%. M.p. 106–110°C	^1H NMR (200.13 MHz) $CDCl_3$: δ 6.69 (d, 1H, Ar); 6.51 (m, 1H, Ar); 6.34 (m, 1H, Ar); 5.90 (s, 2H, OCH_2O); 3.99–3.84 (m, 3H, OCH_2CHOH); 3.44–2.82 (m, 3H, $CHN+NH+OH$, D_2O exchangeable); 1.18 (m, 12H, $C(CH_3)_3+CH_3$)
7c	Chromatography on SiO_2. $CHCl_3/CH_3OH$ gradient: 49:1, 46:4. Yield 52%. M.p. 79–81°C	^1H NMR (200.13 MHz) DMSO-d_6: δ 6.82–6.78 (d, 1H, Ar); 6.62–6.61 (d, 1H, Ar); 6.39–6.34 (dd, 1H, Ar); 5.96 (s, 2H, OCH_2O); 4.93 (s br, 1H, OH, D_2O exchangeable); 3.89–3.78 (m, 3H, alif.); 2.63–2.56 (m, 2H, alif.); 1.41–1.26 (mm, 14H, alif.); 0.90–0.84 (t, 3H, CH_3)
8c	Chromatography on SiO_2. $CHCl_3/CH_3OH$ 45:5. Yield 48%. M.p. 85°C	^1H NMR (200.13 MHz) DMSO-d_6: δ 7.17 (s, 1H, Ar2); 6.96 (s, 1H, Ar5); 6.03 (s, 2H, OCH_2O); 3.93 (m, 3H, OCH_2CHOH); 2.85–2.60 (mm, 2H, CH_2NH); 1.25 (s br, 14H, alif.); 0.90–0.84 (t, 3H, CH_3)
9c	Chromatography on SiO_2. AcOEt/CH_3OH/Et_3N 95:2:3. Yield 60%. M.p. 75–79°C	^1H NMR (200.13 MHz) $CDCl_3$: δ 6.97 (s, 1H, Ar); 6.59 (s, 1H, Ar); 5.94 (s, 2H, OCH_2O); 4.05 (d, 2H, CH_2OAr, J 4.5 Hz); 3.90 (m, 1H, $CHOH$); 2.95 (m, 1H, $CHNH$); 2.80–2.45 (m, 2H, CH_2NH); 2.10 (s broad, 2H, $NH+OH$); 1.45 (m, 2H, $NHCH_2CH_2$); 1.27 (m, 10H, $(CH_2)_5CH_3$); 1.11 (d, 3H, CH_3CH, J 6.8 Hz); 0.88 (t, H, CH_2CH_3, J 6.8 Hz). IR ($CHCl_3$): ν_{max} 2928, 1505, 1476, 1182, 1119, 1040, 935 cm^{-1}

Table 1 (*Continued*)

Compound	Purification	NMR data
10c	Chromatography on SiO_2. AcOEt/CH_3OH 7:3. Yield 47%. Oily	^1H NMR (200.13 MHz) $CDCl_3$: δ 6.69 (d, 1H, Ar); 6.51 (m, 1H, Ar); 6.35 (m, 1H, Ar); 5.90 (s, 2H, OCH_2O); 4.04–3.87 (m, 3H, OCH_2CHOH); 2.94–2.39 (mm, 5H, CHN + NH + OH); 1.50 (m, 2H, NCH_2); 1.30 (m, 12H, $(CH_2)_6$); 1.13 (d, 3H, CH_3CHN); 0.88 (t, 3H, CH_2CH_3)
7d	Chromatography on SiO_2. $CHCl_3$/CH_3OH gradient: 98:2, 8:2. M.p. 130–132°C. Yield 61%. M.p. of salt HCl: 170°C (dec.)	^1H NMR (200.13 MHz) $CDCl_3$: δ 7.31 (m, 5H, Ar.); 6.68 (d, 1H, Ar., J_o 8.5 Hz); 6.51 (d, 1H, Ar., J_m 2.2 Hz); 6.33 (dd, 1H, Ar, J_m 2.2, J_o 8.5 Hz); 5.91 (s, 2H, OCH_2O); 4.01 (m, 1H, CHOH); 3.89 (d, 2H, CH_2Ph, J 5.1 Hz); 3.84 (m, 2H, $ArOCH_2$); 2.85–2.76 (m, 2H, CH_2N); 2.65 (s, 2H, OH + NH)
8d	Chromatography on SiO_2. $CHCl_3$/CH_3OH gradient: 49:1, 48:2, 40:10. Yield 48%. M.p. 112–114°C	^1H NMR (200.13 MHz) DMSO-d_6: δ 7.334–7.308 (m, 5H, Ar); 7.157 (s, 1H, Ar2); 6.941 (s, 1H, Ar5); 6.026 (s, 2H, OCH_2O); 4.955–4.934 (d, 1H, OH, D_2O exchangeable); 3.932 (m, 3H, OCH_2CHOH); 3.739 (s, 2H, CH_2Ph); 2.527 (m, 2H, CH_2N)
9d	Chromatography on SiO_2. Gradient CH_2Cl_2, $CHCl_3$/CH_3OH 98:2. Yield 47%. M.p. 125–128°C	^1H NMR (200.13 MHz) $CDCl_3$: δ 7.25 (m, 5H, Ar); 6.95 (s, 1H, Ar); 6.58 (s, 1H, Ar); 5.95 (s, 2H, OCH_2O); 4.05 (d, 2H, $ArOCH_2$, J 4.5 Hz); 3.95–3.75 (m, 3H, $CH_2Ph + CHOH$); 3.05 (m, 1H, CHNH); 1.69 (s, 2H, OH + NH); 1.18 (t, 3H, CH_2CH_3, J 6.8 Hz). IR ($CHCl_3$): ν_{max} 1505, 1474, 1184, 1119, 040, 935 cm^{-1}
10d	Chromatography on SiO_2. AcOEt/CH_3OH 95:5. Yield 51%. Oily	^1H NMR (200.13 MHz) $CDCl_3$: δ 7.31 (m, 5H, Ph); 6.68 (d, 1H, Ar); 6.51 (m, 1H, Ar); 6.32 (dd, 1H, Ar); 5.91 (s, 2H, OCH_2O); 4.00–3.75 (m, 5H, $OCH_2CHOH + CH_2Ph$); 2.98 (m, 1H, CHN); 1.90 (s broad, 2H, NH + OH); 1.12 (d, 3H, CH_3CHN)
7e	Chromatography on SiO_2. $CHCl_3$/CH_3O 95:5. M.p.56°C. Yield 62%. M.p. of salt HCl: 151–152°C	^1H NMR (200.13 MHz) DMSO-d_6: δ 7.25 (m, 5H, Ph); 6.81 (d, 1H, Ar., J_o 8.7 Hz); 6.64 (d, 1H, Ar., J_m 2.7 Hz); 6.38 (dd, 1H, Ar., J_m 2.7, J_o 8.7 Hz); 5.96 (s, 2H, OCH_2O); 4.91 (s, 1H, OH); 3.84 (m, 3H, $CHOH + CH_2OAr$); 2.76–2.48 (m, 5H, $CH_2N + CH_2Ph + CH_3CHN$); 1.75–1.48 (m, 3H, $NH + CH_2CH_2Ph$); 1.04 (d, 3H, CH_3; J 6.2 Hz)
8e	Chromatography on SiO_2. $CHCl_3$/CH_3OH gradient: 45:5; 43:7. Yield 50%. M.p. 65–68°C	^1H NMR (200.13 MHz) DMSO-d_6: δ 7.311–7.206 (m, 5H, Ar); 7.177 (s, 1H, Ar); 6.966 (s, 1H, Ar); 6.026 (s, 2H, OCH_2O); 5.037 (s broad, 1H, OH, D_2O exchangeable); 3.972–3.842 (m, 3H, OCH_2CHOH); 2.886–2.573 (mm, 3H, alif.); 1.814–1.460 (mm, 3H, alif.); 1.081–1.049 d, 3H, CH_3)
9e	Chromatography on SiO_2. (9:1) CH_2Cl_2–CH_3OH. Yield 49%. Oily	^1H NMR (200.13 MHz) $CDCl_3$: δ 7.25 (m, 5H, Ar); 6.98 (s, 1H, Ar); 6.59 (s, 1H, Ar); 5.94 (s, 2H, OCH_2O); 4.02 (d, 2H, CH_2OAr, J 4.3 Hz); 3.80 (m, 1H, CHOH); 3.05 (m, 1H, $CHCHCH_3$); 2.80 (m, 1H, $NHCHCH_2$); 2.68 (t, 2H, CH_2Ph, J 8.7 Hz); 1.75 (m, 2H, CH_2CH_2Ph); 1.10 (m, 6H, 2 CH_3). IR ($CHCl_3$): ν_{max} 1651, 1541, 1505, 1474, 1182, 1119, 1040 cm^{-1}
10e	Chromatography on SiO_2. (7:3) AcOEt–CH_3OH. Yield 48%. M.p. 62–67°C	^1H NMR (200.13 MHz) $CDCl_3$: δ 7.25 (m, 5H, Ph); 6.69 (d, 1H, Ar); 6.51 (d, 1H, Ar); 6.33 (dd, 1H, Ar); 5.91 (s, 2H, OCH_2O); 3.89 (m, 3H, OCH_2CHOH); 2.99 (m, 1H, CH_3CHN); 2.84–2.62 (m, 3H, $CH_2Ph + NCHCH_2$); 1.82–1.49 (4H, OH + NH + CH_2CH_2Ph); 1.18–1.01 (m, 6H, 2 CH_3)
7f	Chromatography on SiO_2. (95:5) $CHCl_3$–CH_3OH. M.p. 107–108°C. Yield 65%	^1H NMR (200.13 MHz) DMSO-d_6: δ 6.87–6.70 (m, 4H, Ar.); 6.62 (d, 1H, Ar., J_m 2.4 Hz); 6.36 (dd, 1H, Ar., J_m 2.4, J_o 8.4 Hz); 5.97 (s, 2H, OCH_2O); 4.91 (s, 1H, OH); 3.84 (m, 3H, $CHOH + CH_2OAr$); 3.73 (s, 6H, 2 OCH_3); 2.81–2.52 (m, 6H, 2 $CH_2N + CH_2Ar$); 1.84 (s broad, 1H, NH)

Table 1 (*Continued*)

Compound	Purification	NMR data
8f	Chromatography on SiO_2. $CHCl_3$–CH_3OH gradient: (48:2, 45:5). Yield 56%. M.p. 137–39°C	^1H NMR (200.13 MHz) $CDCl_3$: δ 6.956 (s, 1H, Ar2); 6.767–6.715 (m, 3H, Ph); 6.558 (s, 1H, Ar5); 5.927 (s, 2H, OCH_2O); 4.010–3.917 (mm, 3H, OCH_2CHOH); 3.855 (s, 3H, OCH_3); 3.842 (s, 3H, OCH_3); 2.927–2.839 (mm, 4H, alif.); 2.776–2.711 (m, 2H, alif.)
9f	Chromatography on SiO_2. (9:1) $CHCl_3$–CH_3OH. Yield 68%. M.p. 145–150°C.	^1H NMR (200.13 MHz) $CDCl_3$: δ 6.75 (s, 3H, Ar); 6.69 (d, 1H, Ar); 6.55 (s, 1H, Ar); 5.95 (s, 2H, OCH_2O); 4.00 (m, 3H, $CHOH+CH_2OAr$); 3.85 (m, 6H, 2 OCH_3); 3.12–2.75 (m, 5H, CH_2N+CHN CH_2Ar); 2.15 (s, 2H, OH+NH); 1.15 (d, 3H, CH_3, J 6.2 Hz). IR ($CHCl_3$): ν_{max} 2942, 1516, 1474, 1261, 1184, 1040 cm^{-1}
10f	Chromatography on SiO_2. (1:1) AcOEt–CH_3OH. Yield 48%. Oily	^1H NMR (200.13 MHz) $CDCl_3$: δ 6.72 (m, 3H, Ar); 6.70 (d, 1H, Ar, J_o 8.3 Hz); 6.46 (d, 1H, Ar, J_m 2.9 Hz); 6.30 (dd, 1H, Ar, J_m 2.9 Hz, J_o 8.3 Hz); 5.90 (s, 2H, OCH_2O); 4.00–3.84 (m, 9H, $OCH_2CHOH+2$ OCH_3); 3.06–2.70 (m, 5H, CHN+NCH_2CH_2Ar); 2.12 (s broad, 2H, OH+NH); 1.08 (d, 3H, CH_3, J 6.7 Hz)

mixture was stirred and refluxed. After 6 h the excess of $LiAlH_4$ was eliminated by adding a mixture of (1:1) THF–H_2O dropwise. The solution was filtered and extracted with diethyl ether; the ether extracts were collected, dried and evaporated. The residue was loaded onto a column of silica gel 60 and eluted. Yields and spectroscopic data are shown in Table 1.

3.2. Affinity for adrenoceptors

3.2.1. Affinity for β_1-adrenoceptors

Pellets containing β_1-type adrenergic receptors were obtained from turkey erythrocyte membranes as described in the literature [10]. [^{3}H]Dihydroalprenolol ([^{3}H]DHA)(NEN), having a specific activity of 99.9 Ci $mmol^{-1}$ and radiochemical purity $>98.5\%$, was used as the ligand.

The β_1-adrenergic receptor binding assay was determined as follows: 100 µl of membrane (ca. 431 µg ml^{-1} of protein, dilution 1:8 v/v) were incubated for 15 min at 37°C with 100 µl of 6 nM [^{3}H]DHA and 100 µl of various concentrations of the test compounds (dissolved into saline with DMSO 5%) and 12 mM Tris–HCl, pH 7.5 (total volume 1 ml). The incubations were stopped by adding 3 ml of cold buffer (12 mM Tris–HCl) followed by rapid filtration through glass fiber filter disks (Whatman GF/B). The samples were subsequently washed with 4.5 ml of the same buffer and placed into scintillation vials; 10 ml of Filter-Count (Packard) liquid scintillation cocktail was then added to each vial and counting was carried out by scintillation spectrometer (Packard TRI-CARB 300C). Non specific binding was defined as non-displaceable binding in the presence of 100 µl of 10 µM propranolol.

Blank experiments were carried out to determine the effect of the solvent (5%) on binding.

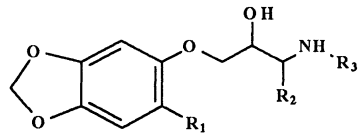
The concentration of the test compound that inhibited [^{3}H]DHA binding by 50% (IC_{50}) were determined by log-probit analysis with seven equivalents of the displacers, each performed in triplicate. The IC_{50} values obtained were used to calculate apparent inhibition constants (K_i) by the method of Cheng and Prusoff [11], from the following equation: $K_i = IC_{50}/(1 + S/K_D)$ where S represents the concentration of the ligand used and K_D is its receptor dissociation constant (K_D value, obtained by Scatchard analysis [12], for [^{3}H]DHA $K_D = 3.6 \times 10^{-9}$).

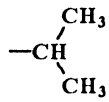
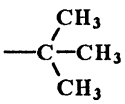
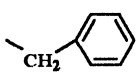
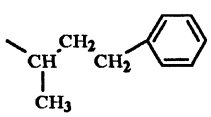
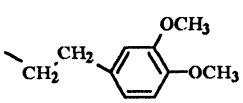
3.2.2. Affinity for β_2-adrenoceptors

Preparation of lung homogenate. Male Spangue–Dawley rats were sacrificed by decapitation. The right lung was removed and freed of the major bronchi. The lungs were homogenized with Brinkman Polytron (setting 5 for 15 s) in 50 volumes of buffer, 75 mM Tris–HCl (pH 7.65), 25 mM $MgCl_2$, and then were centrifuged twice at $30\,000 \times g$ for 10 min. The resulting pellets were suspended in 100 volumes of buffer, 75 mM Tris–HCl (pH 7.65), 25 mM $MgCl_2$, then frozen and stored at -80°C before being assayed [13,14]. [^{3}H]dihydroalprenolol ([^{3}H]DHA) (NEN), having a specific activity of 99.9 Ci $mmol^{-1}$ and radiochemical purity $>98.5\%$, was used as ligand.

The β_2-adrenergic receptor binding assay was determined as follows: 300 µl of lung membrane were incubated for 30 min at 37°C with 50 µl of 6 nM [^{3}H]DHA, 50 µl of Ketanserina 10^{-7} M as 5HT antagonist, 50 µl of practolol 10^{-6} M as β_2-antagonist and 50 µl of various concentrations of the test compounds (dissolved into saline with 5% DMSO or H_2O) and 75 mM Tris–HCl (pH 7.65), 25 mM $MgCl_2$ (total volume 0.5 ml). The samples were subsequently washed with 4.5 ml of the same buffer and placed into scintillation vials; 10

Table 2
β_1- and β_2-adrenoceptor binding affinity (K_i, nM) and selectivity ($K_i\beta_1/K_i\beta_2$) of compounds **7a–f** and **10a–f** [a]



Compound	R_1	R_2	R_3	$K_i\beta_1 \pm SE$	$K_i\beta_2 \pm SE$	$K_i\beta_1/K_i\beta_2$
7a	H	H		320 ± 0.184	67.6 ± 0.212	4.7
8a	Br	H		180 ± 0.224	13 ± 0.221	13.9
9a	Br	CH_3		4500 ± 0.074	56.3 ± 0.229	79.9
10a	H	CH_3		n.a.	303 ± 0.972	–
7b	H	H		830 ± 0.135	63.9 ± 0.265	13.0
8b	Br	H		45 ± 0.720	4.9 ± 0.122	9.2
9b	Br	CH_3		4500 ± 0.103	56.2 ± 0.185	80.1
10b	H	CH_3		n.a.	n.a.	–
7c	H	H		1500 ± 0.310	130 ± 0.224	11.5
8c	Br	H		1900 ± 0.234	24 ± 0.315	79.2
9c	Br	CH_3	$-(CH_2)_7-CH_3$	9000 ± 0.092	22.5 ± 0.331	400.0
10c	H	CH_3		2500 ± 0.260	225 ± 0.473	11.1
7d	H	H		n.a.	n.a.	–
8d	Br	H		600 ± 0.424	270 ± 0.349	2.2
9d	Br	CH_3		n.a.	n.a.	–
10d	H	CH_3		n.a.	n.a.	–
7e	H	H		280 ± 0.155	n.a.	–
8e	Br	H		1.3 ± 0.123	0.094 ± 0.720	13.8
9e	Br	CH_3		900 ± 0.370	112 ± 0.357	80
10e	H	CH_3		n.a.	n.a.	–
7f	H	H		19 ± 0.132	56.3 ± 0.235	0.3
8f	Br	H		20 ± 0.510	1.9 ± 0.201	10.5
9f	Br	CH_3		180 ± 0.228	1.01 ± 0.310	178.2
10f	H	CH_3		450 ± 0.082	105 ± 0.341	4.3
Propranolol				1.9 ± 0.204	2.6 ± 0.204	0.7
ICI-118551				32 ± 0.256	1.09 ± 0.267	29.4

[a] $K_i\beta_1$ vs. 0.6 nM [^{3}H]dihydroalprenolol in turkey erythrocyte and $K_i\beta_2$ versus 1.2 nM [^{3}H]dihydroalprenolol rat lung membranes.

ml of Filter-Count (Packard) liquid scintillation cocktail was then added to each vial and counting was carried out by scintillation spectrometer (Packard TRICARB 300C). Non-specific binding was defined as non-displaceable binding in the presence of 50 μl of 10 μM ICI-118551.

The concentrations of the test compounds that inhibited [^{3}H]DHA binding by 50% (IC_{50}) were determined by log-probit analysis with four equivalents of the displacers, each performed in triplicate. The IC_{50} values obtained were used to calculate apparent inhibition constants (K_i) by the method of Cheng and Prusoff [11], from the following equation: $K_i = IC_{50}/(1 + S/K_D)$ where S represents the concentration of the ligand used and K_D is its receptor dissociation constant (K_D value, obtained by Scatchard analysis [12], for [^{3}H]DHA $K_D = 3.6 \times 10^{-9}$).

Non-specific binding was measured in the presence of 50 μl of 10 μM unlabelled ICI-118551, and specific binding was defined as the difference between total and non-specific binding. Blank experiments were carried out to determine the effect of the solvent (5%) on binding.

4. Discussion and conclusions

The data of the β_1- and β_2-adrenoceptors binding affinities (K_i, nM) and selectivities ($K_i\beta_1/K_i\beta_2$) of compounds **7a**–**f**, **8a**–**f**, **9a**–**f** and **10a**–**f**, synthesized and assayed for this study as racemic mixtures, are shown in Table 2.

It is known that in β-agonists and -antagonists of the arylethanolamine and aryloxypropanolamine series the introduction of a methyl group on the carbon atom α to the amine moiety resulted in an overall reduction in potency, particularly at β_1-receptors. The introduction of

this alkyl group gives rise to two stereoisomers, the *erythro* and *threo*. Generally the *erythro* isomer is more potent than the *threo* one, however both the α-methyl stereoisomers are β_2-selective.

Aliphatic amines, *i*-propylamine and *t*-butylamine, confirm their ability to interact with β-adrenoceptors, whereas the long chain of octylamine decreases affinity especially toward β_1-adrenoceptors. The other amino groups seem to suggest a negative effect of the aromatic ring in compounds where the ring is near to the nitrogen. In fact, while derivatives with a 1-methyl-3-phenylpropyl and 3,4-dimethoxyphenylethyl moiety lead to very active compounds, derivatives with benzyl group are devoid of affinity.

The α methyl group shifts the selectivity toward β_2-adrenoceptors, but meanwhile the affinity for both type of receptors is decreased. Nevertheless the increase in selectivity always comes from a lower affinity for β_1-adrenoceptors.

The introduction of a bromo group in the *ortho* position always leads to an increase in the affinity. Moreover selectivity ($K_i\beta_1/K_i\beta_2$) is significantly enhanced, showing that in all compounds the affinity to β_2-adrenoceptors is increased.

In fact the affinities and selectivity of compounds **8b**, **8f** and **9f** for β_2-adrenoceptors are comparable with the reference compound ICI-118551, while compound **8e** has a better affinity and a similar selectivity. Compounds **9c** and **9f** possess remarkable selectivity toward β_2-adrenoceptors preserving good affinity, and therefore may be useful pharmacological tools to verify the 'epinephrine hypothesis' of essential hypertension.

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