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Synthesis of New Δ^2-Isoxazoline Derivatives and their Pharmacological Characterization as β-Adrenergic Receptor Antagonists

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Abstract—A series of Δ^2-isoxazoline derivatives structurally related to Broxaterol **1** and Falintolol **3** has been prepared and evaluated for their binding affinity to β_1- and β_2-adrenergic receptors. Among the tested compounds only the 3-isopropenyl *anti* derivative **4d** is as active as the reference compounds. An electron-releasing group, probably operating through a $\pi - \pi$ interaction, in the 3-position of the isoxazoline nucleus greatly enhances the affinity of the compounds. Conversely, the closest analogs of Broxaterol (3-bromo Δ^2-isoxazolines **4a** and **5a**) are at least one order of magnitude less active than the model compound **1**. Throughout the series of derivatives the *anti* stereoisomers are invariably more active than their *syn* counterparts.

Introduction

Broxaterol (±)-**1**, a potent and selective β_2-adrenergic agonist,[1,2] has been developed as a potential bronchodilatory agent in asthma therapy.[3] Inspection of the structure of **1** suggests a bioisosterism between its heterocyclic moiety and the catechol group of Isoproterenol **2**, the reference drug (Figure 1). This hypothesis is further supported by the stereochemical requirements of the two compounds. Despite the different notation, the spatial arrangement of the groups around the chiral center is in fact the same in both agonists,[4] along with common high values of the eudismic ratio (ER≥100).[1] Figure 1 reports the absolute configuration of the eutomer of Broxaterol and Isoproterenol.

It is well known that aliphatic oxime ethers exhibit interesting β-adrenergic blocking activities.[5,6] Among a series of derivatives, Falintolol **3** (Figure 1) emerged as a β_1/β_2-adrenergic antagonist.[6] Based on these results, a number of isoxazolinylethanolamines has been designed and evaluated as semi-rigid analogs of oxime ethers, though their structure reproduces an unfavored conformation of the parent open derivatives.[7–9] Since Broxaterol may in turn be regarded as the cyclic form of an oxime ether, we chose **1** as the model compound to plan the synthesis of stereoisomeric Δ^2-isoxazolines **4a–e** and **5a–e** (Figure 1). The substituent on the heterocyclic ring was chosen by taking into account the structure of Broxaterol (i.e., **4a**/**5a**, **4b**/**5b**, and **4e**/**5e**) as well as the structure of Falintolol (i.e., **4c**/**5c** and **4d**/**5d**). This paper deals with the synthesis of derivatives **4a–e** and **5a–e** and their binding affinities to membrane preparations containing β_1- and β_2-adrenergic receptors. We did not evaluate the affinity of our compounds for the β_3-adrenergic receptor because, according to the examples reported in the literature,[10] the selectivity for such a receptor can be achieved by appending a polar group on the substituent at nitrogen.

Chemistry

The synthesis of compounds **4a–e** and **5a–e** was achieved following a general strategy based on the 1,3-dipolar cycloaddition of halogenoximes **6a–c** to an

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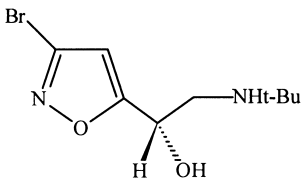
excess of butadiene in the presence of sodium bicarbonate (Scheme 1). Cycloadducts **7a–c** were obtained in fairly good yields. Olefinic compounds **7a–c** were oxidized with 3-chloroperoxybenzoic acid (mCPBA) to produce an equimolar mixture of *anti* **8a–c** and *syn* **9a–c** epoxides, which were separated by column chromatography. A Wittig methylenation carried out on epoxides **8c** and **9c** yielded isopropenyl derivatives **8d** and **9d**, respectively. Aminolysis of epoxides **8a–d** and **9a–d** (Scheme 1) with excess *t*-butylamine in methanol produced an almost quantitative yield of aminoalcohols **4a–d** and **5a–d**. Derivatives **4e** and **5e** were obtained, in excellent yield, by refluxing a methanol solution of **4a** and **5a**, respectively, in the presence of potassium carbonate. Since the transformation of the epoxides into aminoalcohols did not affect the relative configurations of the two chiral centers, *anti* epoxides **8a–d** yielded *anti* aminoalcohols **4a–d** exclusively, in analogy to the *syn* epoxides **9a–d** which produced *syn* derivatives **5a–d** solely.

Compounds **4b** and **5b** are known from literature.[9] The structure of the other *anti* (**4**) and *syn* (**5**) aminoalcohol derivatives was assigned on the basis of the analogy of their ^{1}H NMR pattern with that of the corresponding analogs **4b** and **5b**, respectively. In particular, the value of the coupling constant $J_{5,2'}$ of the *anti* derivatives ($J = 5.0–6.5$ Hz) was always higher than that of the corresponding *syn* stereoisomer ($J = 3.2–3.5$ Hz).

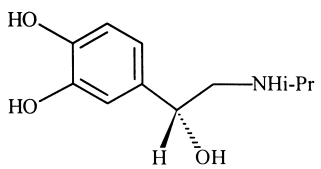
Results and discussion

Broxaterol **1** and isoxazoline derivatives **4a–e** and **5a–e** were assessed for binding affinity to membranes containing β_1 and β_2 adrenergic receptors. Table 1 reports their dissociation constants (K_i-values) obtained in competition experiments with ^{125}I-cyanopindolol (^{125}I-CYP) as a radioligand. Membranes were obtained from CHO cells transfected with the human β_2-adrenergic receptors and from rat C6 glioma cells containing β_1-adrenergic receptors.

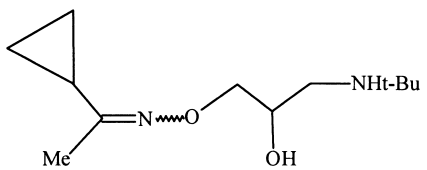
The first point to be addressed is the lack of selectivity observed with Broxaterol, the reference compound. In contrast with in vitro functional data where **1** shows remarkable β_2-selectivity as an agonist,[1] binding data (Table 1) give a very low (1.6) β_1/β_2 ratio. This dichotomy



(S)-(−)-**1**



(R)-(−)-**2**



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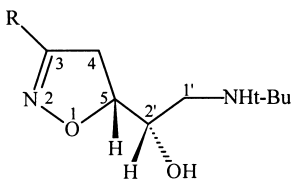
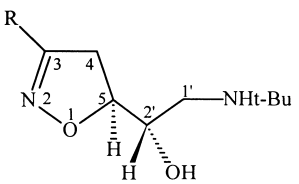
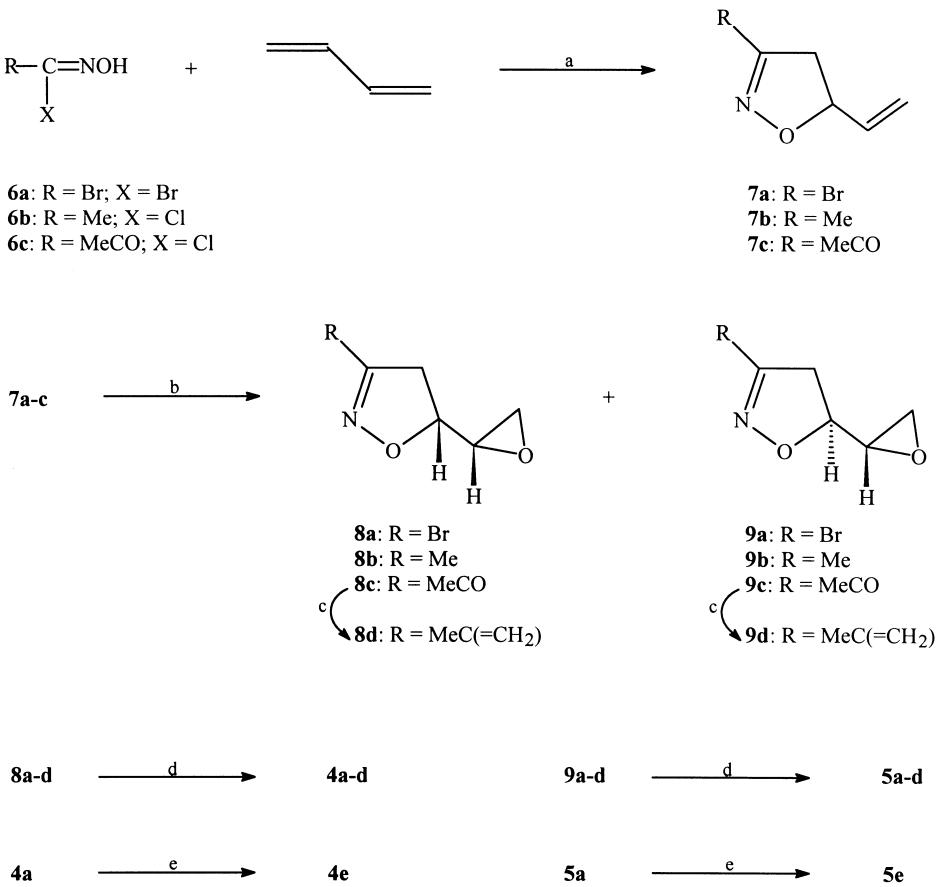


4a: R = Br
4b: R = Me
4c: R = MeCO
4d: R = MeC(=CH_2)
4e: R = MeO



5a: R = Br
5b: R = Me
5c: R = MeCO
5d: R = MeC(=CH_2)
5e: R = MeO

Figure 1.



Scheme 1. (a) $NaHCO_3/CH_3COOEt$; (b) $mCPBA/CH_2Cl_2$; (c) $Ph_3P=CH_2/Et_2O$; (d) *t*-$BuNH_2$/MeOH; (e) $MeOH/K_2CO_3$.

Table 1. Binding affinities of compounds **4a–e** and **5a–e** to rat C6 glioma cells (β_1) and to CHO cells transfected with β_2-adrenergic receptors

Compound	K_i[a](μM) for β_2	K_i[a] (μM) for β_1	β_1/β_2 ratio[b]
1	0.20 (0.13—0.31)[c]	0.32 (0.27—0.37)[c]	1.6
4a	1.44 (0.96—2.14)	2.64 (1.63—4.27)	1.8
5a	36.71 (26.8—50.3)	29.70 (13.7—64.5)	0.8
4b	6.85 (5.55—8.45)	8.64 (6.02—12.4)	1.3
5b	> 100	> 100	
4c	4.53 (3.52—5.82)	12.60 (7.09—22.5)	2.8
5c	28.19 (19.6—40.5)	> 100	> 3.5
4d	0.31 (0.26—0.38)	0.83 (0.66—1.06)	2.7
5d	1.53 (0.87—2.71)	3.66 (2.26—5.91)	2.4
4e	2.06 (1.68—2.54)	4.16 (3.21—5.38)	2.0
5e	8.79 (4.89—15.8)	14.90 (9.16—24.1)	1.7
3[d]	0.35 ± 0.042	0.64 ± 0.18	1.8

[a] K_i values are geometric means of 3–4 experiments.
[b] $1/K_i$ (β_2)/$1/K_i$ (β_1).
[c] The 95% confidence limits are reported in parentheses.
[d] Data taken from Ref. 11; binding affinity to rat lung (β_2) and rabbit heart (β_1).

was examined in detail via competition experiments with the radioligand antagonist ^{125}I-CYP, both in the presence and in the absence of GTP, and by measurement of the activation of adenylate cyclase. In competition experiments with ^{125}I-CYP the binding curves of Broxaterol to β_2-receptors evidenced two affinity states (high and low affinity states), as expected for agonists (Figure 2).

The addition of GTP (100 μM) provoked a shift of the competition curves to the right, abolishing the high affinity state. Similar experiments carried out with β_1-receptors demonstrated a single affinity state both in the presence and the absence of GTP (not shown), suggesting that Broxaterol behaves as an antagonist at this adrenergic receptor subtype. Broxaterol caused about a fourfold stimulation of the adenylate cyclase (Figure 3) via β_2-receptors (10.6 pmol/mg membrane protein/min) when compared to the basal activity (2.9 pmol/mg membrane protein/min). Marginal activation of the enzyme was observed via β_1-receptors (5.5 pmol versus 4.3 pmol) (Figure 3). The EC_{50} values of Broxaterol for β_1- and β_2-adrenergic receptors are 1.3 and 0.17 μM, respectively. Thus, Broxaterol appears to be a full agonist at β_2-receptors and a partial agonist, with a very low intrinsic activity, at β_1-receptors.

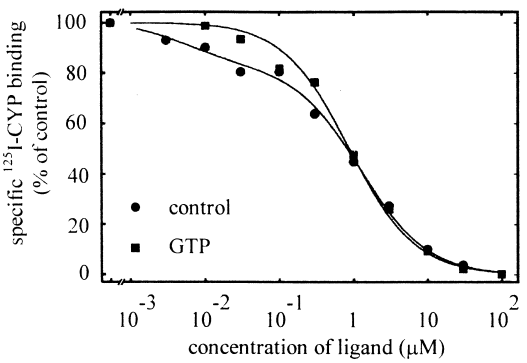


Figure 2. Competition curve of Broxaterol for ^{125}I-CYP binding to β_2-receptors without (●) and in the presence (■) of 100 μM GTP.

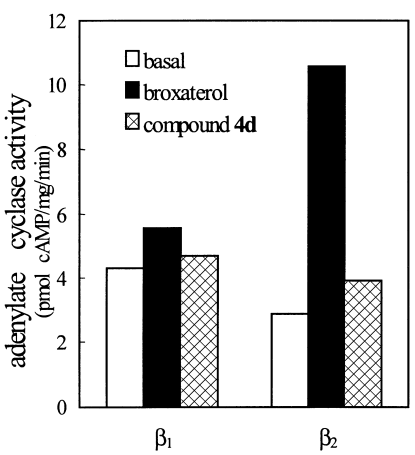


Figure 3. Activity of adenylate cyclase mediated by the stimulation of β_1- and β_2-adrenergic receptors.

The high affinity binding of Broxaterol to β_2-receptors, detected in the absence of GTP, makes it roughly 100-fold more potent at this receptor than at the β_1-subtype. These data provide an explanation for the β_2-selectivity observed in functional experiments, where Broxaterol relaxes spontaneously contracted guinea pig tracheal chain at low doses ($ED_{50} = 6$ nM) and marginally affects the contraction of the guinea pig atria ($ED_{25} = 327$ nM),[1] which could not be confirmed in binding experiments in the presence of GTP.

Inspection of the data reported in Table 1 shows that derivatives **4a** and **5a**, the closest analogs of Broxaterol, are at least one order of magnitude less active than the reference compound. Furthermore, at variance with **1**, which behaves as an agonist at β_2-receptors, compounds **4a** and **5a** appear to be antagonists as suggested by the shape of their binding curve (not shown) in the absence of GTP.

Since the same trend is observed for the other derivatives of the series, it appears that this set of isoxazoline derivatives behaves as antagonists at both β_1- and β_2-receptors and seems to be referred to Falintolol **3** or related linear oxime ethers rather than to Broxaterol. As a consequence, the heterocyclic moiety of Broxaterol can be viewed as the link between catechol, i.e. Isoproterenol, and oxime derivatives. A further analogy among the present isoxazoline derivatives (**4a**–**e** and **5a**–**e**) and linear oxime ethers is the influence of the substituent on adrenergic binding potency. In both series the presence of electron-donating groups, probably operating through a π–π interaction, increases the adrenergic binding potency. The bulkiness of the group also plays an important role in determining the affinity of the ligand for the receptors under investigation. As a matter of fact, Falintolol emerged as the most promising adrenergic antagonist of a huge number of linear aliphatic oxime ethers;[5,6] worth noting is the presence of the cyclopropyl group which is isoelectronic with the vinyl moiety. Isoxazoline **4d** is the most active compound in the set of derivatives we prepared and evaluated. It displays a binding affinity equal to Falintolol and a curve with only the low affinity binding site typical of antagonists. Furthermore, its structure contains the isopropenyl group which can be considered isoelectronic with the cyclopropyl moiety of the reference compound. Falintolol is the racemic form of a mixture of the *anti* **3a**

and *syn* **3b** stereoisomers (Figure 4). The individual stereoisomers have been prepared and evaluated in vitro for adrenergic activity on guinea pig atria (β_1) and trachea (β_2).[12] It emerged that the *syn* and *anti* isomers of **3** display comparable biological effects. Similar potencies have also been observed among the enantiomers of **3**.[12]

An accurate investigation of the conformational profile of linear aliphatic oxime ethers has been carried out.[13] It turned out that there is a certain degree of freedom around the O–CH_2 bond; when τ_2 ranges from 180° to −90° the energy differences vary within 0.12 Kcal/mol. On the other hand, the conformation in which τ_1 is 0° (**3c**) was found to be unfavored with respect to the preferred conformations by ca. 30–40 Kcal/mol. As evident from Figure 4, isoxazoline derivatives **4** and **5** represent the cyclic form of the unfavorable conformation **3c**. Since derivative **4d** is as active as Falintolol **3**, we can deduce that the adrenergic binding potency is not strictly dependent upon the favorable conformations around the N–O bond, but a large degree of conformational freedom is tolerated for the accommodation of the pharmacophoric groups. At this stage, a requirement for the cyclic derivatives is the relative configuration of the two chiral centers, since the ethanolamine side chain needs to be located in a spatial arrangement suitable for the interaction with the complementary receptor subsite. In all the pairs of derivatives **4**/**5** the *anti* stereoisomer **4** was always more active than **5**, the *syn* counterpart. The electronic character as well the bulkiness of the group located in the 3 position of the heterocyclic nucleus greatly affected the affinity for the adrenergic receptors. It remains to be ascertained if the activity of cyclic derivatives **4** and **5** is also affected by the absolute configuration of the chiral centers.

Conclusion

In conclusion, the present results evidence that isoxazolines derivatives **4** and **5** are not related biologically to Broxaterol **1** but more appropriately to aliphatic oxime ethers such as **3**. The influence of the substituent on the biological activity among the two series of derivatives is similar, electron-releasing groups highly enhancing the affinity for the adrenergic receptors. A detailed investigation of the conformational profile of derivatives **4** and **5** is underway and will be reported in due course along with the synthesis and pharmacological characterization of the chiral forms of **4d** and **5d**, and their cyclopropyl analogs.

Experimental

Materials and methods

^1H and ^{13}C NMR spectra were recorded with a Bruker AC-E 200 (200 MHz) spectrometer in $CDCl_3$ solution; chemical shifts (δ) are expressed in ppm and coupling constants (*J*) in Hertz. TLC were performed on commercial silica gel GF_{254} plates; spots were further evidenced by spraying with a dilute alkaline potassium

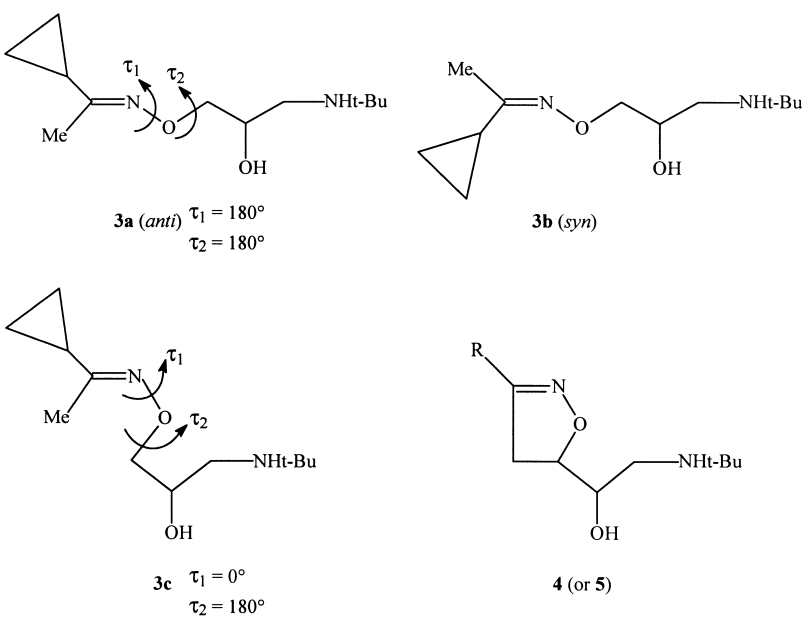


Figure 4.

permanganate solution. Liquid compounds were characterized by the oven temperature for Kugelrohr distillations. Melting points were determined on a Büchi apparatus and are uncorrected. Microanalyses of new compounds agreed with theoretical value to within ± 0.3%. Halogenoximes **6a–c** were prepared following standard procedures.[14–16] Broxaterol was prepared following a published methodology.[4] Aminoalcohols **4b** and **5b** have been previously described.[9]

Synthesis of isoxazoline derivatives 7a–c. To an ethyl acetate solution (70 mL) of halogenoxime **6** (30 mmol), cooled to 0 °C, were added butadiene (10 mL, 0.12 mol) and solid sodium bicarbonate (8.4 g, 0.1 mol). The mixture was stirred at room temperature until disappearance of the halogenoxime; the progress of the reaction was monitored by TLC. (eluant: 20% ethyl acetate/cyclohexane). The slurry was poured in water, the organic layer separated and the aqueous phase was further extracted with ethyl ether (2×20 mL). The organic extracts were dried over anhydrous sodium sulfate and the solvents removed under vacuum. The residue was Kugelrohr distilled to give cycloadducts **7a–c**. Compound **7a** distilled at 95–100 °C/9 mm Hg. Yield: 82%; ^{1}H NMR δ 3.02 (dd, 1, J = 9.6 and 16.8), 3.36 (dd, 1, J = 10.8 and 16.8), 5.09 (ddd, 1, J = 6.8, 9.6 and 10.8), 5.33 (m, 2), 5.94 (m, 1). Compound **7b** distilled at 100–105 °C/20 mm Hg.Yield: 65%;[9] Compound **7c** distilled at 70–75 °C/1 mm Hg.Yield: 75%; ^{1}H NMR δ 2.51 (s, 3), 2.91 (dd, 1, J = 9.6 and 17.0), 3.23 (dd, 1, J = 10.4 and 17.0), 5.15 (ddd, 1, J = 6.6, 9.6 and 10.4), 5.41 (m, 2), 5.96 (m, 1).

General procedure for the synthesis of epoxides 8a–c and 9a–c. To a magnetically stirred solution of cycloadducts **7** (24 mmol) in anhydrous dichloromethane (50 mL) a dichloromethane solution (75 mL) of 55% 3-chloroperoxybenzoic acid (16.7 g, 48 mmol) was added dropwise. The mixture was stirred at room temperature until TLC (eluant: 20% ethyl acetate/cyclohexane) showed the disappearance of the starting material. The mixture was sequentially treated with 20% aqueous solutions of sodium iodide and sodium thiosulfate. The organic layer was separated and the aqueous phase extracted with dichloromethane (3×30 mL). The pooled organic extracts were washed with a 20% potassium carbonate solution, dried over anhydrous sodium sulfate and evaporated to dryness. The crude residue was purified by flash-chromatography (eluant: 20% ethyl acetate/cyclohexane) to yield comparable amounts of *anti* epoxides **8a–c**, as the first fraction, followed by *syn* epoxides **9a–c**. The *anti*/*syn* ratio was in all the examined reactions near to 1. Compound **8a** distilled at 100–105 °C/1.5 mm Hg; yield 37%; ^{1}H NMR δ 2.61 (dd, 1, J = 1.9 and 3.7), 2.87 (dd, 1, J = 1.4 and 3.7), 3.00–3.30 (m, 3), 4.68 (ddd, 1, J = 4.6, 7.8 and 11.2). Compound **9a** distilled at 100–105 °C/1.5 mm Hg; yield 35%; ^{1}H NMR δ 2.84 (m, 2), 3.14 (m, 1), 3.18 (dd, 1, J = 7.4 and 17.6), 3.36 (dd, 1, J = 10.6 and 17.6), 4.72 (ddd, 1, J = 4.1, 7.4 and 10.6). Stereoisomeric epoxides **8b** and **9b** have been previously described.[9] Compound **8c** distilled at 75–80 °C/0.7 mm Hg; yield 41%; ^{1}H NMR δ 2.49 (s, 3), 2.60 (dd, 1, J = 2.5 and 4.3), 2.86 (dd, 1, J = 4.1 and 4.3), 2.95 (dd, 1, J = 7.8 and 17.4), 3.09 (dd, 1, J = 6.1 and 17.4), 3.21 (m, 1), 4.86 (ddd, 1, J = 4.2, 6.1 and 7.8). Compound **9c** distilled at 100–105 °C/1.5 mm Hg; yield 37%; ^{1}H NMR δ 2.47 (s, 3), 2.83 (m, 2), 3.07 (dd, 1, J = 7.8 and 17.4), 3.10 (m, 1), 3.25 (dd, 1, J = 11.4 and 17.4), 4.77 (ddd, 1, J = 3.8, 7.8 and 11.4).

Synthesis of epoxides 8d and 9d. To a stirred suspension of methylenetriphenylphosphorane, generated from methyltriphenyl-phosphonium bromide (1.8 g, 5 mmol) and potassium *t*-butoxide (0.6 g, 4.8 mmol) in anhydrous tetrahydrofuran (50 mL), a solution of epoxide **8c** (or **9c**) in THF (0.5 g, 3.3 mmol–15 mL) was added dropwise and stirring was continued at room temperature overnight. After the usual workup, **8d** (or **9d**) was purified through column chromatography on silica gel (eluant: 20% ethyl acetate/cyclohexane). Compound **8d** distilled at 80–85 °C/1.5 mm Hg. Yield 53%; ^{1}H NMR δ 2.05 (s, 3), 2.65 (dd, 1, J = 2.8 and 4.7), 2.87 (dd, 1, J = 4.2 and 4.4), 3.04 (dd, 1, J = 7.5 and 16.3), 3.12 (m, 1), 3.18 (dd, 1, J = 10.6 and 16.3), 4.59 (ddd, 1, J = 4.2, 7.5 and 10.6), 5.22 (bs, 1), 5.35 (bs, 1). Compound **9d** distilled at 80–85 °C/1.5 mm Hg. Yield 52%; ^{1}H NMR δ 2.05 (s, 3), 2.83 (m, 2), 3.09 (dd, 1, J = 7.9 and 16.6), 3.17 (m, 1), 3.25 (dd, 1, J = 11.0 and 16.6), 4.67 (ddd, 1, J = 4.6, 7.9 and 11.0), 5.21 (bs, 1), 5.35 (bs, 1).

General procedure for the synthesis of aminoalcohols 4a–d and 5a–d. A stirred solution of epoxides **8a–d** or **9a–d** (10 mmol) and *t*-butylamine (60 mmol) in methanol (50 mL) was refluxed until TLC (eluant: 20% ethyl acetate/cyclohexane) evidenced the disappearance of the starting material. The solvent and excess *t*-butylamine were removed under vacuum; the oily residue was dissolved in 6 N HCl (15 mL) and washed with ethyl ether (2×10 mL). The aqueous layer was alkalinized with solid sodium carbonate and extracted with dichloromethane (3×15 mL). The organic layer was separated, dried over anhydrous sodium sulfate, and the solvent evaporated under vacuum. The residue was dissolved in methanol and treated with a threefold excess anhydrous oxalic acid. The salt, separated by addition of anhydrous ethyl ether, was crystallized from the appropriate solvent. ^{1}H and ^{13}C NMR spectra were recorded on the corresponding free bases. Compound **4a**.1/2$C_2H_2O_4$: colorless prisms (from abs ethanol), mp 218–219 °C dec.; ^{1}H NMR δ 1.10 (s, 9), 2.52 (dd, 1, J = 7.7 and 12.1), 2.80 (dd, 1, J = 3.8 and 12.1), 3.24 (dd, 1, J = 10.4 and 16.8), 3.32 (dd, 1, J = 8.1 and 16.8), 3.62 (m, 1), 4.51

(ddd, 1, $J=6.0$, 8.1 and 10.4); ^{13}C NMR δ 30.0 (CMe_3), 44.2 (CH_2N), 44.9 (C-4), 51.3 (NH*C*), 70.8 (*C*HOH), 84.2 (C-5), 139.0 (C-3). Anal $C_{10}H_{18}BrN_2O_4$ (C, H, N). Compound **5a**.$C_2H_2O_4$: colorless needles (from 2-propanol/ethyl ether), mp 188–190 °C; ^{1}H NMR δ 1.08 (s, 9), 2.71 (m, 2), 3.21 (dd, 1, $J=4.9$ and 11.4), 3.29 (dd, 1, $J=3.1$ and 11.4), 3.60 (m, 1), 4.67 (ddd, 1, $J=3.4$, 8.8 and 10.4); ^{13}C NMR δ 29.9 (CMe_3), 44.2 (CH_2N), 44.9 (C-4), 51.3 (NH*C*), 71.1 (*C*HOH), 84.5 (C-5), 138.8 (C-3). Anal $C_{11}H_{19}BrN_2O_6$ (C, H, N). Compound **4b**.1/2$C_2H_2O_4$: colorless prisms (from abs ethanol), mp 233–235 °C dec.; ^{13}C NMR δ 14.0 (Me), 29.9 (CMe_3), 41.4 (CH_2N), 45.4 (C-4), 51.2 (NH*C*), 72.1 (*C*HOH), 82.8 (C-5), 156.8 (C-3). Anal $C_{11}H_{21}N_2O_4$ (C, H, N). Compound **5b**.$C_2H_2O_4$: colorless prisms (from 2-propanol/ethyl ether), mp 129–131 °C; ^{13}C NMR δ 14.0 (Me), 29.9 (CMe_3), 41.7 (CH_2N), 45.6 (C-4), 51.0 (NH*C*), 72.1 (*C*HOH), 82.8 (C-5), 156.8 (C-3). Anal $C_{12}H_{22}N_2O_6$ (C, H, N). Compound **4c**.$C_2H_2O_4$: colorless needles (from 2-propanol), mp 149–151 °C; ^{1}H NMR δ 1.10 (s, 9), 2.47 (dd, 1, $J=8.5$ and 12.2), 2.79 (dd, 1, $J=3.9$ and 12.2), 3.09 (dd, 1, $J=11.3$ and 18.0), 3.19 (dd, 1, $J=8.1$ and 18.0), 3.67 (m, 1), 4.68 (ddd, 1, $J=5.0$, 8.1 and 11.3); ^{13}C NMR δ 27.7 (Me), 30.0 (CMe_3), 34.1 (C-4), 44.8 (CH_2N), 51.3 (NH*C*), 71.0 (*C*HOH), 86.7 (C-5), 159.5 (C-3), 194.0 (C=O). Anal $C_{13}H_{22}N_2O_7$ (C, H, N). Compound **5c**.$C_2H_2O_4$: colorless prisms (from 2-propanol/ethyl ether), mp 178–179 °C dec.; ^{1}H NMR δ 1.08 (s, 9), 2.47 (s, 3), 2.71 (m, 2), 3.14 (d, 2, $J=9.7$), 3.58 (m, 1), 4.74 (ddd, 1, $J=3.2$, 9.9 and 9.9); ^{13}C NMR δ 30.0 (Me and CMe_3), 34.9 (C-4), 45.1 (CH_2N), 51.2 (NH*C*), 71.7 (*C*HOH), 86.9 (C-5), 159.6 (C-3), 194.1 (C=O). Anal $C_{13}H_{22}N_2O_7$ (C, H, N). Compound **4d**.1/2$C_2H_2O_4$: colorless prisms (from abs ethanol), mp 260–265 °C dec.; ^{1}H NMR δ 1.09 (s, 9), 2.03 (s, 3), 2.54 (dd, 1, $J=7.9$ and 12.1), 2.82 (dd, 1, $J=3.7$ and 12.1), 3.13 (dd, 1, $J=10.0$ and 16.9), 3.20 (dd, 1, $J=8.2$ and 16.9), 3.56 (m, 1), 4.50 (ddd, 1, $J=6.5$, 8.2 and 10.0), 5.24 (bs, 1), 5.33 (bs, 1); ^{13}C NMR δ 19.9 (Me), 29.9 (CMe_3), 37.5 (C-4), 45.1 (CH_2N), 51.6 (NH*C*), 71.3 (*C*HOH), 83.9 (C-5), 120.8 (CH_2=C), 136.4 (*C*=CH_2), 160.0 (C-3). Anal $C_{13}H_{23}N_2O_4$ (C, H, N). Compound **5d**.$C_2H_2O_4$: colorless prisms (from 2-propanol/ethyl ether), mp 184–185 °C dec.; ^{1}H NMR δ 1.09 (s, 9), 2.03 (s, 3), 2.72 (m, 2), 3.41 (d, 1, $J=9.6$), 3.59 (m, 1), 4.63 (ddd, 1, $J=3.5$, 9.6 and 9.6), 5.22 (bs, 1), 5.32 (bs, 1); ^{13}C NMR δ 19.9 (Me), 30.0 (CMe_3), 37.2 (C-4), 45.4 (CH_2N), 51.1 (NH*C*), 71.7 (*C*HOH), 84.3 (C-5), 120.6 (CH_2=C), 136.4 (*C*=CH_2), 160.0 (C-3). Anal $C_{14}H_{24}N_2O_6$ (C, H, N).

Synthesis of 4e and 5e. A suspension of **4a** (or **5a**) (0.5 g, 2.3 mmol) and anhydrous potassium carbonate (1.6 g, 11.6 mmol) in methanol (25 mL) was refluxed overnight. Methanol was removed under vacuum and the residue was taken up with water (20 mL) and dichloromethane (25 mL). The organic layer was separated, dried over anhydrous sodium sulfate, and the solvent removed under vacuum. The residue was dissolved in methanol (15 mL) and treated with a threefold excess of anhydrous oxalic acid to yield crude **4e**.$C_2H_2O_4$ (or **5e**.$C_2H_2O_4$). Compound **4e**.$C_2H_2O_4$: colorless needles (from 2-propanol), mp 178–180 °C; ^{1}H NMR δ 1.03 (s, 9), 2.44 (dd, 1, $J=8.2$ and 11.8), 2.68 (dd, 1, $J=3.7$ and 11.8), 2.89 (dd, 1, $J=10.0$ and 16.5), 3.01 (dd, 1, $J=8.0$ and 16.5), 3.64 (m, 1), 3.77 (s, 3), 4.43 (ddd, 1, $J=6.0$, 8.0 and 10.0); ^{13}C NMR δ 29.9 (CMe_3), 35.1 (C-4), 45.3 (CH_2N), 51.2 (NH*C*), 58.2 (OMe), 71.3 (*C*HOH), 84.1 (C-5), 169.0 (C-3). Anal $C_{12}H_{22}N_2O_7$ (C, H, N). Compound **5e**. $C_2H_2O_4$: colorless prisms (from 2-propanol), mp 138–143 °C; ^{1}H NMR δ 1.08 (s, 9), 2.69 (d, 2, $J=5.8$), 2.94 (dd, 1, $J=3.7$ and 16.3), 3.04 (dd, 1, $J=8.7$ and 16.3), 3.59 (m, 1), 3.82 (s, 3), 4.60 (ddd, 1, $J=3.4$, 5.8 and 5.8); ^{13}C NMR δ 29.9 (CMe_3), 35.3 (C-4), 45.2 (CH_2N), 51.1 (NH*C*), 58.2 (OMe), 71.5 (*C*HOH), 84.1 (C-5), 168.9 (C-3). Anal $C_{12}H_{22}N_2O_7$ (C, H, N).

Pharmacology

Materials. ^{125}I-Cyanopindolol (^{125}I-CYP) was obtained from Du Pont NEN, Dreieich, Germany. All other materials were from sources as described earlier.[17] DMSO or water 10 mM stock solutions of the substances under investigation (**4a–e** and **5a–e**) were prepared and then further diluted with binding buffer to the appropriate concentration.

Cells and membranes. CHO cells which do not express endogenous β-adrenergic receptor were stably transfected with the human β_2-adrenergic receptor as described.[17] As a model for β_1-adrenergic receptors rat C6 glioma cells were used. In these cells we could not detect measurable amounts of β_2-receptors as confirmed by competition experiments with the β_1-selective antagonist ICI 118551 for ^{125}I-CYP binding. For binding studies cells were grown on Petri dishes (140 mm) to confluency. Then the medium was removed and cells were washed and frozen in the dishes. The Petri dishes were kept at −25 °C until membranes were prepared. For the preparation of crude membranes, frozen cells were thawed and then scraped off the Petri dishes in the hypotonic buffer (5 mM TRIS/HCl, 2 mM EDTA, pH 7.4). The cell suspension was homogenized with an Ultra-Turrax (IKA) for 2×15 s at full speed and the homogenate was spun for 10 min at 1,000 *g*. The supernatant was then centrifuged for 40 min at 50,000 *g*. The membrane pellet was resuspended in 50 mM TRIS/HCl buffer pH 7.4, frozen in liquid nitrogen at a protein concentration of 1–3 mg/mL and stored at −80 °C.

Radioligand binding. Dissociation constants (K_i-values) of Broxaterol **1** and its analogues **4a–e** and **5a–e** were determined in radioligand competition experiments. The

nonselective antagonist ^{125}I-cyanopindolol was used as the radioligand at a concentration of 15–25 pM. Binding was carried out in a total volume of 200 μL with 20–25 μg of membrane protein in 50 mM TRIS/HCl pH 7.4 in the presence of 50 μM GTP. Samples were incubated for 1 h at 30 °C, filtered with a Scatron cell harvester and washed three times with 4 mL of ice-cold binding buffer. Data were analyzed by nonlinear curve-fitting with the the program SCTFIT.[18]

Adenylate cyclase activity. Adenylate cyclase activity was determined as described by Klotz et al.[19] with minor modifications. The incubation mixture contained 50 μg of membrane protein and about 100,000 cpm of [α-^{32}P]ATP whereas EGTA and NaCl were omitted. The samples were incubated for 20 min at 37 °C.

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