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Bioorganic & Medicinal Chemistry 15 (2007) 2533–2543
Bioorganic & Medicinal Chemistry

Novel chiral isoxazole derivatives: Synthesis and pharmacological characterization at human β-adrenergic receptor subtypes

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Received 27 November 2006; revised 19 January 2007; accepted 31 January 2007
Available online 2 February 2007

Abstract—Isoxazole derivative (±)-**4** and the three pairs of stereoisomeric 3-bromo-isoxazolyl amino alcohols (*S*,*R*)-(−)-**7a**/(*R*,*R*)-(+)-**7b**, (*S*,*R*)-(−)-**8a**/(*R*,*R*)-(+)-**8b**, and (*S*,*R*)-(−)-**9a**/(*R*,*R*)-(+)-**9b** were synthesized and assayed for their affinity and efficacy at human β_1-, β_2-, and β_3-adrenergic receptors (β-ARs) in membranes from Chinese hamster ovary (CHO) cells stably transfected with the respective receptor subtype. Whereas derivative (±)-**4** did not bind at all three β-ARs, stereoisomers (*S*,*R*)-**7a**-(*S*,*R*)-**9a** behaved as high-affinity ligands at β_1- and, particularly, at β_2-ARs (K_i 2.82–66.7 nM). The K_i values of isomers (*R*,*R*)-**7b**-(*R*,*R*)-**9b** at β_1- and β_2-subtypes were about 30–100 times higher than those of their (*S*,*R*)-**7a**–**9a** counterparts, indicating a sizable stereochemical effect. The affinity at β_3-ARs was negligible for all the investigated compounds. When submitted to a functional assay, the three stereoisomeric pairs showed a comparable pattern of efficacy at all three β-AR subtypes. The highest value of efficacy (75–90%) was observed at β_2-ARs, whereas all compounds behaved as partial agonists (30–60%) at the β_3-subtype. The lowest degree of efficacy (15–35%) was found at β_1-ARs. The affinity/efficacy profile of the derivatives under study has been compared with that of the two model compounds, Broxaterol [(±)-**1**] and BRL 37344 [(±)-**6**].

1. Introduction

β-Adrenergic receptors (β-ARs) are among the most thoroughly studied members of G protein-coupled receptors (GPCRs), the largest signaling family of the human genome.[1–4] At present β-ARs are classified as β_1-, β_2-, and β_3-subtypes based on their physiological actions, their pharmacological response to selective ligands as well as cloning of the receptors.[5] Functionally, significant levels of β_1-ARs are found in the heart, kidney, and brain,[6] β_2-ARs are predominant on vascular, uterine, and airway smooth muscles,[7] and β_3-ARs are mainly expressed in adipose tissue.[8] All of them couple primarily to $G\alpha_s$ to stimulate adenylyl cyclase, even though they can also couple to $G\alpha_i$ in some cells under certain conditions.[9]

Among the numerous β-ARs ligands that have found therapeutic application, antagonists ('β-blockers') represent a well-defined class of drugs in clinical use for virtually all major cardiovascular diseases, even though some uncertainties remain concerning their exact mechanisms of action.[3,10] On the other hand, β_2-AR agonists are routinely used in the treatment of asthma[11] owing to their regulatory effect on bronchial tone, and agonists or partial agonists selective for the human β_3-ARs are investigated as potential anti-obesity and anti-diabetes agents as well as novel drugs for urinary bladder dysfunctions.[12,13]

Although a number of amino acids have been identified through different experimental techniques as key binding site residues for a variety of β-AR agonists and antagonists,[14] at present a detailed understanding for β-AR subtype ligand-binding selectivity is far from being clearly established. Moreover, activation of GPCRs seems to be a multistep process further complicated by the existence of these receptors in different putative inactive and active conformations.[15,16] As a consequence, the molecular interaction with agonists

Keywords: Synthesis; Isoxazole derivatives; Hybrid compounds; Human β-adrenergic receptor subtypes; Binding affinity; Efficacy.
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doi:10.1016/j.bmc.2007.01.056

would lead to a stabilization of one or more of these activated states. Such mechanisms complicate the discussion of the relationship between binding affinity and functional efficacy at β-ARs and other GPCRs, however they also open interesting perspectives for the development of novel agonists with the potential of recognizing functionally distinct states of receptor subtypes.[17]

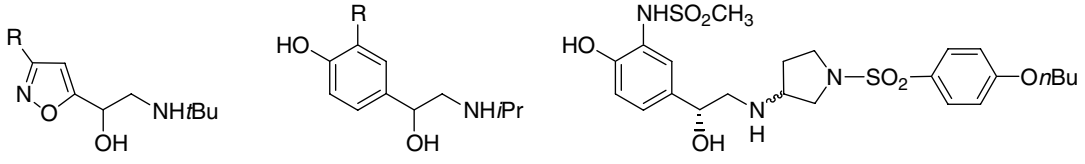
Our interest in the investigation of subtype-selective ligands for the human β-ARs has been focused on a series of Δ^2-isoxazoline and isoxazole derivatives structurally related to Broxaterol (±)-**1** (Fig. 1),[18–20] a β_2-selective agonist initially developed as a potential bronchodilatory agent for the therapy of asthma,[21,22] which was later on discontinued. Interestingly, we found that this ligand, which binds with comparable affinity to all three β-ARs, is an agonist at β_2- and β_3-receptors and an antagonist at the β_1-receptor, thus behaving as a functionally selective β_2-/β_3-AR agonist.[18,23] Despite the qualitatively different pharmacological profile, Broxaterol and Isoproterenol (±)-**2** (Fig. 1), the reference non-selective β-AR agonist, share the same stereochemical preference, since the eutomers of both compounds, that is (*S*)-(−)-**1** and (*R*)-(−)-**2**, respectively, have an identical spatial arrangement of the substituents around the common stereogenic center.[24]

The goal of the present paper was to further investigate the role of the isoxazole moiety in the interaction with the β-AR subtypes. To this end, we initially considered the structure of Soterenol (±)-**3**, a β_2-selective agonist studied about thirty years ago,[25] which is characterized by the replacement of the 3-hydroxy group of the catechol moiety of Isoproterenol with the methanesulfonylamino moiety. Therefore, we planned the synthesis of the 3-methanesulfonylamino-isoxazole derivative (±)-**4** and the evaluation of its affinity at the three β-AR subtypes. Worth mentioning, the skeleton of Soterenol (±)-**3** has recently been incorporated in a number of derivatives, such as **5** (Fig. 1),[26] endowed with the profile of selective β_3-AR agonists.

Next, we considered the structures of (±)-**1** and BRL 37344 (±)-**6** (Fig. 1), one of the first reported β_3-AR agonists[27] and still used as a lead compound,[28] to design hybrid derivatives (*S*,*R*)-(−)-**7a** and (*R*,*R*)-(+)-**7b**. The goal was the evaluation of the impact on the pharmacological profile brought about by the replacement of the 3-chlorobenzene moiety of (±)-**6** with the 3-bromoisoxazole nucleus of Broxaterol (±)-**1**. The related ethyl esters (*S*,*R*)-(−)-**8a** and (*R*,*R*)-(+)-**8b**, and their *O*-*n*-butoxy analogs (*S*,*R*)-(−)-**9a** and (*R*,*R*)-(+)-**9b** (Fig. 1) were also prepared. This paper reports the synthesis of (±)-**4** and of enantiopure 3-bromoisoxazoles (−)-**7a**/(+)-**7b**, (−)-**8a**/(+)-**8b**, and (−)-**9a**/(+)-**9b**, and the evaluation of their binding affinity and efficacy at human β_1-, β_2-, and β_3-receptor subtypes.

2. Chemistry

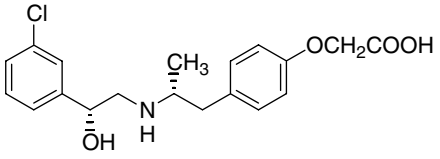
As depicted in Scheme 1, the synthetic sequence to target compound (±)-**4** started with 3-amino-5-ethoxycarbonyl-isoxazole (±)-**10**, in turn prepared according to a published method.[29] The corresponding 3-methanesulfonylamino-isoxazole derivative (±)-**11**, prepared with a standard procedure, was submitted to the Weinreb reaction protocol[30,31] using commercially available *N*,*O*-dimethylhydroxylamine hydrochloride and a THF solution of methylmagnesium chloride to afford methylketone (±)-**12**.[32] Intermediate (±)-**12** was sequentially



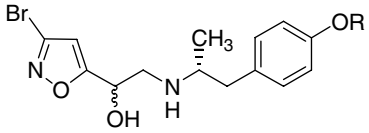
(±)-**1**: R = Br
(±)-**4**: R = $NHSO_2CH_3$

(±)-**2**: R = OH
(±)-**3**: R = $NHSO_2CH_3$

5

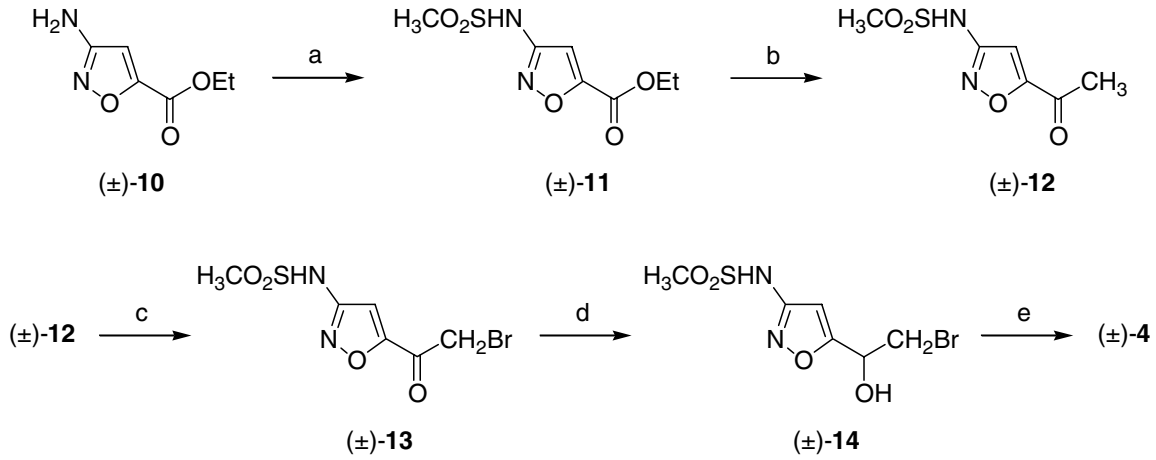


(±)-**6**: (*R**,*R**)-BRL 37344



(*S*,*R*)-(−)-**7a**: R = CH_2CO_2H
(*R*,*R*)-(+)-**7b**: R = CH_2CO_2H
(*S*,*R*)-(−)-**8a**: R = CH_2CO_2Et
(*R*,*R*)-(+)-**8b**: R = CH_2CO_2Et
(*S*,*R*)-(−)-**9a**: R = $(CH_2)_3CH_3$
(*R*,*R*)-(+)-**9b**: R = $(CH_2)_3CH_3$

Figure 1. Model and target compounds in this study.



Scheme 1. Reagents: (a) CH_3SO_2Cl, $Et_3N/Cl(CH_2)_2Cl$; (b) $CH_3ONHCH_3\times$ HCl, CH_3MgCl/THF; (c) $(CH_3)_3C_6H_5N^+Br_3^-$/THF; (d) $NaBH_4$/MeOH; (e) *t*-$BuNH_2$/MeOH.

converted into α-bromoketone (±)-**13** and bromohydrin (±)-**14** which, by treatment with an excess of *tert*-butylamine in refluxing methanol, provided the desired isoxazolyl ethanolamine (±)-**4**.

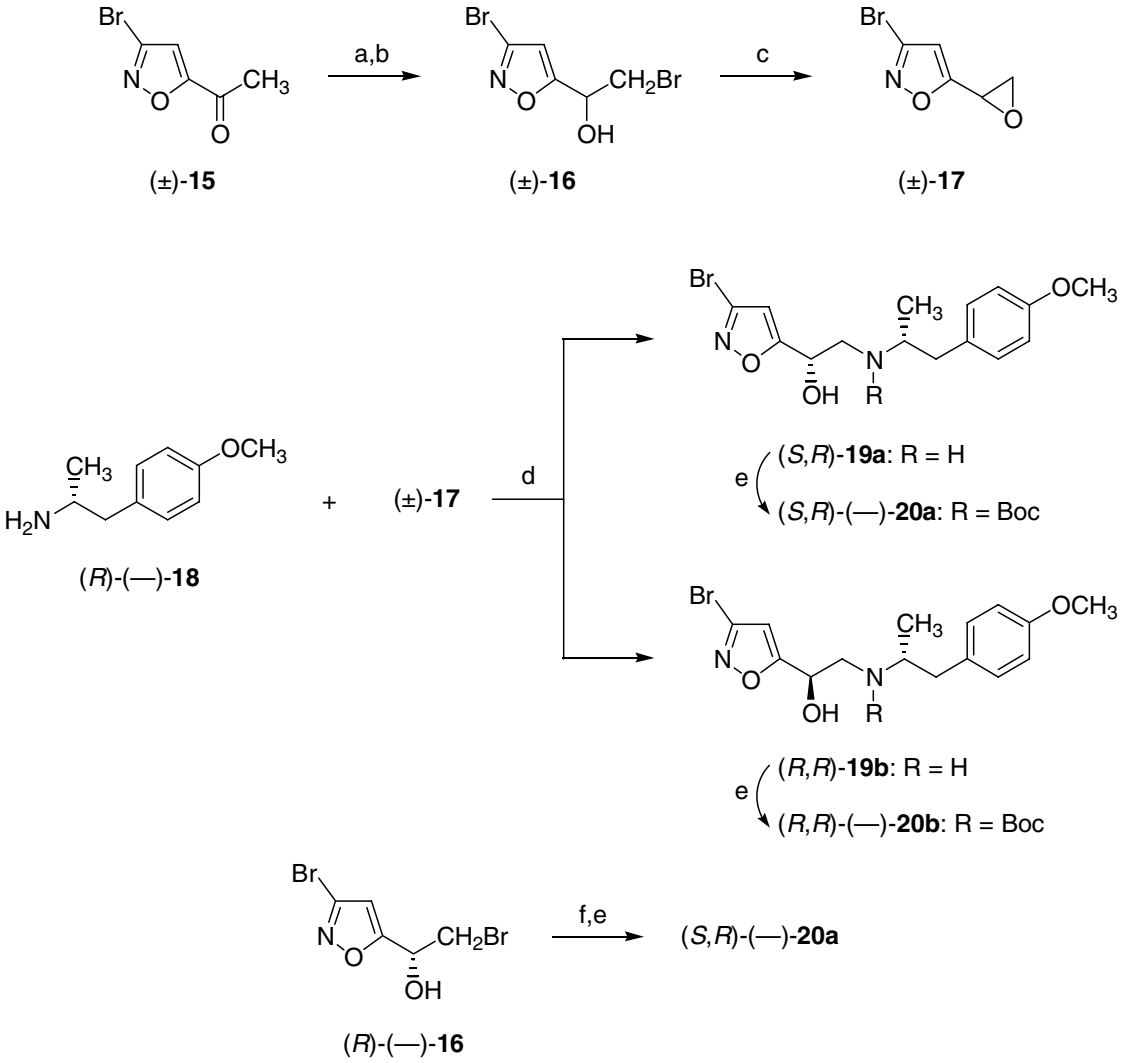
The synthesis of the stereoisomeric couples **7a**–**b**, **8a**–**b**, and **9a**–**b** was accomplished by using the known 3-bromo-5-acetyl-isoxazole (±)-**15**[24] as starting material, which was transformed into the corresponding 3-bromo-5-oxiran-2-yl-isoxazole (±)-**17** through the intermediacy of bromohydrin (±)-**16** (Scheme 2). Epoxide (±)-**17** was subsequently reacted with known (*R*)-(−)-1-methyl-2-(4-methoxyphenyl)ethylamine **18**[33,34] in refluxing acetonitrile under the catalysis of calcium trifluoromethanesulfonate,[35] to produce an almost equimolar mixture of stereomeric amino alcohols (*S*,*R*)-**19a** and (*R*,*R*)-**19b** in 60% overall yield. These experimental conditions proved to be the most convenient among a series of variants. The two stereoisomers could be separated by preparative silica gel chromatography only after their conversion into the corresponding *N*-Boc derivatives (*S*,*R*)-(−)-**20a** and (*R*,*R*)-(−)-**20b**. The absolute configurations were unambiguously assigned to (−)-**20a** and (−)-**20b** by performing, on an analytical scale, the nucleophilic substitution of (*R*)-(−)-**18** onto the enantiopure bromohydrin (*R*)-(−)-**16**[24] in refluxing methanol, which, after insertion of the *N*-Boc group, afforded (*S*,*R*)-(−)-**20a** as the sole isomer.

The synthetic pathway to the final compounds proceeded along the reaction sequence is shown in Scheme 3. Isomers (*S*,*R*)-(−)-**20a** and (*R*,*R*)-(−)-**20b** were separately treated with a 1.0 M solution of boron tribromide in dichloromethane to give intermediates (*S*,*R*)-(−)-**21a** and (*R*,*R*)-(−)-**21b**, respectively. The corresponding *N*-Boc-reprotected derivatives (*S*,*R*)-(−)-**22a** and (*R*,*R*)-(−)-**22b** were then suitably functionalized at the 4-hydroxyphenyl group by treatment with ethyl bromoacetate or 1-bromobutane in a refluxing acetone suspension of potassium carbonate, to produce the pairs of diastereomers (*S*,*R*)-(−)-**23a**/(*R*,*R*)-(−)-**23b** and (*S*,*R*)-(−)-**24a**/(*R*,*R*)-(−)-**24b**, respectively. These diastereomers were then converted into the desired final compounds (*S*,*R*)-(−)-**8a** and (*R*,*R*)-(+)-**8b**, (*S*,*R*)-(−)-**9a** and (*R*,*R*)-(+)-**9b** by removal of the *N*-Boc protecting group with a dichloromethane solution of trifluoroacetic acid. Alternatively, intermediates (*S*,*R*)-(−)-**23a** and (*R*,*R*)-(−)-**23b** were first hydrolyzed to the corresponding carboxylic acids and then submitted to *N*-Boc cleavage to produce target compounds (*S*,*R*)-(−)-**7a** and (*R*,*R*)-(+)-**7b**.

3. Results and discussion

Isoxazole derivative (±)-**4** and the three pairs of stereoisomeric 3-bromo-isoxazolyl derivatives (*S*,*R*)-(−)-**7a**/(*R*,*R*)-(+)-**7b**, (*S*,*R*)-(−)-**8a**/(*R*,*R*)-(+)-**8b**, and (*S*,*R*)-(−)-**9a**/(*R*,*R*)-(+)-**9b** were tested for their binding affinity at human β_1-, β_2-, and β_3-ARs in membranes from CHO cells stably transfected with the respective receptor subtype.[23] Table 1 reports the dissociation constants (K_i values) of the investigated compounds from competition experiments with ^{125}I-cyanopindolol (^{125}I-CYP) as the radioligand. The corresponding K_i values for Broxaterol (±)-**1**[20,23] and the BRL 37344 (±)-**6** have been included for comparison. Since binding experiments were carried out in the presence of 100 μM GTP all K_i values for agonists reflect low-affinity binding.

Inspection of Table 1 reveals the lack of any detectable affinity of derivative (±)-**4** for β-ARs (K_i > 100,000 nM at all three subtypes). Hence, the replacement of the 3-bromo moiety of (±)-**1** by the methanesulfonylamino group prevents any interaction with the binding sites of the β-ARs. Conversely, within the group of structurally related 3-bromoisoxazole derivatives, the (*S*,*R*)-**7a**, (*S*,*R*)-**8a**, and (*S*,*R*)-**9a** analogs behaved as high-affinity ligands at β_1- and, particularly, at β_2-ARs (Table 1). Indeed, in the set of compounds under study, amino alcohol (*S*,*R*)-(−)-**8a**, bearing the ester function on the distal chain, had the highest affinity at β_1 and β_2 subtypes (K_i = 23.2 and 2.82 nM, respectively). The same set of isomers showed a marginal affinity for the β_3-ARs, with the sole exception of the *O*-*n*-butoxy ether (*S*,*R*)-(−)-**9a** which gave a value of K_i equal to 2500 nM. As a general

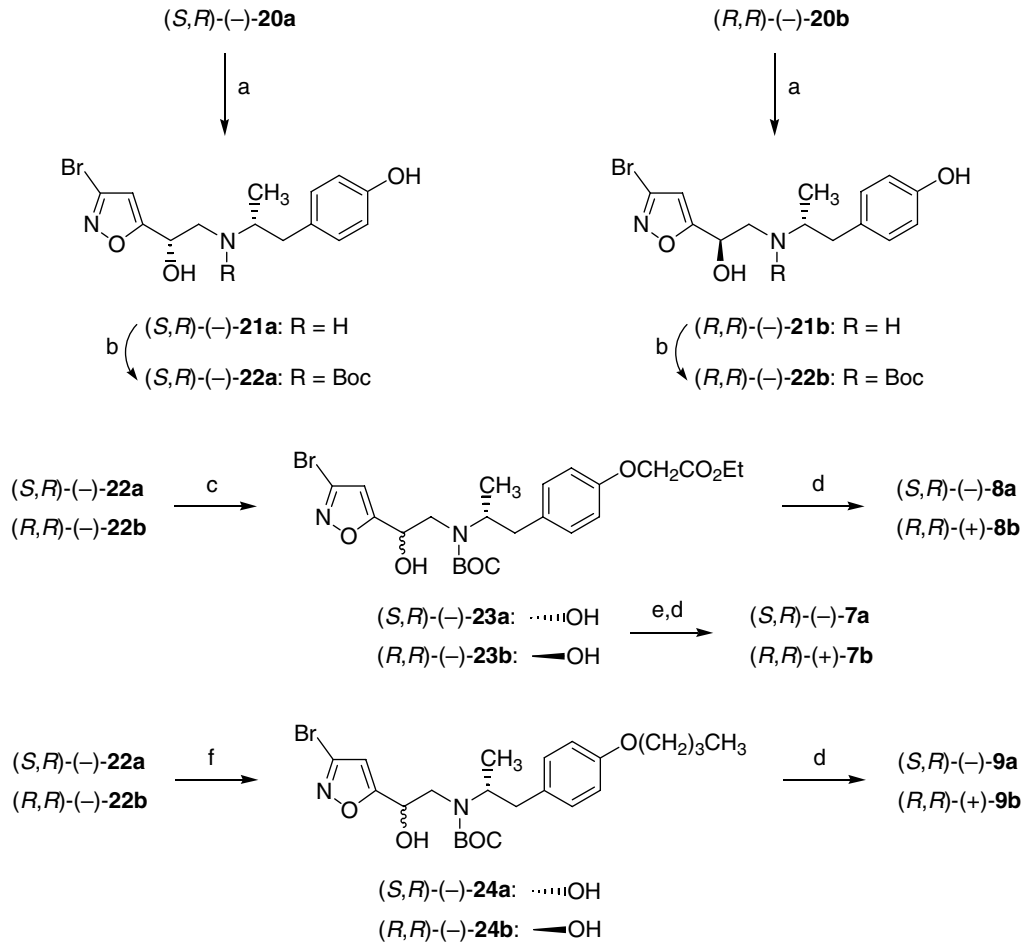


Scheme 2. Reagents: (a) $(CH_3)_3C_6H_5N^+Br_3^-$/THF; (b) $NaBH_4$/MeOH; (c) NaH/toluene; (d) $Ca(OTf)_2$/ CH_3CN; (e) $(Boc)_2O$, Et_3N/CH_2Cl_2; (f) (*R*)-(−)-**18**/MeOH.

trend, the (*R*, *R*)-stereoisomers showed an affinity lower than that of the corresponding (*S*, *R*)-diastereomers. In particular, the K_i values at β_1- and β_2-subtypes were usually 30–100 times higher than those of the corresponding (*S*, *R*)-counterparts, indicating a substantial stereochemical effect.

On the whole, the replacement of the 3-chlorobenzene group of BRL 37344 (±)-**6** with the 3-bromoisoxazole moiety of Broxaterol (±)-**1** brings about a considerable change in the affinity profile, as evidenced by derivative (*S*, *R*)-(−)-**7a**, the hybrid incorporating the structure of the eutomer of Broxaterol, that is (*S*)-(−)-**1**, and retaining the acidic group of BRL 37344 (±)-**6**. As a matter of fact, analogs **7a**–**9a**, characterized by the (*S*, *R*)-configuration at the stereogenic centers, behave as high-affinity ligands at both β_1- and β_2-subtypes (K_i values in the range 2.82–66.7 nM), at variance with (±)-**1** (K_i equal to 1310 nM at β_1-ARs and 1290 nM at β_2-ARs) and, even more, with (±)-**6** (K_i equal to 37,000 nM at β_1-ARs and 2850 nM at β_2-ARs). Within this set of new derivatives, which belong to the class of arylethanolamines, the ligand-receptor recognition process is dictated at β_1-ARs and to a lesser extent at β_2-ARs by the (*S*)-configuration at the stereogenic center localized at the secondary alcohol function. The affinity at β_3-ARs is almost negligible for all the investigated compounds and, consequently, is marginally affected by the absolute configuration at the same stereogenic center.

In order to fully define their pharmacological profile, the six isoxazolyl amino alcohols (*S*, *R*)-**7a**-(*S*, *R*)-**9a** and (*R*, *R*)-**7b**-(*R*, *R*)-**9b** were submitted to a functional assay in which stimulation of adenylyl cyclase (AC) was tested in membranes from stably transfected CHO cells with similar receptor expression for each β-AR subtype.[23] The agonistic activity of these compounds was compared to the maximal stimulation obtained with the full agonist Isoproterenol (100%; basal activity 0%) as the reference. From our previous characterization of the transfected CHO cell clones used in this study, we know that EC_{50} values for agonists tend to be lower than the corresponding K_i values derived from binding experiments.[23] At a K_i-concentration, a signal of 80–90% amplitude is expected from a compound with full



Scheme 3. Reagents: (a) BBr_3/CH_2Cl_2; (b) $(Boc)_2O$, Et_3N/CH_3CN; (c) $BrCH_2CO_2Et$, K_2CO_3/acetone; (d) CF_3COOH/CH_2Cl_2; (e) K_2CO_3/EtOH–H_2O; (f) $Br(CH_2)_3CH_3$, K_2CO_3/acetone.

Table 1. Binding affinities of the novel isoxazole derivatives from competition experiments at human β-adrenergic receptor subtypes

Compound	β_1-receptors		β_2-receptors		β_3-receptors	
	K_i (nM)	95% confidence limits	K_i (nM)	95% confidence limits	K_i (nM)	95% confidence limits
(±)-**4**	$>10^5$	—	$>10^5$	—	$>10^5$	—
(*S*, *R*)-(−)-**7a**	61.1	28.1–133	13.9	5.88–33.1	22,000	8910–54,100
(*R*, *R*)-(+)-**7 b**	6460	4540–9170	501	367–683	>50,000	—
(*S*, *R*)-(−)-**8a**	23.2	11.2–47.9	2.82	1.38–5.75	10,100	3390–30,100
(*R*, *R*)-(+)-**8 b**	1110	708–1750	81.4	68.2–122	21,800	10,700–44,300
(*S*, *R*)-(−)-**9a**	66.7	37.6–118	15.5	9.52–25.4	2485	1820–3390
(*R*, *R*)-(+)-**9 b**	2160	1940–2400	458	333–630	10,700	9680–11,800
(±)-**1**	1310[a]	930–1860	1290[a]	916–1810	3990[a]	3470–4590
(±)-**6**	37,900[a]	34,100–41,800	2850	2120–3830	430[a]	389–475

^{125}I-CYP was used as radioligand at a concentration of 50–80 pM. Experiments were done in the presence of 100 μM GTP. K_i values were calculated with the program SCTFIT and represent geometric mean values of at least three different experiments done in triplicate.
[a] Values are taken from Ref. 23.

agonistic activity. Therefore, compounds were tested at a concentration of 100 μM since a maximal signal may be expected even with the low-affinity interaction at the β_3-AR. The percent values reported in Table 2 for the new isoxazole derivatives show a similar pattern of efficacy at all β-AR subtypes. Higher efficacy values (75–90%) were found at β_2-ARs, whereas at the β_3-subtype all compounds behaved as partial agonists (30–60%). The lowest range of efficacy (15–35%) was found at β_1-ARs. This profile resembles that of Broxaterol (±)-**1,** although this parent compound was previously characterized as an antagonist at β_1-ARs. Depending on the experimental conditions, its efficacy ranged from some inverse agonism (–10%)[23] to negligible partial agonism (+8%).[20] In contrast, BRL 37344 (±)-**6** showed partial agonistic activity at β_3-ARs (28%) and an antagonist profile at the other two subtypes.[23] It is clearly shown that the introduction of the 3-bromoisoxazole for the 3-chlorophenyl moiety increases the efficacy at all β-AR subtypes and thereby converts the β_1- and β_2-antagonist BRL 37344 (±)-**6** into (partial) β_1- and β_2-agonists.

Table 2. Adenylyl cyclase responses of human β-adrenergic receptor subtypes to the listed compounds

Compound	Efficacy % (isoproterenol = 100%)		
	β_1-rec.	β_2-rec.	β_3-rec.
(*S*,*R*)-(−)-**7a**	34 ± 4	74 ± 6	50 ± 4
(*R*,*R*)-(+)-**7b**	27 ± 5	69 ± 0	34 ± 4
(*S*,*R*)-(−)-**8a**	31 ± 4	81 ± 3	56 ± 4
(*R*,*R*)-(+)-**8b**	24 ± 5	77 ± 3	39 ± 9
(*S*,*R*)-(−)-**9a**	24 ± 7	87 ± 7	51 ± 12
(*R*,*R*)-(+)-**9b**	13 ± 3	75 ± 4	31 ± 5
(±)-**1**[a]	8 ± 3	88 ± 7	42 ± 3
(±)-**6**[b]	−5 ± 4	−7 ± 2	28 ± 3

Membranes were prepared from cells with comparable receptor expression level. Adenylyl cyclase stimulation represents the percentage of maximal stimulation achieved by 100 μM isoproterenol (positive values) or percent inhibition of basal activity (negative values) ± SEM.
[a] Values are taken from Ref. 20.
[b] Values are taken from Ref. 23.

Therefore, based on these functional results, the novel hybrid compounds may be classified as full agonists at β_2-ARs and partial agonists at both β_1- and β_3-ARs. The presence of the 3-bromoisoxazole moiety greatly affects their functional profile which, at least at the β_2 and β_3 subtypes, is similar to that of Broxaterol (±)-**1**. At variance with (±)-**1**, which is an antagonist at β_1-ARs, the new derivatives are partial agonists at this receptor subtype.

4. Conclusions

The results of our study pinpoint that the replacement of the 3-chlorobenzene moiety of BRL 37344 (±)-**6** with the 3-bromoisoxazole nucleus of Broxaterol (±)-**1** gives rise to a series of isomeric amino alcohols which are β-AR ligands. These hybrid compounds show a marked change in both the binding and functional patterns at the three β-adrenergic receptor subtypes if compared to the profiles of reference compounds (±)-**1** and (±)-**6**. Most striking is the dramatic increase in β_1- and β_2-affinity of some of the compounds. The stereoisomers with the (*S*)-configuration at the stereocenter bearing the secondary alcohol group turned out to be high-affinity β_1-/β_2-ligands devoid of an appreciable affinity at the β_3-subtype. In addition, all members of the series activated the three β-adrenergic receptor subtypes to various degrees. Since structural modifications in this set of related ligands caused interesting changes in efficacy, these compounds may be valuable tools for studies of receptor activation. They might be particularly useful in studies employing receptor mutants which were recently shown to exhibit different functional responses to compounds like Broxaterol (±)-**1**.[36]

5. Experimental

5.1. Materials and methods

[(*R**,*R**)-(±)-4-[2-[2-(3-chlorophenyl)-2-hydroxyethylamino]propyl] phenoxyacetic acid [BRL 37344, (±)-**6**] was purchased from Tocris/Biotrend (Cologne, Germany). (±)-Broxaterol **1** (free base),[24] 3-aminoisoxazole-5-carboxylic acid ethyl ester (±)-**10**,[29] 2-bromo-1-(3-bromoisoxazol-5-yl)-ethanols (±)-**16**[24] and (*R*)-(−)-**16**,[24] (*R*)-(−)-1-methyl-2-(4-methoxyphenyl)ethylamine **18**,[33] and calcium trifluoromethanesulfonate[35] were all prepared according to published procedures. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Mercury 300 (^{1}H, 300.063; ^{13}C, 75.451 MHz) in $CDCl_3$ (unless otherwise specified) solutions; chemical shifts (δ) are expressed in ppm and coupling constants (*J*) in Hz. Melting points were determined on a Mod. B 540 Büchi apparatus and are uncorrected. Liquid compounds were characterized by the oven temperature for bulb to bulb distillations. Rotary power determinations were carried out with a Jasco J-810 spectropolarimeter coupled with a Haake N3-B thermostat. TLC analyses were performed on commercial silica gel 60 F254 aluminum sheets: spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution. Microanalyses (C, H, N) of new compounds agreed with the theoretical value ±0.4%.

The radioligand (−)3-^{125}I-Iodocyanopindolol (^{125}I-CYP) was purchased from Amersham Biosciences (specific activity, 2200 Ci/mmol). [α-^{32}P]ATP was from Perkin-Elmer LifeScience. Cell culture media and fetal calf serum were from PanSystems, penicillin (100 U/mL), streptomycin (100 μg/mL), L-glutamine, and G-418 were purchased from Gibco-Life Technologies. All other materials were from sources as described earlier.[23]

5.2. Chemical experimental section

5.2.1. *N*-{5-[2-(*tert*-Butylamino)-1-hydroxyethyl]isoxazol-3-yl}methanesulfonamide (±)-4. (A) To a solution of 1.92 g (12.3 mmol) of (±)-**10**[29] in 1,2-dichloroethane (50 mL) were added triethylamine (5.15 mL, 36.9 mmol) and methanesulfonyl cloride (1.43 mL, 18.5 mmol). The reaction mixture was heated at reflux for 1 h, then cooled at rt and washed with water. The organic layer was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. A silica gel column chromatography of the residue (eluant: 30% ethyl acetate/petroleum ether) gave 2.33 g (81% yield) of the desired compound.

Ethyl 3-[(methylsulfonyl)amino]isoxazole-5-carboxylate (±)-**11**: colorless prisms (from ethyl acetate/*n*-hexane), mp 99–101 °C. R_f 0.33 (eluant: 30% ethyl acetate/petroleum ether). ^{1}H NMR: 1.42 (t, 3H, *J* = 6.9), 3.19 (s, 3H), 4.44 (q, 2H, *J* = 6.9), 7.08 (s, 1H), 7.90 (br s, 1H). Anal. Calcd for $C_7H_{10}N_2O_5S$: C, 35.89; H, 4.30; N, 11.96. Found: C, 35.98; H, 4.51; N, 12.16.

(B) To a stirred suspension of *N*,*O*-dimethylhydroxylamine hydrochloride (707 mg, 7.25 mmol) and ethyl ester (±)-**11** (1.40 g, 6.0 mmol) in anhydrous THF (80 mL), maintained at −5 °C under nitrogen, was added dropwise during 1 h 16.2 mL (48.6 mmol) of a 3 M solution of methylmagnesium chloride in anhydrous THF. The reaction mixture was further stirred at a −5 °C for 1 h and at rt for 4 h, then 50 mL of 1 N HCl was added and the organic solvent was evaporated at reduced pressure. After treatment with dichloromethane

(3 × 25 mL), the pooled organic extracts were dried over anhydrous sodium sulfate and concentrated, and the residue was purified by silica gel column chromatography (eluant: 50% ethyl acetate/petroleum ether) to give 625 mg (51% yield) of the wanted methyl ketone.

N-(5-Acetylisoxazol-3-yl)methanesulfonamide (±)-**12**: colorless prisms (from ethyl acetate/*n*-hexane), mp 160–162 °C. R_f 0.36 (eluant: 80% ethyl acetate/petroleum ether). ^{1}H NMR: 2.60 (s, 3H), 3.18 (s, 3H), 7.02 (s, 1H). 7.80 (br s, 1H). Anal. Calcd for $C_6H_8N_2O_4$ S: C, 35.29; H, 3.95; N, 13.72. Found: C, 35.08; H, 4.18; N, 13.90.

(C) To a stirred solution of (±)-**12** (429 mg, 2.10 mmol) in anhydrous THF (20 mL), trimethylphenylammonium tribromide (790 mg, 2.10 mmol) was added portionwise at rt in 30 min. The mixture was further stirred for about 30 min until disappearance of the orange color, then water (25 mL) was added, the solvent was evaporated at reduced pressure, and the residue was extracted with ethyl acetate (3 × 20 mL). After the usual work-up, the crude bromoketone (±)-**13** was used in the next step without further purification.

(D) To a stirred solution of crude (±)-**13** (505 mg, 1.78 mmol) in methanol (20 mL) sodium borohydride (202 mg, 5.34 mmol) was added portionwise at 0 °C. After completion of the reduction (30 min, rt), the solvent was removed under vacuum, the residue was treated with dilute HCl (20 mL) and extracted with ethyl acetate (3 × 20 mL). After the usual work-up, the desired bromohydrin (287 mg, 48% overall yield) was obtained through purification on a silica gel column (eluant: 45% ethyl acetate/petroleum ether).

N-[5-(2-Bromo-1-hydroxyethyl)isoxazol-3-yl]methanesulfonamide (±)-**14**: colorless viscous oil, bp 195–200 °C/0.2 mmHg. R_f 0.30 (eluant: 45% ethyl acetate/petroleum ether). ^{1}H NMR: 1.92 (br s, 1H), 3.17 (s, 3H), 3.72 (dd, 1H, J = 6.2 and 10.6), 3.79 (dd, 1H, J = 4.0 and 10.6), 5.05 (dd, 1H, J = 4.0 and 6.2), 6.52 (s, 1H), 7.86 (br s, 1H). Anal. Calcd for $C_6H_9BrN_2O_4S$: C, 25.28; H, 3.18; N, 9.83. Found: C, 24.90; H, 3.41; N, 9.53.

(E) A stirred solution of bromohydrin (±)-**14** (250 mg, 0.88 mmol) and *tert*-butylamine (925 μL, 8.80 mmol) in methanol (10 mL) was refluxed until TLC evidenced the disappearance of the starting material (about 2 h). The solvent and excess reagent were removed under vacuum, and the residue was directly purified on a silica gel column (eluant: 30% methanol/ dichloromethane/0.1% concentrated ammonia), which afforded the zwitterionic final derivative (120 mg, 49% yield).

Compound (±)-**4**: pale pink prisms (from methanol), mp 232–234 °C. R_f 0.22 (eluant: 20% methanol/dichloromethane/0.1% concentrated ammonia). ^{1}H NMR (D_2O): 1.21 (s, 9H), 2.83 (s, 3H), 3.17 (dd, 1H, J = 9.2 and 13.2), 3.27 (dd, 1H, J = 3.3 and 13.2), 4.87 (dd, 1H, J = 3.3 and 9.2), 5.94 (s, 1H). ^{13}C NMR (D_2O): 24.9, 39.4, 44.7, 57.9, 62.7, 98.3, 164.8, 169.2. Anal. Calcd for $C_{10}H_{19}N_3O_4S$: C, 43.31; H, 6.91; N, 15.15. Found: C, 43.55; H, 7.12; N, 15.37.

5.2.2. 1-(3-Bromoisoxazol-5-yl)-2-{[2-(4-methoxyphenyl)-1-methylethyl]*tert*-butoxycarbonylamino}ethanols (*S, R*)-(−)-20a and (*R, R*)-(−)-20b. (A) To a suspension of 222 mg (9.23 mmol) of sodium hydride in dry toluene (80 mL) was dropped a solution of bromohydrin (±)-**16**[24] (2.50 g, 9.23 mmol) in dry toluene (15 mL) under nitrogen at rt After stirring for 1 h at rt, the suspension was filtered and the concentrated residue was purified by silica gel column chromatography (eluant: 5% ethyl acetate/petroleum ether) giving 1.54 g (88% yield) of the epoxide.

3-Bromo-5-oxiran-2-ylisoxazole (±)-**17**: colorless oil, bp 120–125 °C/0.5 mmHg. R_f 0.49 (eluant: 20% ethyl acetate/petroleum ether). ^{1}H NMR: 3.12 (dd, 1H, J = 2.6 and 5.5), 3.20 (dd, 1H, J = 4.3 and 5.5), 3.96 (dd, 1H, J = 2.6 and 4.3), 6.34 (s, 1H). Anal. Calcd for $C_5H_4BrNO_2$: C, 31.61; H, 2.12; N, 7.37. Found: C, 31.24; H, 2.42; N, 7.59.

(B) To a solution of epoxide (±)-**17** (1.51 g, 7.95 mmol) in acetonitrile (50 mL) were added calcium trifluoromethanesulfonate (1.35 g, 3.99 mmol) and (*R*)-(−)-1-methyl-2-(4-methoxyphenyl)ethylamine **18**[33](1.31 g, 7.95 mmol). The reaction mixture was stirred and heated at reflux under nitrogen for 24 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (eluant: 2% methanol/ dichloromethane) to afford 1.72 g (61% overall yield,) of a mixture of stereoisomeric amino alcohols (*S, R*)-**19a** and (*R, R*)-**19b**, R_f 0.54 (eluant: 10% methanol/dichloromethane). The ^{1}H NMR analysis allowed evaluation of the relative amounts of the two isomers, by considering the two singlets resonating at 6.21 δ [(*R, R*)-**19b**, 53%] and at 6.30 δ [(*S, R*)-**19a**, 47%], which corresponded to the H-4 proton of the isoxazole nucleus for each isomer.

(C) To a stirred and ice cooled mixture of (*S, R*)-**19a** and (*R, R*)-**19b** (3.27 g, 9.21 mmol) dissolved in dichloromethane (75 mL) were sequentially added di-*tert*-butyl dicarbonate (2.21 g, 10.1 mmol) and triethylamine (2.82 mL, 20.2 mmol). The reaction mixture was left under stirring for 24 h at rt, then washed with 3 × 25 mL of diluted HCl (pH 5). After the usual work-up, the residue of the organic phase was submitted to a silica gel column chromatography (eluant: 5% ethyl acetate/petroleum ether), which allowed the isolation of the two stereoisomers (*S, R*)-(−)-**20a** (1.36 g) and (*R, R*)-(−)-**20b** (1.74 g) in 72% overall yield.

Isomer (*S, R*)-(−)-**20a**: thick pale yellow oil, R_f 0.48 (eluant: 20% ethyl acetate/petroleum ether). ^{1}H NMR: 1.22 (d, 3H, J = 6.6), 1.37 (s, 9H), 2.58 (dd, 1H, J = 5.9 and 13.6), 2.68 (dd, 1H, J = 8.1 and 13.6), 3.41 (dd, 1H, J = 2.4 and 14.7), 3.51 (dd, 1H, J = 8.4 and 14.7), 3.77 (s, 3H), 4.06 (m, 1H), 4.81 (m, 1H), 5.77 (br s, 1H), 6.39 (s, 1H), 6.80 (d, 2H, J = 8.1), 7.00 (d, 2H, J = 8.1). $[\alpha]_D^{20}$ −61.6 (*c* 0.950, $CHCl_3$). Anal. Calcd for $C_{20}H_{27}BrN_2O_5$: C, 52.75; H, 5.98; N, 6.15. Found: C, 53.12; H, 5.65; N, 6.37.

Isomer (*R, R*)-(−)-**20b**: thick pale yellow oil, R_f 0.40 (eluant: 20% ethyl acetate/petroleum ether). ^{1}H NMR: 1.24

(d, 3H, $J = 6.6$), 1.37 (s, 9H), 2.65 (dd, 1H, $J = 6.6$ and 13.6), 2.77 (dd, 1H, $J = 8.4$ and 13.6), 3.44 (dd, 1H, $J = 2.7$ and 14.7), 3.50 (dd, 1H, $J = 7.4$ and 14.7), 3.78 (s, 3H), 4.09 (m, 1H), 4.88 (m, 1H), 5.74 (br s, 1H), 6.35 (s, 1H), 6.83 (d, 2H, $J = 8.4$), 7.06 (d, 2H, $J = 8.4$). $[\alpha]_D^{20}$ −39.6 (*c* 0.900, $CHCl_3$). Anal. Calcd for $C_{20}H_{27}BrN_2O_5$: C, 52.75; H, 5.98; N, 6.15. Found: C, 52.87; H, 5.61; N, 6.50.

(D) A solution of bromohydrin (*R*)-(−)-**16**[24] (271 mg, 1 mmol) and amine (*R*)-(−)-**18**(165 mg, 1.00 mmol) in methanol (5 mL) was stirred and heated at reflux under nitrogen for 24 h. The solvent was removed under vacuum, the brown oily residue was dissolved in 3 N HCl (10 mL) and washed with ethyl ether (2 × 10 mL). The aqueous layer was alkalinized with solid sodium carbonate and extracted with dichloromethane (4 × 10 mL). After the usual work-up, the residue was dissolved in dichloromethane (5 mL) and treated with di-*tert*-butyl dicarbonate (218 mg, 1.00 mmol) and triethylamine (280 μL, 2.00 mmol). After stirring for 24 h at rt, the reaction mixture was washed with a solution of diluted HCl (pH 5) and, after the usual work-up, the residue of the organic phase was purified on a silica gel column chromatography (eluant: 10% ethyl acetate/petroleum ether), which allowed isolation of compound (*S*, *R*)-(−)-**20a** (118 mg, 26% overall yield) as a thick pale yellow oil, $[\alpha]_D^{20}$ −62.15 (*c* 0.900, $CHCl_3$). The ^1H NMR data matched those above reported for the same isomer.

5.2.3. Ethyl {4-[2-{[2-(3-bromoisoxazol-5-yl)-2- hydroxyethyl]amino}propyl]phenoxy} acetates (*S, R*)-(−)-8a and (*R, R*)-(+)-8b. (A) To a stirred solution of (*S*, *R*)-(−)-**20a** (2.16 g, 4.74 mmol) in dichloromethane (50 mL) was added dropwise 25 mL of a 1 M solution of boron tribromide. After stirring for 2 h at rt, water (30 mL) was added to the reaction mixture cooled at −5 °C. The aqueous phase was separated, alkalinized (pH 8), and extracted with ethyl acetate (4 × 25 mL). After the usual work-up, the residue was purified by silica gel column chromatography (eluant: 2% methanol/ dichloromethane) to give the desired intermediate (*S*, *R*)-(−)-**21a** (1.34 g, 83% yield).

4-[2-{[2-(3-Bromoisoxazol-5-yl)-2-hydroxyethyl]amino}-propyl]phenol (*S*, *R*)-(−)-**21a**: thick colorless oil, R_f 0.49 (eluant: 10% methanol/dichloromethane). ^1H NMR (CD_3OD): 1.07 (d, 3H, $J = 6.2$), 2.52 (dd, 1H, J =7 .7 and 13.2), 2.72 (dd, 1H, $J = 6.4$ and 13.2), 3.00 (m, 3H), 4.88 (dd, 1H, $J = 5.7$ and 8.4), 6.47 (s, 1H), 6.71 (d, 2H, $J = 8.4$), 7.00 (d, 2H, $J = 8.4$). $[\alpha]_D^{20}$ −29.0 (*c* 1.080, CH_3OH). Anal. Calcd for $C_{14}H_{17}BrN_2O_3$: C, 49.28; H, 5.02; N, 8.21. Found: C, 49.02; H, 5.21; N, 8.48.

The same procedure was applied to the transformation of a comparable amount of isomer (*R*, *R*)-(−)-**20b** which produced the free base (*R*, *R*)-(−)-**21b** in 94% yield.

4-[2-{[2-(3-Bromoisoxazol-5-yl)-2-hydroxyethyl]amino}-propyl]phenol (*R*, *R*)-(−)-**21b**: thick colorless oil, R_f 0.60 (eluant: 10% methanol/dichloromethane). ^1H NMR (CD_3OD): 1.06 (d, 3H, $J = 6.2$), 2.58 (dd, 1H, $J = 5.8$ and 12.5), 2.62 (dd, 1H, $J = 7.1$ and 12.5), 2.80-2.95 (m, 2H), 3.02 (dd, 1H, $J = 4.0$ and 12.1), 4.85 (dd, 1H, $J = 4.2$ and 9.2), 6.41 (s, 1H), 6.70 (d, 2H, $J = 8.4$), 7.01 (d, 2H, $J = 8.4$). $[\alpha]_D^{20}$ −4.25 (*c* 1.040, CH_3OH). Anal. Calcd for $C_{14}H_{17}BrN_2O_3$: C, 49.28; H, 5.02; N, 8.21. Found: C, 49.51; H, 4.82; N, 8.42.

(B) To a stirred and ice cooled mixture of (*S*, *R*)-(−)-**21a** (1.31 g, 3.84 mmol) dissolved in acetonitrile (15 mL) was sequentially added a solution of di-*tert*-butyl dicarbonate (1.09 g, 4.99 mmol) in acetonitrile (10 mL) and triethylamine (1.18 mL, 8.45 mmol). The reaction mixture was left under stirring for 6 h at rt, then concentrated at reduced pressure, treated with 20 mL of diluted HCl (pH 5), and extracted with dichloromethane (3 × 20 mL). After the usual work-up, the residue of the organic phases was submitted to a silica gel column chromatography (eluant: 5% ethyl acetate/petroleum ether), which afforded 1.47 g of carbamate (*S*, *R*)-(−)-**22a** (87% yield).

4-[2-{[2-(3-Bromoisoxazol-5-yl)-2-hydroxyethyl]*tert*-butoxycarbonylamino}propyl]phenol (*S*, *R*)-(−)-**22a**: thick pale yellow oil, R_f 0.26 (eluant: 20% ethyl acetate/petroleum ether). ^1H NMR: 1.23 (d, 3H, $J = 6.9$), 1.38 (s, 9H), 2.57 (dd, 1H, $J = 6.2$ and 13.2), 2.64 (dd, 1H, $J = 8.4$ and 13.2), 3.39 (dd, 1H, $J = 2.2$ and 15.0), 3.53 (dd, 1H, $J = 7.7$ and 15.0), 4.04 (m, 1H), 4.80 (m, 1H), 5.0 (br s, 1H), 5.78 (br s, 1H), 6.40 (s, 1H), 6.74 (d, 2H, $J = 8.4$), 6.95 (d, 2H, $J = 8.4$). $[\alpha]_D^{20}$ −69.2 (*c* 0.910, $CHCl_3$). Anal. Calcd for $C_{19}H_{25}BrN_2O_5$: C, 51.71; H, 5.71; N, 6.35. Found: C, 52.07; H, 5.95; N, 6.08.

Similarly, isomer (*R*, *R*)-(−)-**21b** was converted into the corresponding carbamate (*R*, *R*)-(−)-**22b** in 77% yield.

4-[2-{[2-(3-Bromoisoxazol-5-yl)-2-hydroxyethyl]*tert*-butoxycarbonylamino}propyl]phenol (*R*, *R*)-(−)-**22b**: thick colorless oil, R_f 0.39 (eluant: 30% ethyl acetate/petroleum ether). ^1H NMR: 1.26 (d, 3H, $J = 6.9$), 1.45 (s, 9H), 2.65 (dd, 1H, $J = 7.2$ and 13.8), 2.78 (dd, 1H, $J = 8.1$ and 13.8), 3.46 (m, 2H), 4.08 (m, 1H), 4.85 (m, 2H), 5.80 (br s, 1H), 6.35 (s, 1H), 6.75 (d, 2H, $J = 8.4$), 7.02 (d, 2H, $J = 8.4$). $[\alpha]_D^{20}$ −36.25 (*c* 0.960, $CHCl_3$). Anal. Calcd for $C_{19}H_{25}BrN_2O_5$: C, 51.71; H, 5.71; N, 6.35. Found: C, 51.47; H, 6.05; N, 6.52.

(C) To a stirred solution of (*S*, *R*)-(−)-**22a** (487 mg, 1.10 mmol) in acetone (15 mL) were added potassium carbonate (381 mg, 2.76 mmol) and ethyl bromoacetate (122 μL, 1.10 mmol). The suspension was stirred and heated at reflux for 6 h, then cooled at rt and concentrated under vacuum. Water (10 mL) was added and the aqueous phase was extracted with ethlyl acetate (4 × 20 mL). After the usual work-up, the crude reaction mixture was purified by silica gel column chromatography (eluant: 15% ethyl acetate/petroleum ether), affording 489 mg (84% yield) of ester (*S*, *R*)-(−)-**23a**.

Ethyl {4-[2-{[2-(3-bromoisoxazol-5-yl)-2-hydroxyethyl]*tert*-butoxycarbonylamino}propyl] phenoxy}acetate (*S*, *R*)-(−)-**23a**: thick pale yellow oil, R_f 0.66 (eluant:

15% ethyl acetate/petroleum ether). ^{1}H NMR: 1.22 (d, 3H, J = 6.9), 1.29 (t, 3H, J = 7.3), 1.37 (s, 9H), 2.52-275 (m, 2H), 3.40 (dd, 1H, J = 2.6 and 14.8), 3.48 (dd, 1H, J = 7.2 and 14.8), 4.09 (m, 1H), 4.26 (q, 2H, J = 7.3), 4.58 (s, 2H), 4.79 (m, 1H), 5.77 (br s, 1H), 6.40 (s, 1H), 6.83 (d, 2H, J = 8.0), 7.02 (d, 2H, J = 8.0). $[\alpha]_D^{20}$ −59.9 (c 0.950, $CHCl_3$). Anal. Calcd for $C_{23}H_{31}BrN_2O_7$: C, 52.38; H, 5.92; N, 5.31. Found: C, 52.47; H, 6.15; N, 5.50.

The same procedure was applied to the transformation of a comparable amount of isomer (*R*,*R*)-(−)-**22b**, which produced ester (*R*,*R*)-(−)-**23b** in 75% yield.

Ethyl {4-[2-{[2-(3-bromoisoxazol-5-yl)-2-hydroxyethyl]*tert*-butoxycarbonylamino}propyl] phenoxy}acetate (*R*,*R*)-(−)-**23b**: thick pale yellow oil, R_f 0.48 (eluant: 30% ethyl acetate/petroleum ether). ^{1}H NMR: 1.27 (d, 3H, J = 6.9), 1.30 (t, 3H, J = 7.3), 1.44 (s, 9H), 2.66 (dd, 1H, J = 7.0 and 13.9), 2.77 (m, 1H), 3.45 (dd, 1H, J = 2.6 and 14.7), 3.52 (m, 1H), 4.09 (m, 1H), 4.25 (q, 2H, J = 7.3), 4.59 (s, 2H), 4.86 (m, 1H), 5.73 (br s, 1H), 6.35 (s, 1H), 6.83 (d, 2H, J = 8.4), 7.07 (d, 2H, J = 8.4). $[\alpha]_D^{20}$ −26.4 (c 0.940, $CHCl_3$). Anal. Calcd for $C_{23}H_{31}BrN_2O_7$: C, 52.38; H, 5.92; N, 5.31. Found: C, 52.59; H, 5.65; N, 5.60.

(D) The *N*-Boc protected amino ester (*S*,*R*)-(−)-**23a** (211 mg, 0.40 mmol) was treated with a 30% dichloromethane solution (1 mL) of trifluoroacetic acid (308 μL, 4.15 mmol) at 0 °C. The solution was stirred at rt for 12 h until disappearance of the starting material (TLC: 15% ethyl acetate/petroleum ether). The volatiles were removed under vacuum and the thick oily residue was taken up with anhydrous diethyl ether and dried under vacuum to afford 175 mg (81% yield) of the desired trifluoroacetic acid salt.

(*S*,*R*)-(−)-**8a** trifluoroacetate: gummy colorless solid. ^{1}H NMR: 1.22 (d, 3H, J = 6.9), 1.28 (t, 3H, J = 7.2), 2.58 (dd, 1H, J = 6.9 and 13.8), 2.66 (dd, 1H, J = 6.6 and 13.8), 2.89 (m, 1H), 3.0 (m, 2H), 3.63 (br s, 2H), 4.24 (q, 2H, J = 7.2), 4.57 (s, 2H), 4.76 (m, 1H), 6.30 (s, 1H), 6.83 (d, 2H, J = 8.4), 7.06 (d, 2H, J = 8.4). ^{13}C NMR: 14.4, 20.1, 42.4, 50.1, 55.1, 61.3, 65.1, 65.7, 105.5, 115.0, 130.5, 131.9, 140.7, 156.7, 169.3, 175.5. $[\alpha]_D^{20}$ −21.5 (c 1.150, CH_3OH). Anal. Calcd for $C_{20}H_{24}BrF_3N_2O_7$: C, 44.38; H, 4.47; N, 5.18. Found: C, 44.68; H, 4.21; N, 5.35.

Similarly, isomer (*R*,*R*)-(−)-**23b** was converted into the corresponding trifluoroacetic acid salt of (*R*,*R*)-(+)-**8b** in 72% yield.

(*R*,*R*)-(+)-**8b** trifluoroacetate: gummy pale yellow solid. ^{1}H NMR: 1.23 (d, 3H, J = 6.9), 1.29 (t, 3H, J = 7.2), 2.72 (dd, 1H, J = 8.1 and 13.9), 3.18 (dd, 1H, J = 5.5 and 13.9), 3.23 (m, 1H), 3.44 (m, 2H), 4.23 (q, 2H, J = 7.2), 4.58 (s, 2H), 4.83 (br s, 2H), 5.29 (m, 1H), 6.38 (s, 1H), 6.81 (d, 2H, J = 8.4), 7.02 (d, 2H, J = 8.4). ^{13}C NMR: 14.4, 15.9, 38.6, 48.8, 57.3, 61.8, 62.9, 65.5, 106.4, 115.4, 128.5, 130.6, 141.0, 157.4, 169.3, 172.5. $[\alpha]_D^{20}$ +2.1 (c 1.180, CH_3OH). Anal. Calcd for $C_{20}H_{24}BrF_3N_2O_7$: C, 44.38; H, 4.47; N, 5.18. Found: C, 44.09; H, 4.71; N, 5.39.

5.2.4. {4-[2-{[2-(3-Bromoisoxazol-5-yl)-2-hydroxyethyl]-amino}propyl]phenoxy}acetic acids (*S*,*R*)-(−)-7a and (*R*,*R*)-(+)-7b. (A) A solution of ethyl ester (*S*,*R*)-(−)-**23a** (260 mg, 0.49 mmol) in methanol (5 mL) was treated with 5 mL of a 10% aqueous solution of potassium carbonate. The reaction mixture was stirred at rt for 1 h until disappearance of the starting material (TLC: 15% ethyl acetate/petroleum ether), then the solvent was evaporated under vacuum. After addition of water (10 mL) and treatment with diethyl ether (3 × 5 mL), the residual aqueous phase was acidified with diluted HCl (pH 4) and extracted with ethyl acetate (3 × 5 mL). After the usual work-up, the thick oily residue of the pooled organic extracts (215 mg, 87% yield) was used in the following step without further purification.

(B) The crude above prepared *N*-Boc protected amino acid (215 mg, 0.43 mmol) was treated with a 30% dichloromethane solution of trifluoroacetic acid at 0 °C, following the procedure described for (*S*,*R*)-(−)-**8a**. The volatiles were removed under vacuum and the oily residue was taken up with anhydrous diethyl ether and dried under vacuum giving 185 mg (84% yield) of the wanted trifluoroacetic acid salt.

(*S*,*R*)-(−)-**7a** trifluoroacetate: colorless prisms (from 50% methanol/diethyl ether), mp 159–160 °C, dec ^{1}H NMR (CD_3OD): 1.22 (d, 3H, J = 6.9), 2.71 (dd, 1H, J = 10.3 and 13.2), 3.10 (dd, 1H, J = 4.3 and 13.2), 3.40–3.56 (m, 3H), 4.59 (s, 2H), 5.18 (m, 1H), 6.64 (s, 1H), 6.92 (d, 2H, J = 8.4), 7.18 (d, 2H, J = 8.4). ^{13}C NMR (CD_3OD): 14.2, 38.2, 56.3, 62.4, 65.4, 65.7, 105.8, 114.8, 128.6, 128.9, 130.3, 140.6, 157.8, 173.6. $[\alpha]_D^{20}$ −31.7 (c 0.900, CH_3OH). Anal. Calcd for $C_{18}H_{20}BrF_3N_2O_7$: C, 42.12; H, 3.93; N, 5.46. Found: C, 42.31; H, 4.12; N, 5.19.

In a parallel way, isomer (*R*,*R*)-(−)-**23b** (180 mg, 0.34 mmol) was transformed into the corresponding trifluoroacetate of (*R*,*R*)-(+)-**7b** (116 mg, 66% overall yield).

(*R*,*R*)-(+)-**7b** trifluoroacetate: colorless prisms (from 20% methanol/diethyl ether), mp 152–153 °C. ^{1}H NMR (CD_3OD): 1.24 (d, 3H, J = 6.9), 2.66 (dd, 1H, J = 9.9 and 13.2), 3.11 (dd, 1H, J = 4.5 and 13.2), 3.37–3.49 (m, 3H), 4.60 (s, 2H), 5.15 (m, 1H), 6.67 (s, 1H), 6.92 (d, 2H, J = 8.4), 7.18 (d, 2H, J = 8.4). ^{13}C NMR (CD_3OD): 14.9, 37.6, 56.4, 62.5, 64.8, 65.0, 105.9, 114.9, 128.7, 129.0, 130.3, 140.7, 157.7, 173.4. $[\alpha]_D^{20}$ +1.5 (c 0.830, CH_3OH). Anal. Calcd for $C_{18}H_{20}BrF_3N_2O_7$: C, 42.12; H, 3.93; N, 5.46. Found: C, 41.95; H, 4.18; N, 5.58.

5.2.5. 1-(3-Bromoisoxazol-5-yl)-2-{[2-(4-butoxyphenyl)-1-methylethyl]amino}ethanols (*S*,*R*)-(−)-9a and (*R*,*R*)-(+)-9b. (A) To a stirred solution of (*S*,*R*)-(−)-**22a** (300 mg, 0.68 mmol) in acetone (10 mL) were added potassium carbonate (235 mg, 1.70 mmol) and

1-bromobutane (330 μL, 3.07 mmol). The suspension was stirred and heated at reflux for 2 d, then cooled at rt and filtered under vacuum. The concentrated filtrate was purified by silica gel column chromatography (eluant: 10% ethyl acetate/petroleum ether) to produce 287 mg (85% yield) of ether (*S*,*R*)-(−)-**24a**.

1-(3-Bromoisoxazol-5-yl)-2-{[2-(4-butoxyphenyl)-1-methylethyl]*tert*-butoxycarbonylamino} ethanol (*S*,*R*)-(−)-**24a**: thick pale yellow oil, R_f 0.24 (eluant: 10% ethyl acetate/petroleum ether). ^{1}H NMR: 0.96 (t, 3H, *J* = 7.4), 1.22 (d, 3H, *J* = 6.6), 1.36 (s, 9H), 1.47 (m, 2H), 1.74 (m, 2H), 2.58 (dd, 1H, *J* = 5.9 and 13.5), 2.67 (dd, 1H, *J* = 8.0 and 13.5), 3.42 (dd, 1H, *J* = 1.8 and 15.0), 3.52 (dd, 1H, *J* = 7.3 and 15.0), 3.92 (t, 2H, *J* = 6.6), 4.04 (m, 1H), 4.80 (m, 1H), 5.80 (br s, 1H), 6.40 (s, 1H), 6.80 (d, 2H, *J* = 8.4), 6.98 (d, 2H, *J* = 8.4). $[\alpha]_D^{20}$ −58.0 (*c* 1.050, $CHCl_3$). Anal. Calcd for $C_{23}H_{33}BrN_2O_5$: C, 55.54; H, 6.69; N, 5.63. Found: C, 55.80; H, 6.41; N, 5.88.

The same procedure was applied to the transformation of a comparable amount of isomer (*R*,*R*)-(−)-**22b**, which produced ether (*R*,*R*)-(−)-**24b** in 89% yield.

1-(3-Bromoisoxazol-5-yl)-2-{[2-(4-butoxyphenyl)-1-methylethyl]*tert*-butoxycarbonylamino} ethanol (*R*,*R*)-(−)-**24b**: thick pale yellow oil, R_f 0.48 (eluant: 20% ethyl acetate/petroleum ether). ^{1}H NMR: 0.97 (t, 3H, *J* = 7.4), 1.26 (d, 3H, *J* = 6.6), 1.44 (s, 9H), 1.55 (m, 2H), 1.75 (m, 2H), 2.66 (dd, 1H, *J* = 7.0 and 13.9), 2.76 (dd, 1H, *J* = 7.9 and 13.9), 3.45 (dd, 1H, *J* = 2.2 and 14.6), 3.56 (m, 1H), 3.93 (t, 2H, *J* = 6.6), 4.09 (m, 1H), 4.87 (m, 1H), 5.75 (br s, 1H), 6.35 (s, 1H), 6.80 (d, 2H, *J* = 8.4), 7.04 (d, 2H, *J* = 8.4). $[\alpha]_D^{20}$ −34.5 (*c* 1.020, $CHCl_3$). Anal. Calcd for $C_{23}H_{33}BrN_2O_5$: C, 55.54; H, 6.69; N, 5.63. Found: C, 55.29; H, 6.88; N, 5.90.

(B) The *N*-Boc protected amino ether (*S*,*R*)-(−)-**24a** (220 mg, 0.44 mmol) was treated with a 30% dichloromethane solution of trifluoroacetic acid at 0 °C following the procedure described for (*S*,*R*)-(−)-**8a**. The volatiles were removed under vacuum and the thick oily residue was taken up with *n*-hexane and dried under vacuum to afford 142 mg (63% yield) of the corresponding trifluoroacetic acid salt.

(*S*,*R*)-(−)-**9a** trifluoroacetate: colorless powder (from *n*-hexane), mp 87–88 °C. R_f 0.38 (eluant: 5% methanol/dichloromethane). ^{1}H NMR: 0.97 (t, 3H, *J* = 7.3), 1.35 (d, 3H, *J* = 6.6), 1.51 (m, 2H), 1.75 (m, 2H), 2.80 (dd, 1H, *J* = 8.8 and 13.6), 3.09 (dd, 1H, *J* = 5.3 and 13.6), 3.30–3.53 (m, 3H), 3.93 (t, 2H, *J* = 6.6), 5.32 (m, 1H), 5.78 (br s, 1H), 6.42 (s, 1H), 6.85 (d, 2H, *J* = 8.4), 7.06 (d, 2H, *J* = 8.4). ^{13}C NMR: 14.1, 15.5, 19.4, 31.5, 38.8, 48.6, 57.4, 62.8, 67.9, 106.5, 115.2, 126.9, 130.4, 141.1, 158.8, 172.4. $[\alpha]_D^{20}$ −25.8 (*c* 0.960, $CHCl_3$). Anal. Calcd for $C_{20}H_{26}BrF_3N_2O_5$: C, 46.98; H, 5.13; N, 5.48. Found: C, 47.18; H, 5.31; N, 5.20.

Isomer (*R*,*R*)-(+)-**9b** was similarly obtained as trifluoroacetic acid salt in 73% yield.

(*R*,*R*)-(+)-**9b** trifluoroacetate: gummy colorless solid, R_f 0.47 (eluant: 5% methanol/ dichloromethane). ^{1}H NMR: 0.97 (t, 3H, *J* = 7.3), 1.39 (d, 3H, *J* = 6.6), 1.49 (m, 2H), 1.76 (m, 2H), 2.82 (dd, 1H, *J* = 8.4 and 13.9), 3.04-3.19 (m, 2H), 3.36 (m, 1H), 3.52 (m, 1H), 3.93 (t, 2H, *J* = 6.6), 5.32 (m, 1H), 5.50 (br s, 1H), 6.38 (s, 1H), 6.86 (d, 2H, *J* = 8.4), 7.06 (d, 2H, *J* = 8.4). ^{13}C NMR: 14.1, 16.0, 19.5, 31.5, 38.7, 48.8, 57.5, 62.9, 67.9, 106.4, 115.3, 126.6, 130.4, 141.0, 158.8, 172.5. $[\alpha]_D^{20}$ +3.1 (*c* 1.0, $CHCl_3$). Anal. Calcd for $C_{20}H_{26}BrF_3N_2O_5$: C, 46.98; H, 5.13; N, 5.48. Found: C, 46.72; H, 5.41; N, 5.60.

5.3. Pharmacological experimental section

5.3.1. Cell culture and membrane preparation. CHO cells expressing the stably transfected human β-adrenergic receptor subtypes were grown adherently and maintained in Dulbecco's modified Eagle's medium with nutrient mixture F12 (DMEM/F12), containing 10% fetal calf serum, penicillin (100 U/mL), streptomycin (100 μg/mL), L-glutamine (2 mM), and Geneticin (G-418, 0.2 mg/mL) at 37 °C in 5% CO_2/95% air. Details have been described by Hoffmann et al.[23] Membranes for radioligand binding were prepared from frozen cells in a two-step procedure as described previously.[37] For the measurement of adenylyl cyclase a one-step procedure from fresh cells was used.[23,37]

5.3.2. Radioligand binding studies and adenylyl cyclase activity. Radioligand binding experiments were carried out as described recently.[23] In brief, membranes from CHO cells stably transfected with human β-AR subtypes (β_1, β_2 about 5 μg, β_3 about 25 μg of protein) were incubated with the antagonist ^{125}I-CYP in a concentration of about 50 pM in the case of β_1- and β_2-receptors, or about 80 pM ^{125}I-CYP for β_3-receptors. Assays were carried out in 50 mM Tris/HCl, pH 7.4 (assay buffer), containing 100 μM GTP, in a total volume of 200 μL. GTP was added to achieve monophasic binding curves for agonists. Membranes were incubated for 90 min at 30 °C. Bound ligand was separated from free ligand by filtration through Whatman GF/C filters, which were then washed three times with ice-cold assay buffer. Non-specific binding was determined in the presence of 10 μM alprenolol. K_i values were calculated by non-linear curve fitting with the program SCTFIT.[38]

The activity of adenylyl cyclase in cell membranes was determined as described recently.[23] Briefly, the conversion of [α-^{32}P]ATP to [^{32}P]cAMP was measured in an assay mixture containing membranes from CHO cells expressing human β-AR subtypes (about 50 μg of protein), 100 μM cAMP, 0.2% BSA, 10 μM GTP, 100 μM ATP, 1 mM $mgCl_2$, 100 μM isobutylmethylxanthine, and an ATP-regenerating system consisting of 15 mM phosphocreatine and 300 U/ml of creatine kinase in 50 mM Tris/HCl, pH 7.4. The reaction was allowed to proceed for 20 min at 37 °C. The reaction was stopped by precipitation with zinc acetate and sodium carbonate. After centrifugation [^{32}P]cAMP and remaining [α-^{32}P]ATP in the supernatant were separated by chromatography over (neutral) alumina columns and the

amount of [^{32}P]cAMP was determined in a β-counter. The efficacy of compounds under investigation was tested at a concentration of 100 μM and compared to the stimulation by 100 μM isoproterenol (100%) over basal adenylyl cyclase activity (0%). Inverse agonistic activity is expressed as percent inhibition of basal activity.

Acknowledgments

This work was financially supported by the Università degli Studi di Milano (FIRST 2004 and 2005). The expert technical assistance of Sonja Kachler and Nico Falgner is gratefully acknowledged. The financial support from the Vigoni program (to C.D.M. and K.-N.K.), which encourages German–Italian scientific collaborations, is gratefully acknowledged.

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