Arch Pharm Res Vol 28, No 12, 1317-1323, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

Benzisothiazoles and β-Adrenoceptors: Synthesis and Pharmacological Investigation of Novel Propanolamine and Oxypropanolamine Derivatives in Isolated Rat Tissues

Giovanni Morini, Enzo Poli[1], Mara Comini, Alessandro Menozzi[1], and Cristina Pozzoli[1]

University of Parma, School of Pharmacy, Pharmaceutical Department, Viale delle Scienze 27A, 43100 Parma, ITALY and [1]School of Medicine, Department of Human Anatomy, Pharmacology and Medico-Forensic Sciences, Section of Pharmacology, Via Volturno 39, I-43100 Parma-ITALY

(Received August 18, 2005)

In an attempt to examine the ability of benzisothiazole-based drugs to interact with β-adrenoceptors, a series of 1,2-benzisothiazole derivatives, which were substituted with various propanolamine or oxypropanolamine side chains in the 2 or 3 position, were synthesised and tested. The pharmacological activity of these compounds at the β-adrenoceptors was examined using isolated rat atria and small intestinal segments, which preferentially express the β_1- and β_3-adrenoceptor-mediated responses, respectively. None of these products showed any β-adrenoceptor agonistic activity. In contrast, the 2- and 3-substituted isopropyl, *tert*-butyl, benzyl, and piperonyl derivatives **2a-d** and **3a-d** elicited surmountable inhibition of the isoprenaline-induced chronotropic effects in the atria, suggesting competitive antagonism at the β_1-recognition site. The pA_2 values revealed *tert*-butyl **3b** and the isopropyl substituted piperonyl derivatives **3a** to be the most effective. Remarkably, many of the 2-substituted propanolamines were less active than the corresponding 3-substituted oxypropanolamines. With the exception of compound **3b**, none of these drugs antagonised the muscle relaxant activity of isoprenaline in the intestine, suggesting no effect on the β_3-adrenoceptors. These results confirm the ability of the benzisothiazole ring to interact with the β-adrenoceptors, and demonstrate that 2-substitution with propanolamine or 3-substitution with oxypropanolamine groups yields compounds with preferential antagonistic activity at the cardiac β_1-adrenoceptors. The degree of antagonism depends strongly on both the nature of the substituent and its position on the benzisothiazole ring.

Key words: 1,2-Benzisothiazoles, Benzisothiazolpropanolamines, Benzisothiazoloxypropanolamines, Rat ileum, Rat atria, Cardiac β_1-adrenoceptors

INTRODUCTION

Many compounds that act on the β-adrenoceptors contain the catechol nucleus, which typically occurs in natural catecholamines, norepinephrine, and epinephrine, as well as in most (semi)synthetic compounds, including isoprenaline and colterol, which have agonistic activity at these receptors (Kusayama *et al.*, 1994; Strosberg, 1997).

Evidence of heterogeneity in the β-adrenoceptor family has allowed the catechol nucleus to be replaced with isosteric groups (Von Franke, 1980), in an attempt to identify new groups that can distinguish between the different β-adrenoceptor subtypes, particularly the newer β_3 and β_4 (Blin *et al.*, 1993; Strosberg, 1997).

Previous studies aimed at examining the ability of benzisothiazole-based compounds to interact with different aminergic receptors have demonstrated that several substituted benzisothiazoles can block the nicotinic, muscarinic, histamine H_1, and serotonine $5HT_3$ receptors (Molina *et al.*, 1974; Ishibashi *et al.*, 1996; Mos *et al.*, 1997; Morini *et al.*, 1999). In addition, a series of 1,2-benzisothiazole derivatives, which were substituted with different oxypropanolamines in the 5- or 7- positions of the ring, were found to recognise the β-adrenoceptors (Mingiardi *et al.*, 1983; Barocelli *et al.*, 1992; Morini *et al.*, 2005). Among these compounds, 5-substituted compounds such

Correspondence to: Enzo Poli, University of Parma, School of Medicine, Department of Human Anatomy, Pharmacology and Medico-Forensic Sciences, Section of Pharmacology, Via Volturno, 39, I-43100 Parma, ITALY
Tel: 39-0521-903869, Fax: 39-0521-903852
E-mail: enzo.poli@unipr.it

as isopropyl and the cyclohexyl derivatives have a greater antagonistic effect at the cardiac β_1-adrenoceptors than the corresponding 7-substituted compounds. Functional analysis of their activity in the smooth muscle from a rat bladder or intestine highlighted their inability to interfere with both the β_2- or β_3-adrenoceptors (Morini *et al.*, 2005), suggesting preferential antagonistic activity at the β_1-adrenoceptor recognition site.

The overall aim of this study was to better understand the activity of benzisothiazoles at the β-receptors (Mingiardi *et al.*, 1983; Barocelli *et al.*, 1992). A series of 1,2-benzisothiazoles (benzo[d]isothiazoles) substituted with various propanolamine **2a-f** or oxypropanolamine **3a-f** side chains at the 2- or 3-positions (Table I) were prepared and tested for their pharmacological activity. Some already known compounds **2a**, **3a**, **2b**, and **3b** (Mingiardi *et al.*, 1983) were re-synthesized and are included in this study for comparison. The aim was to confirm the ability of the benzisothiazole nucleus with substituents at the 2 or 3 positions to interfere with the β-adrenoceptors, as was previously demonstrated for 5- or 7-substituted (Morini *et al.*, 2005) and 4- or 5-substituted compounds (Von Franke *et al.*, 1980). The aim was to determine if the nature of substituent could influence the type of interaction with such receptors. Previous studies on the benzisothiazole family revealed that small substituents, such as isopropyl or cyclohexyl groups, produce stronger β_1-blockers than bulkier substituents (Morini *et al.*, 2005). Preliminary studies revealed that compound **3a** competitively interacts with the cardiac β_1-adrenoceptors (Barocelli *et al.*, 1992). It is also important to determine if the nature of the substituents can influence the specificity of the receptor interaction on this series. Accordingly, a phenylethanolamine side chain linked to a catechol ring is one of the structural prerequisites in most compounds identified as specific β_3-ligands (Strosberg, 1997). Therefore, the possible discriminative activities of the target compounds among the β_1- *vs.* β_3-adrenoceptor subtypes need to be determined.

MATERIALS AND METHODS

The melting points were determined using a Gallenkamp melting point apparatus and were uncorrected. Elemental analyses were performed using a ThermoQuest FlashEA 1112 Elemental Analyzer, and agreed with the theoretical values to within ±0.4%. The ^{1}H-MNR spectra were recorded on a Bruker 300 spectrometer (300 MHz). The chemical shifts are reported in ppm (δ scale) relative to tetramethylsilane (TMS, δ 0.0) internal standard. The data are reported in the following order: multiplicity, approximate coupling constant (*J* value) in hertz (Hz) and number of protons. The signals were characterised as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet), br s (broad signal), bit (benzo[d]isothiazole), pip (piperidine), a (axial), and e (equatorial). The IR spectra were obtained using a Jasco FT-IR 300E spectrophotometer (Jasco Ltd., Tokyo, Japan). The data (not reported) were in agreement with the expected structures. The mass spectra were recorded using a Finningan MAT SSQ 710 instrument. The reactions were monitored by TLC on Kieselgel 60 F 254 (DC-Alufolien, Merk). The final compounds were purified by preparative Gilson medium pressure liquid chromatography apparatus using a SiO_2 column (LiChoprep, Si 60, 25-40 mm, Merck). When indicated, gaseous NH_3 was added to the methanolic phase (MeOH) to obtain a 5% w/w solution [MeOH(NH_3)]. The commercial reagents were purchased from SIGMA-ALDRICH and used without further purification.

General method of preparation of the final compounds (2a-2f and 3a-3f)

The preparation of the title derivatives was carried out as described below. The suitable amine (0.84 mmol) was added to a solution of the appropriate epoxide (0.2 g, 0.84 mmol) in DMF (2 mL), and the mixture was stirred overnight at 80°C. The products were purified by chromatography. Elution with AcOEt:MeOH(NH_3) = 95:5 v/v afforded the compounds **2c**, **2d**, **2f**, **3c**, **3d**, and **3f**. The remaining derivatives were purified using a 95:5 v/v CH_2Cl_2: MeOH(NH_3) mixture =. The propanolamines **2a** and **2b** and oxypropanolamines **3a** and **3b** are described elsewhere (Mingiardi *et al.*, 1983). The physical and spectroscopic data for the original compounds are listed below.

(*R,S*)-2-(3-Benzylamino-2-hydroxypropyl)benzo[d]isothiazol-3-one (2c)

Recrystallized from EtOH-H_2O, 69% yield, m.p. 84-86°C. ^{1}H-NMR (DMSO-d_6) δ 2.47-2.54 (m, 2H, C$\underline{H_2}$NH), 3.71 (s, 2H, NHC$\underline{H_2}$Ph), 3.72-4.01 (m, 3H, C$\underline{H}$OH, bitCH_2), 5.17 (d, *J* = 2.7 Hz, 1H, OH), 7.16-7.38 (m, 5H, Ph), 7.42 (t, *J* = 7.6 Hz, 1H, bit), 7.67 (t, *J* = 7.6 Hz, 1H, bit), 7.87 (d, *J* = 7.8 Hz, 1H, bit), 7.93 (d, *J* = 7.5 Hz, 1H, bit); MS (CI) 314 [M+1]$^+$. Anal. Calcd for $C_{17}H_{18}N_2O_2S$: C, 64.94; H, 5.77; N, 8.89. Found: C, 64.69; H, 5.88; N, 8.65.

(*R,S*)-2-{3-[(Benzo[1,3]dioxol-5-ylmethyl)amino]-2-hydroxypropyl}-benzo[d]isothiazol-3-one (2d)

Recrystallized from EtOH, 67% yield, m.p. 153-151°C. ^{1}H-NMR (DMSO-d_6) δ 2.47-2.53 (m, 2H, C$\underline{H_2}$NH), 3.61 (s, 2H, NHC$\underline{H_2}$Ph), 3.70-4.01 (m, 3H, C$\underline{H}$OH, bitCH_2), 5.14 (d, *J* = 3.9 Hz, 1H, OH), 5.95 (s, 2H, OCH_2O), 6.71-6.83 (m, 2H, Ph-5,6H), 6.90 (s, 1H, Ph-4H), 7.42 (t, *J* = 7.6 Hz, 1H, bit), 7.67 (t, *J* = 7.6 Hz, 1H, bit), 7.86 (d, *J* = 8.1 Hz, 1H, bit), 7.93 (d, *J* = 8.1 Hz, 1H, bit); MS (CI) 358 [M+1]$^+$.

Anal. Calcd for $C_{18}H_{18}N_2O_4S$: C, 60.32; H, 5.06; N, 7.81. Found: C, 60.06; H, 4.97; N, 7.96.

(*R,S*)-(4-{2-[2-Hydroxy-3-(3-oxo-3H-benzo[d]isothiazol-2-yl)propylamino]ethyl}phenyl)sulfinamic acid 4-methoxyphenyl ester (2e)

Recrystallized from EtOH-H_2O, 64% yield, m.p. 84-86°C. ^{1}H-NMR (DMSO-d_6) δ 2.50-2.75 (m, 6H, C$\underline{H}_2$NH, C$\underline{H}_2$C$\underline{H}_2$Ph), 3.65-3.95 (m, 6H, C$\underline{H}$OH, bitC$\underline{H}_2$, PhOC$\underline{H}_3$), 5.17 (br s, 1H, OH), 6.93-7.09 (m, 6H, NHPh-2,3,5,6$\underline{H}$, CH$_3$OPh-3,5$\underline{H}$), 7.42 (t, *J* = 7.6 Hz, 1H, bit), 7.60-7.71 (m, 3H, CH$_3$OPh-2,6$\underline{H}$, bit), 7.85 (d, *J* = 7.8 Hz, 1H, bit), 7.93 (d, *J* = 8.1 Hz, 1H, bit); MS (CI) 513 $[M+1]^+$. Anal. Calcd for $C_{25}H_{27}N_3O_5S_2 \cdot 1/2H_2O$: C, 57.45; H, 5.39; N, 8.03. Found: C, 57.76; H, 5.56; N, 7.79.

(*R,S*)-4-[2-Hydroxy-3-(3-oxo-3H-benzo[d]isothiazol-2-yl)-propylamino]-piperidine-1-sulfinic acid 4-methoxyphenyl ester (2f)

Recrystallized from EtOH, 50% yield, m.p. 196-197°C. ^{1}H-NMR (DMSO-d_6) (hydrochloride) δ 1.53-1.75 (m, 2H, pip-3,5H$_a$), 2.02-2.25 (m, 4H, pip-3,5H$_e$, pip-2,6H$_a$), 2.75-2.91 (m, 1H, pip-4H), 2.94-3.12 (m, 2H, CH-C$\underline{H}_2$-NH), 3.62-3.71 (m, 2H, pip-2,6H$_e$), 3.82-3.94 (m, 5H, Ph-O-CH$_3$, bitCH$_2$), 4.14-4.26 (m, 1H, C$\underline{H}$OH), 6.02 (d, *J* = 5.1 Hz, 1H, OH) 7.15 (d, *J* = 8.7 Hz, 2H, Ph H-3,5), 7.43 (t, *J* = 8.4 Hz, 1H, bit), 7.62-7.73 (m, *J* = 8.7 Hz, 3H, Ph-2,6H, bit), 7.86 (d, *J* = 7.5 Hz, 1H, bit), 7.98 (d, *J* = 8.4 Hz, 1H, bit), 8.88 (br s, 1H, NH$^+$), 9.23 (br s, 1H, NH$^+$); MS (CI) 477 $[M+1]^+$. Anal. Calcd for $C_{22}H_{27}N_3O_5S_2 \cdot 1/2H_2O$: C, 54.30; H, 5.79; N, 8.63. Found: C, 54.59; H, 5.66; N, 8.81.

(*R,S*)-1-(Benzo[d]isothiazol-3-yloxy)-3-benzylaminopropan-2-ol (3c)

Recrystallized from EtOH-H_2O, 72% yield, m.p. 124-126 °C. ^{1}H-NMR (DMSO-d_6) δ 2.58-2.74 (m, 2H, C$\underline{H}_2$NH), 3.73 (s, 2H, NHC$\underline{H}_2$Ph), 3.97-4.08 (m, 1H, C$\underline{H}$OH), 4.36-4.55 (m, 2H, OCH$_2$), 5.05 (d, *J* = 5.1 Hz, 1H, OH), 7.14-7.35 (m, 5H, Ph), 7.46 (t, *J* = 8.1 Hz, 1H, bit), 7.62 (t, *J* = 7.6 Hz, 1H, bit), 7.89 (d, *J* = 7.8 Hz, 1H, bit), 8.06 (d, *J* = 8.1 1H, bit); MS (CI) 314 $[M+1]^+$. Anal. Calcd for $C_{17}H_{18}N_2O_2S$: C, 64.94; H, 5.77; N, 8.90. Found: C, 65.16; H, 5.84; N, 8.62.

(*R,S*)-1-[(Benzo[1,3]dioxol-5-ylmethyl)amino]-3-(benzo[d]isothiazol-3-yloxy) propan-2-ol (3d)

Recrystallized from EtOH, 58% yield, m.p. 133-134°C. ^{1}H-NMR (DMSO-d_6) δ 2.56-2.72 (m, 2H, C$\underline{H}_2$NH), 3.65 (s, 2H, NHC$\underline{H}_2$Ph), 3.96-4.09 (m, 1H, C$\underline{H}$OH), 4.36-4.54 (m, 2H, OCH$_2$), 5.05 (br s, 1H, OH), 5.94 (s, 2H, OCH$_2$O), 6.73-6.82 (m, 2H, Ph-5,6H), 6.90 (s, 1H, Ph-4H), 7.46 (t, *J* = 7.3 Hz, 1H, bit), 7.61 (t, *J* = 7.7 Hz, 1H, bit), 7.88 (d, *J* = 8.1 Hz, 1H, bit), 8.05 (d, *J* = 7.8 Hz, 1H, bit); MS (CI) 358 $[M+1]^+$. Anal. Calcd for $C_{18}H_{18}N_2O_4S$: C, 60.34; H, 5.02; N, 7.81. Found: C, 60.13; H, 5.02; N, 7.91.

(*R,S*)-(4-{2-[3-(Benzo[d]isothiazol-3-yloxy)-2-hydroxypropylamino]ethyl}phenyl)sulfinamic acid 4-methoxyphenyl ester ·HCl (3e)

Recrystallized from abs EtOH-Et_2O, 44% yield, m.p. 179-181°C. ^{1}H-NMR (D_2O) δ 2.54-2.75 (m, 6H, C$\underline{H}_2$NH, C$\underline{H}_2$C$\underline{H}_2$Ph), 3.73 (s, 3H, PhOC$\underline{H}_3$), 3.90-4.03 (m, 1H, C$\underline{H}$OH), 4.29-4.46 (m, 2H, OCH$_2$), 6.90-7.06 (m, 6H, NHPh-2,3,5,6$\underline{H}$, CH$_3$OPh-3,5$\underline{H}$), 7.45 (t, *J* = 7.8 Hz, 1H, bit), 7.53-7.67 (m, 3H, CH$_3$OPh-2,6$\underline{H}$, bit), 7.90 (d, *J* = 8.1 Hz, 1H, bit), 7.98 (d, *J* = 8.1 Hz, 1H, bit); MS (CI) 513 $[M+1]^+$. Anal. Calcd for $C_{25}H_{27}N_3O_5S_2 \cdot HCl$: C, 54.59; H, 5.13; N, 7.64. Found: C, 54.56; H, 5.25; N, 7.43.

(*R,S*)-4-[3-(Benzo[d]isothiazol-3-yloxy)-2-hydroxypropylamino]-piperidine-1-sulfinic acid 4-methoxyphenyl ester (3f)

Recrystallized from EtOH-H_2O, 40% yield, m.p. 125-126 °C. ^{1}H-NMR (DMSO-d_6) δ 1.19-1.38 (m, 2H, pip-3,5H$_a$), 1.75-1.88 (m, 2H, pip-3,5H$_e$), 2.30-2.45 (m, 3H, pip-2,6H$_a$, pip-4H), 2.52-2.72 (m, 2H, C$\underline{H}_2$-NH), 3.32-3.43 (m, 2H, pip-2,6H$_e$), 3.83 (s, 3H, Ph-O-CH$_3$), 3.86-3.98 (m, 1H, C$\underline{H}$OH), 4.32-4.48 (m, 2H, OCH$_2$), 5.04 (br s, 1H, OH), 7.13 (d, *J* = 8.7 Hz, 2H, Ph H-3,5), 7.46 (t, *J* = 7.3 Hz, 1H, bit), 7.57-7.68 (m, *J* = 8.7 Hz, 3H, Ph-2,6H, bit), 7.90 (d, *J* = 8,1 Hz, 1H, bit), 8.05 (d, *J* = 8.1 Hz, 1H, bit); MS (CI) 477 $[M+1]^+$. Anal. Calcd for $C_{22}H_{27}N_3O_5S_2$: C, 55.33; H, 5.70; N, 8.80. Found: C, 55.11; H, 5.69; N, 8.62.

Pharmacology

Isolated rat tissues, atria, and small intestine, which preferentially but not exclusively express the β_1- and β_3-adrenoceptor-mediated responses, respectively, were used in this study (Kaumann and Molenaar, 1996; Roberts *et al.*, 1999).

The experiments performed on the isolated tissues were in accordance with the European Guidelines for the use of animals. The protocols were approved by the National Research Committee of the Italian Ministry of Health.

Preparation of isolated atria

The classical method used in this study followed the methodology reported elsewhere (Bertaccini *et al.*, 1986). Male albino rats (250-400 g) were sacrificed by cervical dislocation after light anaesthesia with ether. The heart was quickly removed and placed in cold Krebs-Henseleit solution of the following composition (mmol/L): NaCl 113; KCl 4.7; $CaCl_2 \cdot 2H_20$ 2.5; KH_2PO_4 1.2; $MgSO_4 \cdot 7H_20$ 1.2; $NaHCO_3$ 25 and dextrose 11.5, at 30°C, bubbled with 5% CO_2 in O_2, in order to maintain a constant oxygen level

and pH value in the range 7.1-7.3.

The atria were isolated with an intact sinus node, tied to both auricles and set up in an isolated organ chamber, which is suitable for measuring the spontaneous beating activity. The preparation was connected to an isometric transducer and the developed contractile force was recorded on a pen-writing polygraph. The frequency was measured by counting the number of contraction cycles from the recorded mechanogram over a 10 sec period.

Smooth muscle preparations

The smooth muscle tissue was prepared using the methodology reported elsewhere (Coruzzi and Poli, 1987). The small intestine (with the exception of the duodenum and of the terminal ileum) was rapidly isolated and placed into a dissection plate containing the above-described Krebs-Heinseleit solution. Segments of the whole intestine (~25 mm in length) were set-up into 10 mL organ chambers at 37°C, containing above-described Krebs-Heinseleit solution and suspended from isotonic transducers under a passive stretch of 0.4-0.5 g. The smooth muscle contractions were recorded on a pen writing polygraph (Basile, Milano, Italy).

Experimental protocols

The preparations were left to equilibrate after setting them up for 30-45 min, during which the solution in the bath was rinsed every 10 min.

In order to obtain a complete concentration-response curve relative to the increase in beating frequency, the isolated atria were stimulated with isoprenaline in a cumulative fashion.

The intestinal preparations were contracted by applying KCl (40 mmol/L) every 15 min to obtain a stable plateau response. Smooth muscle relaxants were subsequently administered in a cumulative manner and left to act until the maximum muscle relaxant effect had been reached. The plateau responses to KCl were obtained in atropine (1 μM)-treated preparations in order to prevent the effects of any endogenous acetylcholine mobilised by excess of K^+ ions and to obtain muscle contractions that were closely dependent on the extracellular Ca^{2+} level (Coruzzi and Poli, 1987).

The antagonistic effects of the drugs was measured by determining the concentration-response curve to isoprenaline this time in presence of the antagonist, and the tissues were incubated for 25 min before administering the β-adrenoceptor agonist.

Data analysis

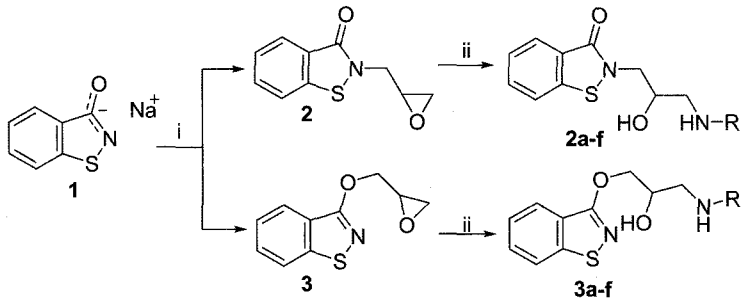
Values presented as a mean ± SEM of 4-8 experiments. The agonistic activity of isoprenaline on the right atrium is expressed as pD_2 values (-Log of the concentration giving 50% of its maximum effect), whereas the relaxant effect on the intestinal muscle is expressed as EC_{50} values (concentration of an agonist giving 50% of the maximum relaxation observed, Kenakin, 1987). The data was then converted to the corresponding logarithmic values ($-LogEC_{50}$) in order to calculate the arithmetic means and for statistical analysis. The Student's *t* test for paired or unpaired data was used to compare two sets of data . A *P* value <0.05 was considered significant.

The pA_2 value for the antagonistic activity of the compounds was estimated using the Gaddum's equation: $pA_2 = - Log [B] + Log (CR-1)$, where [B] represents the concentration of the antagonist and CR is the concentration-ratio at the EC_{50} level of the agonist (isoprenaline) concentration-response curve, which was measured in the presence or absence of the antagonist (Kenakin, 1987).

RESULTS AND DISCUSSION

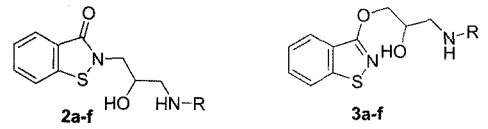
Chemistry

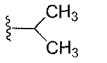
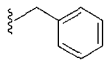
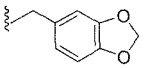
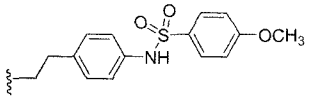
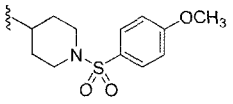
The synthesis of the target compounds **2a-f** and **3a-f** is outlined in Scheme 1. All the compounds were prepared as racemic mixtures. The 1,2-Benzisothiazol-3-one (benzo [d]isothiazol-3-one) sodium salt (**1**) was obtained in quantitative yield beginning from the commercially available 1,2-benzisothiazol-3-one and sodium hydride. The reaction of the sodium salt **1** with epichlorohydrin using the methodology reported elsewhere (Mingiardi *et al.*, 1983) generated the isomeric epoxides **2** and **3**, which that were



Scheme 1. Synthesis of the target compounds; reagents and conditions: i) epichlorohydrin, DMSO, RT, 12 h; ii) amine, DMF, 80°C, 14 h.

Table I. Structure of the target compounds. Compounds **2a, 2b, 3a,** and **3b** were reported previously (Mingiardi *et al.*, 1983).



Compounds	R	Compounds
2a		3a
2b		3b
2c		3c
2d		3d
2e		3e
2f		3f

separated and purified by column chromatography. The opening of the epoxides **2** and **3** with the suitable amine yielded the derivatives listed in Table I. The compounds prepared have been divided into two structural groups for the convenience of discussion: propanolamine derivatives **2a-f** and oxypropanolamine derivatives **3a-f**. The amines used to prepare the compounds **2a-d** and **3a-d** are commercially available. The amine intermediate [4-(2-amino-ethyl)-phenyl]-sulfinamic acid 4-methoxy-phenyl ester was synthesised in good yield in our laboratory (Morini *et al.*, 2005). The remaining amine was prepared using the conditions reported elsewhere (Sum *et al.*, 2003).

Pharmacology

Rat atria

As already described in a similar model (Barocelli *et al.*, 1992; Morini *et al.*, 2005), the non-specific β-adrenoceptor agonist, isoprenaline, induced an increase in the spontaneous beating frequency of the rat atria (from 161±15 to 268±15 beats per min, n = 24). The isoprenaline-induced effect was antagonised by propranolol in a surmountable way, showing a pA_2 value of 9.24±0.21, which is compatible with a competitive antagonism at the β_1-adrenoceptors (Table II). At concentrations up to 30 μM, none of the compounds, **2a-f** and **3a-f**, produced any change in the spontaneous rate, excluding the agonistic activities at cardiac β-adrenoceptors, nor any other direct effects on the mechanisms controlling the sinus node activity (not shown). At higher concentrations (100 μM), most compounds elicited a bradycardic effect, which was not modified by either propranolol (up to 1 μM), or the preferential β_3-adrenoceptor antagonist, bupranolol (up to 10 μM) (Kaumann and Molenaar, 1996) (data not shown). This feature excludes the involvement of the inhibitory β_3-adrenoceptors (Arch, 2001).

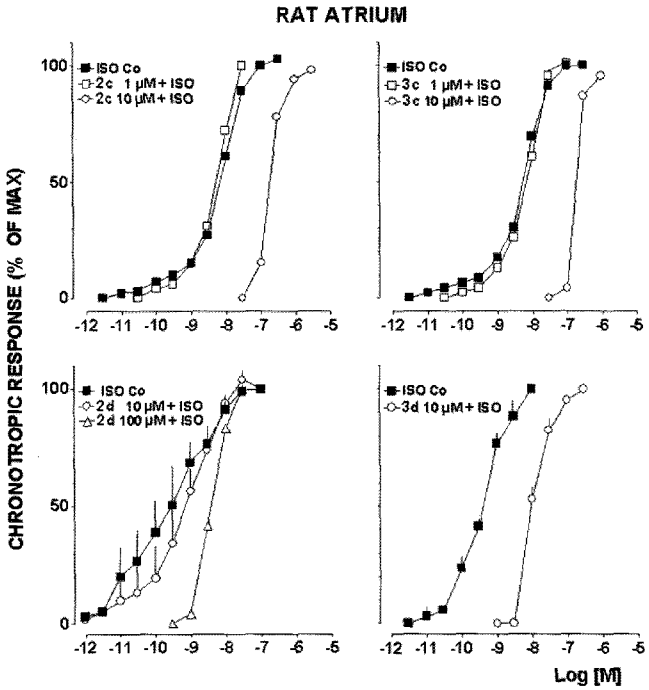


Fig. 1. *Spontaneously-beating rat atria.* Positive chronotropic effect of isoprenaline (ISO) and its antagonism by some representative compounds **2c**, **3c**, **2d**, and **3d**. The concentrations are expressed in mmol/L. The data is reported as a mean ± SEM of 5-6 observations. *In abscissa*: molar concentration of ISO; *in ordinates*: percent of the maximum chronotropic response to ISO, taken as 100%.

Most of the compounds cause a rightward shift in the concentration-response curve of isoprenaline (Fig. 1 and Table II), without affecting the positive chronotropic effects induced by histamine (not shown). Such features suggest specific, surmountable antagonism at the β_1-adrenoceptors. Surprisingly, the pA_2 value calculated for the isopropyl derivative compound **3b** is similar to that of propranolol (P=0.48, n=4). In contrast, compounds **2e-f** and **3e-f** showed no antagonistic activity up to 100 μM.

Rat ileum

Isoprenaline elicits a concentration-dependent relaxation in the KCl-precontracted ileal preparations, which were antagonised in a surmountable fashion by the preferential β_3-adrenoceptor antagonist, bupranolol (pA_2 value 7.30 ± 0.18, Kaumann and Molenaar, 1996) (Fig. 2) and by propranolol (pA_2 value 7.02±0.15, Morini *et al.*, 2005).

Compounds **2d** and **3d** had a bupranolol- and propranolol-insensitive muscle-relaxant effect, which is likely

Table II. Antagonistic activity of the target compounds at the cardiac â$_1$-adrenoceptors along with their muscle-relaxant activity on the KCl-precontracted rat ileum

Compound	pA_2±SEM (atrium)	$-LogEC_{50}$±SEM (ileum)
Propranolol	9.24±0.12*	6.71±0.03*#
2a	6.92±0.23	<4
3a	7.56±0.24**	<4
2b	5.69±0.28	<4
3b	9.39±0.08	<4
2c	6.17±0.33	<4
3c	6.43±0.04	<4
2d	5.45±0.28	4.74±0.08
3d	6.67±0.25	4.77±0.07
2e	N.E.	<4
3e	N.E.	<4
2f	N.E.	<4
3f	N.E.	<4

The data represents the mean of 5-6 experiments. *Values are from Morini *et al.*, 2005; **from Barocelli *et al.*, 1992. #Value refers to the racemate; the value relative to the active enantiomer, (S)-propranolol, is <4. N.E.: not effective up to 100 μM.

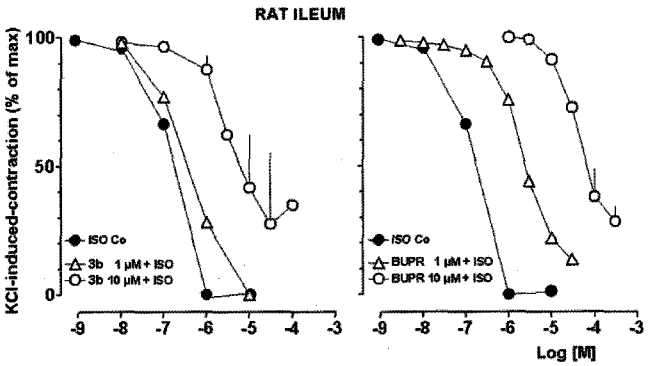


Fig. 2. *KCl-precontracted rat ileum.* Antagonistic activity of compound **3b** (*left panel*) and bupranolol (BUPR) (*right panel*) on the muscle relaxation induced by isoprenaline (ISO). The concentrations of antagonists are expressed in mmol/L. The data is reported as a mean ±SEM of 4-6 observations. *In abscissa*: molar concentration of ISO; *in ordinates*: percent relaxation of the KCl-induced plateau response, taken as 100%.

due to a direct, β-adrenoceptor-independent interaction with the contractile machinery, as has been reported for other members of the family (Molina *et al.*, 1974). The concentrations requested to produce muscle relaxation are higher than those needed to antagonise the β_1-adrenoceptors in the atria, (Table II). All the other compounds produce erratic and partial relaxation, which were measured at very high concentrations (>100 μM). Overall, the poor muscle relaxant effects of these compounds reduce their ability to act as Ca^{2+} channel blockers or as anaesthetic-like drugs (Amoretti *et al.*, 1972; Molina *et al.*, 1974; Morini *et al.*, 2005).

When tested at concentrations up to 10 μM, none of the compounds except compound **3b** affected the relaxant effect evoked by isoprenaline (not shown), which indicates that that they are devoid of antagonistic activity at the β_3-adrenoceptors in the smooth muscle. Only compound **3b** causes a rightward displacement of the isoprenaline concentration-response curve, showing a bupranolol-like effect (Fig. 2). The antagonistic activity of compound **3b** was concentration-dependent and straightforward, which suggests competitive antagonism at the β_3-adrenoceptor recognition site, showing a pA_2 value of 6.28±0.09 (n=5).

CONCLUSIONS

1,2-benzisothiazole derivatives, which were substituted with propanolamine or oxypropanolamine side chains, had antagonistic activity at the cardiac β_1-adrenoceptors without any major effect on the spontaneous sinus rate. These results are in accordance with previous findings using a different series of benzisothiazole derivatives substituted in the 5 and 7 positions of the ring instead (Morini *et al.*, 2005). This finding also agrees with the general view that bulky substituents on the oxypropanolamine chain preclude an optimum interaction of the moieties with the β_1-adrenoceptor recognition site (Benfield *et al.*, 1986; Wadworth *et al.*, 1991). Accordingly, the compounds showing the highest pA_2 values were compound **3a**, where the substituent is the small isopropyl group (also present in isoprenaline as well as in several structures of the β-adrenoceptor blocking drugs, including metoprolol, atenolol, and propranolol itself, Benfield *et al.*, 1986; Wadworth *et al.*, 1991), and compound **3b**, which contains a *tert*-butyl group. Moreover, the differences in the pA_2 values between the corresponding 2- and 3-substituted compounds demonstrate that the position of the substituent on the ring plays an important role in determining the degree of β_1-adrenoceptor activity of the compounds.

The lack of activity at β_3 receptors is surprising considering that the aryloxypropanolamine class includes preferential or specific β_3-adrenoceptor agonists, such as LY-377604 or cyanopindolol and CGP-12177 (Candelore *et al.*, 1999), and that bulky substitutions on the structures isosteric with respect to that of the catechol nucleus have been used to create novel ligands for this subtype (Arch *et al.*, 2001). On the other hand, the compound with β_3-blocking properties, compound **3b**, showed a more favourable β_1/β_3 ratio in terms of the respective pA_2 values (9.39 *vs.* 6.28) for the β_1-adrenoceptor blockade compared with the prototypical β-receptor blocker, propranolol (9.24 *vs.* 7.02). Preliminary investigations of compound **3b** on the

rat bladder excluded any effect on the β_2-adrenoceptors (not shown), which confirms the hypothesis of the specific effects at cardiac β_1-adrenoceptors.

Based on these findings, the benzisothiazole ring is not an appropriate moiety for the design of novel compounds acting on the β_3-adrenoceptors.

Although the therapeutic armamentarium in the field of β-blocking agents is well known, further "in silico" and preclinical studies will be needed to identify potential applications of benzisothiazole-based compounds that can specifically affect the cardiac β_1-adrenoceptors.

REFERENCES

Amoretti, L., Catellani, P. L., Impicciatore, M., and Cavaggioni, A., Biological properties of 1,2-benzisothiazole compounds. Local anaesthetic activity of amides and esters of 1,2-benzisothiazol-3-ylcarboxylic acid. *Il Farmaco*, 27, 855-865, (1972).

Arch, J. R. S., The β_3-adrenergic system and b_3-adrenergic agonists. *Rev. Endocr. Metab. Dis.*, 2, 385-393 (2001).

Barocelli, E., Chiavarini, M., Ballabeni, V., Mingiardi, M. R., Morini, G., Ronchini, R., and Impicciatore, M., Pharmacological properties of new 1,2-benzisothiazolyl oxypropanolamines on cardiac and tracheal β-receptors. *Boll. Soc. It. Biol. Sperim.*, 68, 445-451 (1992).

Benfield, P., Clissold, S. P., Brogden, R. N., Metoprolol. An updated review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy, in hypertension, ischaemic heart disease and related cardiovascular disorders. *Drugs*, 31, 376-429 (1986).

Bertaccini, G., Coruzzi, C., Poli, E., and Adami, M., Pharmacology of the novel H_2 antagonist famotidine: *in vitro* studies. *Agents Actions*, 19, 180-187 (1986).

Blin, N., Camion, L., Magret, B., and Strosberg, A. D., Structural and conformational features determining selective signal transduction in the β_3-adrenergic receptor. *Mol. Pharmacol.*, 44, 1094-1104 (1993).

Candelore, M. R., Deng, L., Tota, L., Xiao-Ming, G., Amend, A., Liu, Y., Newbold, R., Cascieri, M. A., and Weber, A. E., Potent and selective human β-adrenergic receptor antagonists. *J. Pharm. Exp. Ther.*, 290, 649-655 (1999).

Coruzzi, G. and Poli, E., Changes in duodenal contractility induced by "calcium antagonists" with different modes of action. *Gen. Pharmacol.*, 18, 69-74 (1987).

Ishibashi, T., Ikeda, K., Ishida, K., Yasui, J., Tojima, R., Nakamura, M., and Ohno, Y., Contrasting effects of SM-9018, a potential atypical antipsychotic, and haloperidol on *c*-fos mRNA expression in the rat striatum. *Eur. J. Pharmacol.*, 303, 247-251 (1996).

Kaumann, A. J. and Molenaar, P., Differences between the third cardiac β-adrenoceptor and the colonic β_3-adrenoceptor in the rat. *Br. J. Pharmacol.*, 118, 2085-2098 (1996).

Kenakin, T. P., Drug antagonism, in Kenakin, T. P. (Ed.), Pharmacologic analysis of drug-receptor interaction. Raven Press, New York, pp. 205-225 (1987).

Kusayama, T., Oka, J., Yabana, H., Adaki-Akahane, and S., Nagao, T., Binding of a catechol derivative of denopamine (T.0509) and N-tert-butylnoradrenaline (Colterol) to β_1- and β_2-adrenoceptors. *Biol. Pharm. Bull.*, 17, 1023-1027 (1994).

Mingiardi, M. R., Maggiali, C. A., and Ronchini, F., 1,2-Benzisothiazole derivatives potentially active as β-blockers. *Ateneo Parmense, Acta Nat.*, 19, 145-151 (1983).

Molina, E., Zappia, L., Amoretti, L., and Catellani, P. L., Biological properties of 1,2-benzisothiazole compounds. Spasmolytic activity of benzisothiazol-3-ylcarboxyamides. *Ateneo Parmense, Acta Bio-Med.*, 45, 183-189 (1974).

Morini, G., Pozzoli, C., Adami, M., Poli, E., and Coruzzi, G., Synthesis of 1,2-benzisothiazole derivatives and investigation of their putative histaminergic activity. *Il Farmaco*, 54, 740-746 (1999).

Morini, G., Pozzoli, C., Menozzi, A., Comini, M., and Poli, E., Synthesis of 1,2-benzisothiazolyloxypropanolamine derivatives and investigation of their activity at β-adrenoceptors. *Il Farmaco*, 60, 810-817 (2005).

Mos, J., Van-Hest, A., Van Drimmelen, M., Herremans, A. H., and Olivier, B., The putative 5-HT_{1A} receptor antagonist DU125530 blocks the discriminative stimulus of the 5-HT_{1A} receptor agonist flesinoxan in pigeons. *Eur. J. Pharmacol.*, 325, 145-153 (1997).

Roberts, S. J., Papaioannou, M., Evans, B. A., and Summers, R, J., Characterisation of β-adrenoceptor mediated smooth muscle relaxation and the detection of mRNA for β_1-, β_2-, and β_3-adrenoceptors in rat ileum. *Br. J. Pharmacol.*, 127, 949-961 (1999).

Strosberg, A. D., Structure and function of the b_3-adrenergic receptor. *Annu. Rev. Pharmacol. Toxicol.*, 37, 421-450 (1997).

Sum, F. W., Wong, V., Han, S., Largis, E., Mulvey, R., and Tillett, J., Cyclic amine sulfonamides as linkers in the design and synthesis of novel human β_3 adrenergic receptor agonists. *Bioorg. Med. Chem. Lett.*, 13, 2191-2194 (2003).

Von Franche, A., Frickel, F. F., Gries, J., Lehman, H. D., Lenke, D., and Ohnsorge, U., New β-sympatholytic agents: synthesis and pharmacological activity of isomeric benzothiazole and benzoxazole derivatives. *Arzneim. Forsch. Drug Res.*, 30, 1831-1835 (1980).

Wadworth, A. N., Murdoch, D., and Brogden, R. N., Atenolol. A reappraisal of its pharmacological properties and therapeutic use in cardiovascular disorders. *Drugs*, 42, 468-510 (1991).