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New α- and β-adrenoceptor blockers

Synthesis and in vitro pharmacological activity of oxypropanol analogs of labetalol

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Abstract

New oxypropanol α- and β-adrenoceptor blocking agents, analogs of labetalol, were synthesised and studied in vitro in left atria and aorta for α and β activity.

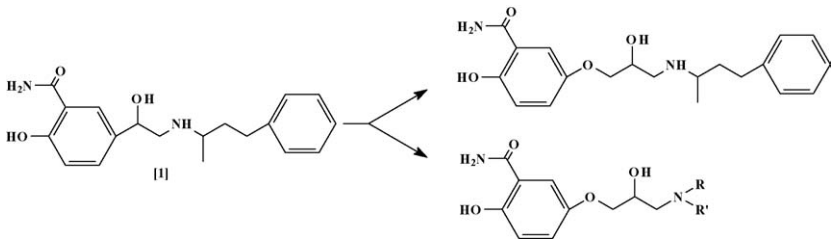
Keywords: Adrenoceptor blockers; Cardiovascular diseases; Antihypertensives; Labetalol

1. Introduction

At present α- and β-adrenoceptors are classified into α_1/α_2 and β_1/β_2 (and also β_3) [1,2] subtypes on the basis of structural and pharmacological studies. Both α- and β-adrenoceptor antagonists were proved to be effective and useful in the treatment of hypertension. β-Blockers, especially those selectively blocking heart β-adrenoceptors (β_1) with little or no activity on bronchial or vascular adrenoceptors (β_2), have been widely used and tested on a numbers of patients in all major multicentre intervention trials performed during past years [3–5]. α-Adrenoceptor blocking agents have been less widely applied, although selective α_1-adrenoceptor antagonists, like prazosin, have been introduced as useful antihypertensive agents. Therapeutically, the combination of α_1 and β_1-adrenoceptor antagonists is a logical one [6]. These observations led to the synthesis of several compounds combining both α- and β-adrenergic antagonist activities, and labetalol [1] is the first example of combined α- and β-adrenoceptor antagonistic drug [7].

Arylethanolamines and aryloxypropanolamines were the two main structures, which exerted β-adrenoceptor antagonism. In various recent examples of antihypertensive β-adrenoceptor blocking agents the substitution of the central ethanolaminic moiety with an aryloxypropanolaminic chain was proved to be effective [8].

All these considerations prompted us to modify labetalol structure from arylethanolamine into aryloxypropanolamine and to introduce arylalkylamines that have been shown to lend cardioselectivity or to increase affinity for α-adrenoceptors.



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2. Chemistry

The synthesis proceed as illustrated in Scheme 1 by way of the synthetic intermediate [**III**], prepared in three steps according to the literature procedure [9]. The epoxyde [**IV**], obtained starting from [**III**] and epichlorohydrin by phase-transfer reaction in sodium hydroxide and toluene with tetrabutylammonium hydrogen sulphate as catalyst at room temperature, was refluxed in 2-propanol with a slight excess of each amine (**a–e**) to yield the derivatives [**Va–e**]. Acidic hydrolysis (dioxan/acetic acid/water) of compounds [**Va–e**] gave the final products [**VIa–e**].

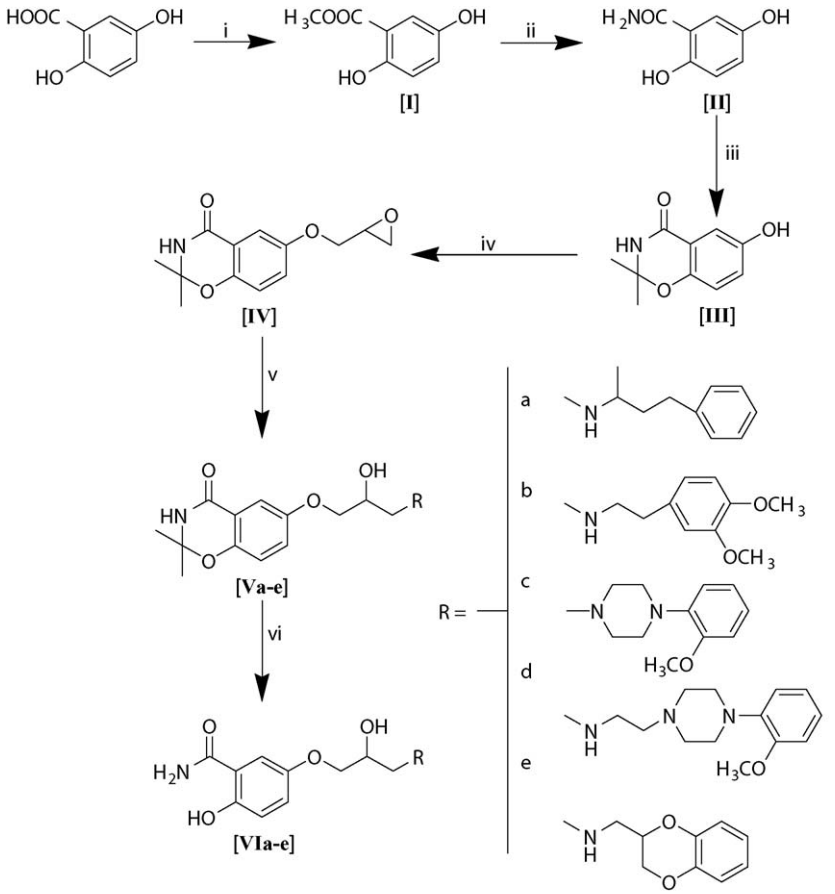
All the final compounds were obtained as a mixture of two or four stereoisomers and were tested as such.

Nevertheless, on account of great importance of stereochemistry for many drugs, particularly for β-blockers, and considering the results of pharmacological tests of racemic compounds (see Table 3), we have planned the synthesis of the isomers R and S of compounds **VIb** and **VIc**, which retain α_1- and β_1-adrenergic blocking activity. The synthesis of the two couples of enantiomers, Scheme 2, starts with the compound [**III**] which was reacted in a Mitsunobu reaction, respectively, with (*R*)-(+)-glycidol and (*S*)-(−)-glycidol obtaining the two epoxydes, namely [**VII**] and [**VIII**]. Reaction of the two epoxydes [**VII**] and [**VIII**] with the amine **b** gave compounds [**IX**] and [**X**], while the same reaction with the amine **c** gave compounds [**XI**] and [**XII**], which were deprotected to the final products [**XIII**], [**XIV**], [**XV**] and [**XVI**]. The four final compounds were submitted to the pharmacological tests as the racemic compounds.

3. Experimental

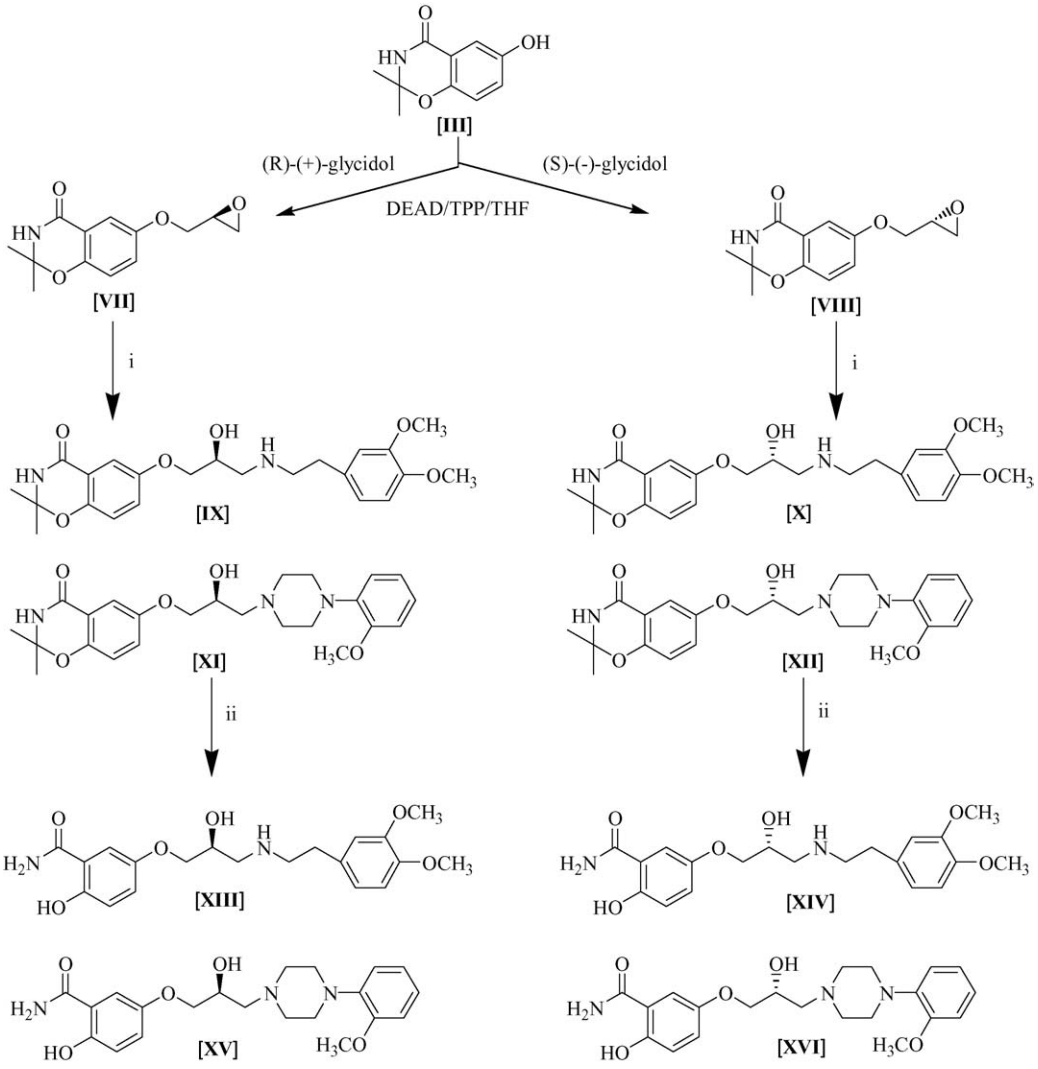
3.1. Chemistry

Melting points were determined using a Köfler block and are uncorrected. Elemental analyses of all synthesised compounds were performed by our analytical laboratory in a Perkin-Elmer elemental analyser Mod. 240 for C, H, N, and the data are within ± 0.4% of the theoretical values. Optical rotations were measured with a Perkin-Elmer 343 automatic polarimeter in a 0.1 dm tube (c = g/ml). ^{1}H NMR spectra was recorded at 25 °C on a Brucker AC200F and chemical shifts are expressed as δ (ppm). FTIR spectra were recorded on a Perkin-Elmer Mod. 398 spectrometer. Mass spectral data were



i) CH_3OH/H^+; ii) NH_3; iii) 2,2-dimethoxypropane/H^+; iv) NaOH/toluene, epichlorohydrin, tetrabutylammonium sulphate; v) amine, isopropanol, reflux; vi) dioxan, acetic acid,water (1/6/2), reflux.

Scheme 1.



i) amine, anhydrous toluene, reflux; ii) dioxane/acetic acid/water (1/6/2), reflux.

Scheme 2.

determined by direct insertion at 70 eV with a VG70 spectrometer. All the compounds were checked for purity by T.L.C. on Merck 60 F_{254} silica plates. For column chromatography Merck 60 silica gel, 230–400 mesh, was used. Flash chromatography system Biotage, with columns 12.25 mm, packed with KP-Sil, 60A, 32–63 μM was used for flash chromatography.

3.1.1. (±)-2,3-dihydro-2,2-dimethyl-6-oxyranilmethoxy-(4H)-1,3-benzoxazin-4-one [IV]

A mixture of 50 ml of NaOH 1 N, 20 ml of toluene, 50 mg of tetrabutylammonium hydrogen sulphate, 5 g (25.9 mmol) of [**III**] and 6.3 ml (80 mmol) of epichlorohydrin was stirred at r.t. for 5–6 h, extracted with $CHCl_3$, dried on Na_2SO_4, evaporated and the residue was purified by column chromatography on silica gel ($CHCl_3$:MeOH/47:3). Recrystallization of the product from dilute ethanol gave 4.5 g (70%) of [**IV**]. White solid. M.p. 139–42 °C.

^{1}H NMR (200.13 MHz): (DMSO-d_6) δ 8.62 (s, 1H, NH); 7.37 (d, 1H, Ar-5, J = 3.00 Hz); 7.04 (dd, 1H, Ar-7, J = 3.00 Hz, J = 8.85 Hz); 6.81 (d, 1H, Ar-8, J = 8.89 Hz); 4.23 (dd, 1H, OCH_2, J = 2.93 Hz, J = 11.01 Hz); 3.92 (dd, 1H, OCH_2, J = 5.72 Hz, J = 11.04 Hz); 3.32–3.28 (m, 1H, CH); 2.88–2.84 (m, 1H, OCH_2 oxiranic); 2.73–2.70 (m, 1H, OCH_2 oxiranic); 1.60 (s, 6H, 2 CH_3). I.R. (KBr, cm^{-1}): ν 3205 (NH), ν 1685 (CO).

3.1.2. (±)-2-hydroxy-5-[2-hydroxy-3-(substituted-amino)]-propoxybenzamide [VIa,e]

A solution of 2 g (8.0 mmol) of [**IV**] and 8.5 mmol of the appropriate amine in 30 ml of 2-propanol was stirred and refluxed for 4 h. After work up of the reaction, products [**Va,e**] (yields, purification procedures, melting points, and spectroscopic data are reported in Table 1) were submitted to acidic hydrolysis refluxing in dioxan/acetic acid/water (1:6:2) [10] for 24 h. After neutralisation with sodium bicarbonate, the solution was extracted with chloroform; extracts were col-

Table 1
Yields, physical and spectroscopic data of the racemic compounds **Va–e**

Compound	Yield (%)	Purification	M.p. (°C)	^{1}H NMR (200.13 MHz) DMSO-d$_6$, (*J* in Hz)
Va	69	Chromatography on SiO_2 $CHCl_3$/MeOH (40/10) and recrystallized by MeOH/diethyl ether	197–9	δ 8.63 (s br, 1H, NHCO); 7.32–7.09 (mm, 7H, Ar); 6.92 (d, 1H, Ar, *J* = 8.85); 4.90 (s br, 1H OH); 4.03–3.88 (m, 3H, OCH_2C*H*OH); 3.49–3.20 (mm, 4H, CH_2NHC*H*); 2.70–2.52 (mm, 4H, CH_2CH_2Ar); 1.53 (s, 6H, 2 CH_3); 1.06 (d, 3H, CHCH_3, *J* = 6.32)
Vb	33	Chromatography on SiO_2 $CHCl_3$/MeOH (40/10)	166–9	δ 8.63 (s, 1H, CONH); 7.22–6.66 (mm, 6H, Ar); 4.90 (s br, 1H, OH); 4.05–3.95 (m, 2H, C*H*OH, CH_2O); 3.84 (s, 6H, 2 OCH_3); 3.80–3.77 (m, 1H, CH_2O); 3.34 (s br, 1H, NH); 3.00–2.80 (mm, 6H, CHCH_2NHCH_2CH_2Ar); 1.50 (s, 6H, 2 CH_3)
Vc	59	Crystallized by MeOH	144–6	δ 8.61 (s, 1H, CONH); 7.20–6.86 (mm, 7H, Ar); 4.87–4.85 (s br, 1H, OH); 4.20–4.10 (m, 1H, C*H*OH); 4.10–4.00 (m, 2H, CH_2O); 3.74 (s, 3H, OCH_3); 3.15–3.10 (m, 4H, 2 CH_2 piperaz.); 2.94–2.55 (mm, 6H, CH_2N + 2 CH_2 piperaz.); 1.48 (s, 6H, 2 CH_3).
Vd	28	Chromatography on SiO_2 $CHCl_3$/MeOH (35/15) and recrystallized by MeOH/diethyl ether	119–20	δ 8.62 (s, 1H, CONH); 7.36 (d, 1H, Ar., *J* = 2.80); 7.18 (dd, 1H, Ar., *J* = 2.80, *J* = 8.91); 6.96–6.82 (mm, 5H, Ar.); 5.84 (s br, 1H, OH); 4.05 (m, 3H, C*H*OH + OCH_2); 3.78 (s, 3H, OCH_3); 3.49–3.41 (m, 4H, 2 CH_2 piperaz.); 2.90–2.84 (mm, 4H, CH_2NHCH_2); 2.75–2.69 (m, 3H, CH_2 + NH); 2.58–2.53 (m, 4H, CH_2 piperaz.); 1.51 (s, 6H, 2 CH_3)
Ve	49	Chromatography on SiO_2 $CHCl_3$/MeOH (40/10)	184–8	δ 8.60 (s, 1H, CONH); 7.26 (d, 1H, Ar, *J* = 2.90); 7.11 (dd, 1H, Ar, *J* = 3.10, *J* = 8.80); 6.96–6.80 (mm, 5H, Ar); 5.01 (d, 1H, OH, *J* = 4.10); 4.37–4.22 (m, 3H, OCH_2CHOH + CH_2C*H*O); 4.04–3.87 (mm, 4H, C*H*OH + CHCH_2O + NH); 2.85–2.51 (mm, 4H, CH_2NHCH_2); 1.59 (s, 6H, 2 CH_3)

lected, dried, and evaporated to obtain the final compounds [**VIa,e**]. Yields, purification procedures, melting points, and spectroscopic data are shown in Table 2.

3.1.3. (S)-(+)-2,3-dihydro-2,2-dimethyl-6-oxyranilmethoxy-(4H)-1,3-benzoxazin-4-one [VII]

A solution of 0.588 g (530 μl, 3.37 mmol) of diethylazodicarboxylate in anhydrous THF was added to a solution of [**III**] (0.650 g, 3.37 mmol), (*R*)-(+)-glycidol (0.250 g, 225 μl, 3.37 mmol) and triphenylphosphine (0.884 g, 3.37 mmol) in anhydrous THF. The resulting mixture was stirred at r.t. for 36 h and then concentrated, diluted with chloroform and washed with saturated brine. The raw material was purified by column chromatography on silica gel first with *n*-hexane/ethylacetate (1/1) and then with $CHCl_3$/MeOH (48/2). Yield 60%. White solid. M.p. 140–42 °C.

^{1}H NMR (200.13 MHz): ($CDCl_3$) δ 7.37 (d, 1H, Ar-5, *J* = 3.00 Hz); 7.07 (dd, 1H, Ar-7, *J* = 2.40 Hz, *J* = 8.72 Hz);

Table 2
Yields, purification, physical and spectroscopic data of the racemic compounds **VIa–e**

Compound	Yield (%)	Purification	M.p. (°C)	^{1}H NMR (200.13 MHz) DMSO-d$_6$, (*J* in Hz)
VIa	61	Flash chromatography ($CHCl_3$ 40/MeOH 10)	113–5	δ 12.36 (s, 1H, Ar-*OH*); 8.48 (s br, 1 H, NH_2); 7.81 (s br, 1H, NH_2); 7.54 (d, 1H, Ar., *J* = 2.50); 7.31–7.15 (mm, 5H, Ar.); 7.03 (dd, 1H, Ar., *J* = 2.50, *J* 9.00); 6.80 (d, 1H, Ar., *J* = 9.00); 4.18 (s br, 1H, CHO*H*); 3.97–3.95 (m, 3H, OCH_2C*H*OH); 3.13–3.07 (mm, 3H, CH_2N*H*); 2.97–2.92 (m, 1H, C*H*CH_3); 2.70–2.48 (m, 2H, 2 CH_2Ar); 1.88–1.74 (m, 2H, CHCH_2); 1.27 (d, 3H, CH_3, *J* = 5.00)
VIb	78	Flash chromatography ($CHCl_3$ 40/MeOH 10) recrystallized by MeOH/diethyl ether	84–6	δ 12.37 (s, 1H, Ar-*OH*); 8.43 (s, 1H, $CONH_2$); 7.85 (s, 1H, $CONH_2$); 7.46 (d, 1H, Ar., *J* = 2.80); 7.06 (dd, 1H, Ar., *J* = 2.80, *J* = 8.90); 6.88–6.81 (m, 3H, Ar.); 6.76–6.72 (m, 1H, Ar); 4.85 (s br, 1H, *CHOH*); 3.94–3.86 (m, 3H, OCH_2C*H*OH); 3.76 (s, 3H, OCH_3); 3.74 (s, 3H, OCH_3); 2.79–2.54 (mm, 7H, *CH*$_2$*NH* *CH*$_2$*CH*$_2$)
VIc	62	Flash chromatography ($CHCl_3$ 40/MeOH 10)	155–7	δ 12.39 (s, 1H, Ar-*OH*); 8.32 (s, 1H, $CONH_2$); 7.76 (s, 1H, $CONH_2$); 7.40 (d, 1H, Ar., *J* = 2.80); 7.02 (dd, 1H, Ar., *J* = 2.80, *J* = 8.90); 6.93–6.74 (mm, 5H, Ar.); 4.74 (s br, 1H, CH-*OH*); 3.92–3.77 (m, 3H, OCH_2C*H*OH); 3.72 (s, 3H, OCH_3); 2.91 (m, 4H, piper.); 2.56 (m, 4H, piper.); 2.46–2.43 (m, 2H, CH_2N)
VId	50	Flash chromatography ($CHCl_3$ 40/MeOH 10) recrystallized by MeOH/diethyl ether	121–3	δ 12.42 (s, 1H, Ar-*OH*); 8.51 (s, 1H, $CONH_2$); 7.87 (s, 1H, $CONH_2$); 7.56 (d, 1H, Ar., *J* = 2.80); 7.08 (dd, 1H, Ar., *J* = 2.80, *J* = 8.90); 7.02–6.82 (mm, 5H, Ar.); 5.24 (s broad, 1H, CHO*H*); 3.91 (m, 3H, OCH_2C*H*OH); 3.77 (s, 3H, OCH_3); 3.46–3.39 (m, 4H, piper.); 2.85–2.79 (mm, 4H, CH_2CH_2); 2.72–2.64 (m, 3H, CH_2NH); 2.54–2.51 (m, 4H, piper.)
VIe	60	Flash chromatography ($CHCl_3$ 40/MeOH 10)	87–91	δ 12.38 (s, 1H, Ar-*OH*); 8.31 (s, 1H, $CONH_2$); 7.77 (s, 1H, $CONH_2$); 7.40 (d, 1H, Ar, *J* = 2.70); 7.05–6.87 (m, 2H, Ar); 6.82–6.72 (m, 4H, Ar); 4.83 (s br, 1H, CH*OH*); 4.34–4.22 (m, 1H, CH_2C*H*O); 4.02–3.86 (mm, 5H, OCH_2CHOH + CHCH_2O + NH); 3.40–3.26 (m, 1H, C*H*OH); 2.92–2.48 (mm, 4H, *CH*$_2$NHCH_2)

Table 3
In vitro effects of labetalol and structurally related new synthesised racemic compounds

Compound	β_1-Adrenergic antagonism activity IC_{50} (M)	α_1-Adrenergic antagonism activity IC_{50} (M)
Labetalol	$4.8 \times 10^{-7} \pm 0.8 \times 10^{-7}$	$1.5 \times 10^{-6} \pm 0.5 \times 10^{-6}$
VIa	$3.5 \times 10^{-6} \pm 0.9 \times 10^{-6}$	N.d.[a]
VIb	$4.9 \times 10^{-6} \pm 0.4 \times 10^{-6}$	$9.3 \times 10^{-8} \pm 0.2 \times 10^{-8}$
VIc	$2.6 \times 10^{-6} \pm 0.6 \times 10^{-6}$	$1.2 \times 10^{-7} \pm 0.3 \times 10^{-7}$
VId	$8.4 \times 10^{-6} \pm 4.8 \times 10^{-6}$	N.d.[a]
VIe	N.d.[a]	$2.7 \times 10^{-7} \pm 0.2 \times 10^{-7}$

The IC_{50} values are reported as mean ± SEM of three different experiments.
[a] Not detectable.

6.82 (d, 1H, Ar-8, *J* = 8.85 Hz); 6.15 (s br, 1H, NHCO); 4.24 (dd, 1H, OCH_2, *J* = 2.60 Hz, *J* = 10.76 Hz); 3.92 (dd, 1H, OCH_2, *J* = 5.84 Hz, *J* = 10.82 Hz); 3.34–3.31 (m, 1H, CH); 2.87 (t, 1H, OCH_2 oxiranic, *J* = 4.23 Hz); 2.74 (m, 1H, OCH_2 oxiranic); 1.61 (s, 6H, 2 CH_3). $[\alpha]_D^{20} = +15.0^\circ$ (*c* = 0.013; acetone).

*3.1.4. (R)-(–)-2,3-dihydro-2,2-dimethyl-6-oxyranilmethoxy-(4H)-1,3-benzoxazin-4-one [**VIII**]*

This compound has been obtained following the same procedure used for [**VII**], but starting from [**III**] and (*S*)-(–)-glycidol. Yield 55%. White solid. M.p. 140–42 °C.

^{1}H NMR (200.13 MHz): ($CDCl_3$) δ 7.39 (d, 1H, Ar-5, *J* = 3.03 Hz); 7.07 (dd, 1H, Ar-7, *J* = 2.99 Hz, *J* = 8.85 Hz); 6.84 (d, 1H, Ar-8, *J* = 8.86 Hz); 6.15 (s br, 1H, NHCO); 4.25 (dd, 1H, OCH_2, *J* = 2.94 Hz, *J* = 11.32 Hz); 3.93 (dd, 1H, OCH_2, *J* = 5.64 Hz, *J* = 11.22 Hz); 3.37–3.31 (m, 1H, CH); 2.89 (t, 1H, OCH_2 oxiranic, *J* = 4.45 Hz); 2.74 (dd, 1H, OCH_2 oxiranic, *J* = 2.30 Hz, *J* = 4.90 Hz); 1.62 (s, 6H, 2 CH_3). $[\alpha]_D^{20} = -15.0^\circ$ (*c* = 0.025; acetone).

*3.1.5. Representative procedure for obtaining compounds [**IX**], [**X**], [**XI**] and [**XII**]*

To the appropriate epoxyde (1.0 eq.) dissolved in anhydrous toluene the corresponding amine (**b** and **c**, 1.2eq.) was added, refluxing the resulting solution under nitrogen atmosphere for 12 h. After cooling, the reaction mixture has been concentrated and the residue dissolved in chloroform; the organic layer was washed with saturated brine and dried. Filtration and evaporation of solvent gave a crude material that was purified by flash chromatography using $CHCl_3$/MeOH (45/5) as eluent to give pure products.

*3.1.5.1. (S)-(+)-2,3-dihydro-2,2-dimethyl-6-{2-hydroxy-3-[2-(3,4-dimethoxyphenyl)ethylamino]propoxy}-(4H)-1,3-benzoxazin-4-one [**IX**].* ^{1}H NMR (200.13 MHz): ($CDCl_3$) δ 7.38–6.68 (m, 6H, Ar); 6.64 (s br, 1H, NHCO); 4.03–3.93 (m, 2H, C*H*OH and CH_2O); 3.85 (s, 3H, OCH_3); 3.83 (s, 3H, OCH_3); 3.79–3.77 (m, 1H, CH_2O); 3.20 (s br, 2H, NH and OH); 2.94–2.70 (m, 6H, $CHCH_2NH$ and $NHCH_2CH_2Ar$); 1.60 (s, 6H, 2 CH_3). Pale yellow oil (70%). MS (EI) *m/z*: 431 $[M + 1]^+$. $[\alpha]_D^{20} = +16.0^\circ$ (*c* = 0.01; methanol).

*3.1.5.2. (R)-(–)-2,3-dihydro-2,2-dimethyl-6-{-hydroxy-3-[2-(3,4-dimethoxyphenyl)ethylamino]propoxy}-(4H)-1,3-benzoxazin-4-one [**X**].* ^{1}H NMR (200.13 MHz): ($CDCl_3$) δ 7.39–6.70 (m, 6H, Ar); 6.54 (s br, 1H, NHCO); 4.03–3.94 (m, 2H, C*H*OH and CH_2O); 3.85 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 3.79–3.78 (m, 1H, CH_2O); 3.31–2.71 (m, 6H, $CHCH_2NH$ and $NHCH_2CH_2Ar$); 1.60 (s, 6H, $2CH_3$). Pale yellow oil (65%). MS (EI) *m/z*: 431 $[M + 1]^+$. $[\alpha]_D^{20} = -16.0^\circ$ (*c* = 0.008; methanol).

*3.1.5.3. (S)-(+)-2,3-dihydro-2,2-dimethyl-6-{2-hydroxy-3-[4-(2-methoxyphenyl)-1-piperazinyl]propoxy}-(4H)-1,3-benzoxazin-4-one [**XI**].* ^{1}H NMR (200.13 MHz): ($CDCl_3$) δ 7.42 (d, 1H, Ar, *J* = 2.97 Hz); 7.11–6.51 (m, 6H, Ar); 6.50 (s br, 1H, NHCO); 4.16–4.07 (m, 1H, C*H*OH); 4.06–3.97 (m, 2H, CH_2O); 3.86 (s, 3H, OCH_3); 3.50 (s br, 1H, OH); 3.11–3.09 (m, 4H, 2 CH_2 piperaz.); 2.93–2.83 (m, 2H, CH_2N); 2.71–2.59 (m, 4H, 2 CH_2 piperaz.); 1.62 (s, 6H, 2 CH_3). Crystallized from diethylether, white powder (70%). MS (EI) *m/z*: 442 $[M + 1]^+$. M.p. 144–46 °C. $[\alpha]_D^{20} = +13.0^\circ$ (*c* = 0.01; methanol).

*3.1.5.4. (R)-(–)-2,3-dihydro-2,2-dimethyl-6-{2-hydroxy-3-[4-(2-methoxyphenyl)-1-piperazinyl]propoxy}-(4H)-1,3-benzoxazin-4-one [**XII**].* ^{1}H NMR (200.13 MHz): ($CDCl_3$) δ 7.41 (d, 1H, Ar, *J* = 2.88 Hz); 7.11–6.81 (m, 6H, Ar); 6.23 (s br, 1H, NHCO); 4.15–4.09 (m, 1H, C*H*OH); 4.07–3.98 (m, 2H, CH_2O); 3.85 (s, 3H, OCH_3); 3.50 (s br, 1H, OH); 3.12–2.96 (m, 4H, 2 CH_2 piperaz.); 2.92–2.84 (m, 2H, CH_2N); 2.68–2.56 (m, 4H, 2 CH_2 piperaz.); 1.61 (s, 6H, 2 CH_3). Crystallized from ether as a white solid (85%). M.p. 144–46 °C. MS (EI) *m/z*: 442 $[M + 1]^+$. $[\alpha]_D^{20} = -13.0^\circ$ (*c* = 0.009; methanol).

*3.1.6. Representative procedure for obtaining compounds [**XIII**], [**XIV**], [**XV**] and [**XVI**]*

Compounds [**IX**], [**X**], [**XI**] and [**XII**] were dissolved in the least quantity of dioxan, added of a double volume of water/acetic acid (1/3) and the solution heated at reflux for 24 h while stirring. After cooling, the reaction mixture was diluted with water, neutralised by sodium carbonate and extracted with chloroform; the organic layer was dried on sodium sulphate anhydrous, filtered and finally evaporated obtaining a raw material, purified by flash chromatography with $CHCl_3$/MeOH (45/5) as eluent.

*3.1.6.1. (S)-(–)-2-hydroxy-5-{2-hydroxy-3-[2-(3,4-dimethoxyphenyl)ethylamino]propoxy}benzamide [**XIII**].* ^{1}H NMR (200.13 MHz): ($CDCl_3$) δ 7.45 (s br, 2H, NH_2); 7.38 (d, 1H, Ar, *J* = 2.87 Hz); 7.08 (s br, 1H, ArOH); 7.00 (dd, 1H, Ar, *J* = 2.86 Hz, *J* = 9.00 Hz); 6.88–6.70 (m, 4H, Ar); 4.31–4.03 (m, 1H, C*H*OH); 4.00–3.83 (m, 2H, OCH_2); 3.74 (s, 3H, OCH_3); 3.72 (s, 3H, OCH_3); 3.32–3.19 (m, 2H, $NHCH_2CH_2$); 3.13–2.73 (m, 4H, $CHCH_2NH$ and CH_2Ar). Crystallized from MeOH/diethylether as a white solid (30%). M.p. 84–86 °C. MS (EI) *m/z*: 413 $[M + Na]^+$, 391 $[M + 1]^+$. $[\alpha]_D^{20} = -10.0^\circ$ (*c* = 0.014; methanol).

*3.1.6.2. (R)-(+)-2-hydroxy-5-{2-hydroxy-3-[2-(3,4-dimethoxyphenyl)ethylamino]propoxy}benzamide [**XIV**].* ^{1}H NMR (200.13 MHz): ($CDCl_3$) δ 7.62 (s br, 2H, NH_2); 7.38 (d, 1H, Ar, J = 2.87 Hz); 7.08 (s br, 1H, ArOH); 7.00 (dd, 1H, Ar, J = 2.94 Hz, J = 8.88 Hz); 6.86–6.69 (m, 4H, Ar); 4.32–4.23 (m, 1H, C*H*OH); 4.09–3.89 (m, 2H, OCH_2); 3.74 (s, 3H, OCH_3); 3.73 (s, 3H, OCH_3); 3.40–3.36 (m, 2H, NHCH_2CH_2); 3.12–2.82 (m, 4H, CHCH_2NH and CH_2Ar). Crystallized from MeOH/diethylether as a white solid (35%). M.p. 84–86 °C. MS (EI) *m/z*: 413 [M + Na]$^+$, 391 [M + 1]$^+$. $[\alpha]_D^{20}$ = + 10.0° (c = 0.005; methanol).

*3.1.6.3. (S)-(+)-2-hydroxy-5-{2-hydroxy-3-[4-(2-methoxyphenyl)-1-piperazinyl]propoxy}benzamide [**XV**].* ^{1}H NMR (200.13 MHz): ($CDCl_3$) δ 7.13 (d, 1H, Ar, J = 2.71 Hz); 7.04–6.82 (m, 6H, Ar); 6.71 (s br, 3H, $CONH_2$ and OH); 4.23–4.17 (m, 1H, C*H*OH); 3.94 (d, 2H, OCH_2, J = 4.87 Hz); 3.83 (s, 3H, OCH_3); 3.14–3.10 (m, 4H, 2 CH_2 piperaz.); 3.04–2.97 (m, 2H, CH_2N); 2.88–2.64 (m, 4H, 2 CH_2 piperaz.). White solid (85%). M.p. 155–57 °C. MS (EI) *m/z*: 402 [M + 1]$^+$. $[\alpha]_D^{20}$ = + 10.0° (c = 0.05; methanol).

*3.1.6.4. (R)-(–)-2-hydroxy-5-{2-hydroxy-3-[4-(2-methoxyphenyl)-1-piperazinyl]propoxy}benzamide [**XVI**].* ^{1}H NMR (200.13 MHz): ($CDCl_3$) δ 7.13 (d, 1H, Ar, J = 2.69 Hz); 7.05–6.84 (m, 6H, Ar); 5.56 (s br, 3H, $CONH_2$ and OH); 4.24–4.20 (m, 1H, C*H*OH); 3.97 (d, 2H, OCH_2, J = 4.91 Hz); 3.85 (s, 3H, OCH_3); 3.20–3.17 (m, 4H, 2 CH_2 piperaz.); 3.08–3.01 (m, 2H, CH_2N); 2.93–2.74 (m, 4H, 2 CH_2 piperaz.). White solid (50%). M.p. 155–57 °C. MS (EI) *m/z*: 402 [M + 1]$^+$. $[\alpha]_D^{20}$ = − 10.0° (c = 0.02; methanol).

3.2. Pharmacology

The animal protocols used were reviewed and approved by the Animal Care and Ethics Committee of the Università degli Studi di Siena, Italy.

3.2.1. Measurement of contractile force in isolated rat left atria

Experiments were performed following the method described by Doggrell [11] with slight modifications.

Male Wistar rats (250–350 g) were anaesthetised with a mixture of Ketavert® (Gellini, Italy) and Rompum® (Bayer, Germany), sacrificed and the hearts were quickly removed and rinsed in ice-cold Tyrode's solution. Then, left atria were dissected and mounted at 0.5 g resting tension on stainless steel hooks in a 50 ml organ bath, and bathed at 37 °C in physiological saline solution containing (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24, glucose 11. The bath was aerated with 95% O_2 and 5% CO_2 mixture. One end of the preparation was fixed to the bottom of the bath and the other end was connected by a hook to the level of a force–displacement transducer (Ugo Basile, Comero, Italy). Tissues were electrically stimulated at 1 Hz, 5 ms duration, via two platinum electrodes placed on both sides of the muscle and were always allowed to equilibrate for 90 min before the experiments were begun. After 10 min of stimulation a cumulative challenge with (1×10^{-10} to 1×10^{-6}) isoprenaline was made. This procedure was repeated three times, after that a dose of antagonist was added to the organ bath, equilibrated for 5 min and the isoprenaline cumulative curves were repeated.

3.2.2. Measurement of contractile force in isolated rat aorta

Experiments were performed following the method described previously [12].

Male Wistar rats (250–350 g) were anaesthetised with a mixture of Ketavert® (Gellini, Italy) and Rompum® (Bayer, Germany), sacrificed and aorta was isolated, immediately removed, cleaned of connective tissue, and cut into 1.5 mm rings. Each arterial ring was mounted over two rigid parallel stainless-steel tubes, one fixed in place and the other attached to an isometric transducer (Basile, Varese, Italy). The preparation was immersed in a water-jacketed organ bath (37 °C), containing 5 ml of a modified Krebs–Henseleit physiological salt solution (PSS) (composition, mM: NaCl, 124; KCl, 4; $CaCl_2$, 1.8; $MgCl_2$, 1.1; KH_2PO_4, 0.4; $NaHCO_3$, 25; glucose, 5.5) bubbled with a 95% O_2 5% CO_2 gas mixture to give a pH of 7.4.

The vessel segments were allowed to equilibrate for 1 h at a resting tension of 1 g. Under these conditions maximal plateau levels of active tension of about 400 mg were obtained following full depolarization with 80 mM KCl or 10^{-6} M phenylephrine (EC_{90}). The α_1-antagonism were assayed by preincubating for 5 min the vessel preparations in presence of novel compounds and measuring the response promoted by 10^{-6} M phenylephrine.

4. Results and conclusion

In the present study cardiac electrical stimulation responses were not significantly altered by the compounds used at different concentration (1×10^{-8} to 5×10^{-6}). On the contrary, the inotropic response of isoprenaline was inhibited at different extent by the tested compounds. The highest inhibition was observed when **VIc** compound was present in the perfusion solution. The compound inhibited the 10^{-6} M isoprenaline left atria contractions with an IC_{50} value five times higher than that observed with labetalol. The compound promoted a parallel rightward shift of isoprenaline response curve and no effect on the maximum response of the adrenergic compound. Furthermore **VIc** counteracted also the contractions of rat aorta rings promoted by 10^{-6} M α-agonist phenylephrine (Table 3).

A similar behaviour on left atria was observed by **VId,** which inhibited the contractions promoted by isoprenaline in competitive manner with an IC_{50} value one order of magnitude greater to that observed with labetalol. However, this compound did not present α-adrenergic antagonism. The other

Table 4
In vitro effects of labetalol and enantiomeric compounds

Compound	β_1-Adrenergic antagonism activity IC_{50} (M)	α_1-Adrenergic antagonism activity IC_{50} (M)
Labetalol	$8 \times 10^{-7} \pm 0.5 \times 10^{-7}$	$5 \times 10^{-6} \pm 0.2 \times 10^{-6}$
XIII [(*S*)-(–)-VIb]	$3.5 \times 10^{-6} \pm 0.9 \times 10^{-6}$	N.d.[a]
XIV [(*R*)-(+)-VIb]	N.d.[a]	$1.2 \times 10^{-8} \pm 0.7 \times 10^{-8}$
XV [(*S*)-(+)-VIc]	N.d.[a]	$4.6 \times 10^{-7} \pm 0.7 \times 10^{-7}$
XVI [(*R*)-(–)-VIc]	$5.3 \times 10^{-6} \pm 0.8 \times 10^{-6}$	$3.6 \times 10^{-7} \pm 0.7 \times 10^{-7}$

The IC_{50} values are reported as mean ± SEM of three different experiments.
[a] Not detectable.

two compounds, namely **VIa** and **VIb**, affected the isoprenaline response in non-competitive manner, with similar IC_{50} values. However, **VIb** presented a marked inhibition of phenylephrine promoted contractions on aorta rings, while **VIa** had not activity.

VIe did not counteracted the isoprenaline response on the isolated atria, while presented a marked inhibition of the phenylephrine response on vascular preparations.

Since compounds **VIb** and **VIc** seemed the most interesting derivatives maintaining an acceptable β_1-adrenoceptor blocking activity with an enhanced affinity for α_1-adrenoceptors, we have synthesised their enantiomers, compounds **XIII** [(*S*)-(–)-VIb], **XIV** [(*R*)-(+)-VIb], **XV** [(*S*)-(+)-VIc] and **XVI** [(*R*)-(–)-VIc]. These pure enantiomers were assayed, as the racemic compounds, for their α_1- and β_1-blocking activity and results were reported in Table 4.

Only compounds **XVI** [(*R*)-(–)-VIc] and **XIII** [(*S*)-(–)-VIb] have been shown a β_1-antagonist activity with a IC_{50} comparable to that observed with the respective racemic compounds. On the contrary the α_1-antagonist activity has been still observed in both pure enantiomers of **VIc**, while only **XIV** [(*R*)-(+)-VIb] was able to inhibit the phenylephrine promoted contractions on aorta rings.

In conclusion, replacement of the ethanol structure with the oxypropanol moiety into compound **VIa**, the labetalol analog, had determined an unexpected decrease of the β_1-blocking activity. Moreover, this compound is also devoid of any α_1-blocking activity. On the contrary replacement of 1-methyl-3-phenylpropylamine with other amines had led, with the exception of compound **VId**, to an increase of the α_1-blocking activity.

Results of these tests on the four enantiomers showed that only compound **XVI** [(*R*)-(–)-VIc] retain both antagonistic activity on α_1- and β_1-adrenoreceptors and then may be a good candidate for an in vivo study of antihypertensive activity.

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