Arch. Pharm. Chem. Life Sci. 2016, *349*, 1–8

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Full Paper

Synthesis and *In Vitro* Pharmacological Evaluation of 5-(Alkoxymethyl)-2-(3-alkylamino-2-hydroxypropoxy)-phenylethanones Related to Acebutolol and Celiprolol

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The structure–activity relationships of 13 analogs of aryloxyaminopropanol type derived from 2-hydroxyphenylethanone as potential β-blockers are described. The synthesized compounds possess an isopropyl or a *tert*-butyl group in the hydrophilic part of the molecule and an alkoxymethyl substitution in the lipophilic moiety. The target compounds were prepared by an established four-step method and their structures were confirmed by interpretation of their UV, IR, ^{1}H NMR and ^{13}C NMR spectra, and by elemental analysis. The β-adrenolytic efficacy of the prepared racemic compounds was determined on isolated guinea pig atria (β_1) and trachea (β_2) and expressed as pA_2 values against isoprenaline tachycardia. The assumed cardioselectivity was expressed as β_1/β_2 ratio and the values of compounds with an alkoxy group (CH_3O, iC_3H_5O, $C_5H_{11}O$, $CH_2{=}CHCH_2O$, $CH_3OCH_2CH_2O$) in the lipophilic part and with *tert*-butyl in the hydrophilic part of the molecule were found to be comparable or higher than those of the standards acebutolol and celiprolol. All evaluated substances at a concentration of 10^{-7} mol/dm^3 showed also negative chronotropic effects.

Keywords: 2-Hydroxyphenylethanone / Cardioselectivity / Isolated atria / Isolated trachea

Received: May 6, 2016; Revised: June 8, 2016; Accepted: June 22, 2016

DOI 10.1002/ardp.201600136

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Introduction

β-Adrenergic blocking agents (β-blockers) have been well established in the therapy of various cardiovascular disorders for more than four decades [1]. These agents also have established therapeutic benefits beyond the original cardiovascular application, namely in the treatment of glaucoma [2], anxiety [3], thyreotoxicosis [4], prophylaxis of migraine [5], hemangioma [6], and osteoporosis [7]. With respect to their clinical utility, the classification of β-blockers is usually based on their selectivity for different subtypes of the β-adrenoceptor. While nonselective β-blockers (propranolol, pindolol) antagonize both β_1- and β_2-adrenoreceptors, selective drugs, i.e., acebutolol, celiprolol, and metoprolol, have a much higher binding affinity for β_1-adrenoceptors. Selective β_1-blockers are preferred for patients in whom β_2-adrenoceptor antagonism may be associated with an increased risk of adverse effects (asthma, diabetes) [8–10].

The developmental history of β-adrenoceptor blockers confirms that the pharmacological activity of these compounds is based on an essential aryloxyaminopropanol structure [11, 12]. Numerous derivatives have been synthesized and the basic relationship between their chemical structure and biological activity has been established [13, 14]. Our previous studies demonstrated that cardioselectivity can be conferred to aryloxyaminopropanols by appropriate

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substitution in the 4-position of the phenyl ring or on the amino nitrogen [15–17]. In recent years, a number of selected compounds have been evaluated for their basic β-adrenolytic activities [18–21]. The pharmacological classification of antagonists as selective is usually based on comparison of their pA_2 values in two tissues considered to possess β_1- or β_2-adrenergic receptors. For this purpose, guinea pig atria and trachea are frequently used. The obtained values show that most of the compounds are very effective and their pharmacological activity is similar to clinically used β-blockers, i.e., acebutolol and celiprolol which have also acyl group. A fraction of derivatives of 4-hydroxyacetophenones have also been evaluated for their antidysrhythmic efficacy in guinea pigs against ouabain-, calcium-, and barium-induced heart rate disturbances [22–24].

This study describes the synthesis of 13 new derivatives of aryloxyaminopropanol with an acyl group in the 2-position next to the aminopropanol group. The synthesized compounds possess isopropyl or *tert*-butyl group in the hydrophilic part of the molecule and alkoxymethyl substitution in the lipophilic moiety. The antiisoprenaline activity of these newly synthesized derivatives was studied on isolated guinea pig atria and trachea. The results were expressed as pA_2 values and the cardioselectivity ratio was calculated as β_1/β_2.

Results and discussion

Chemistry

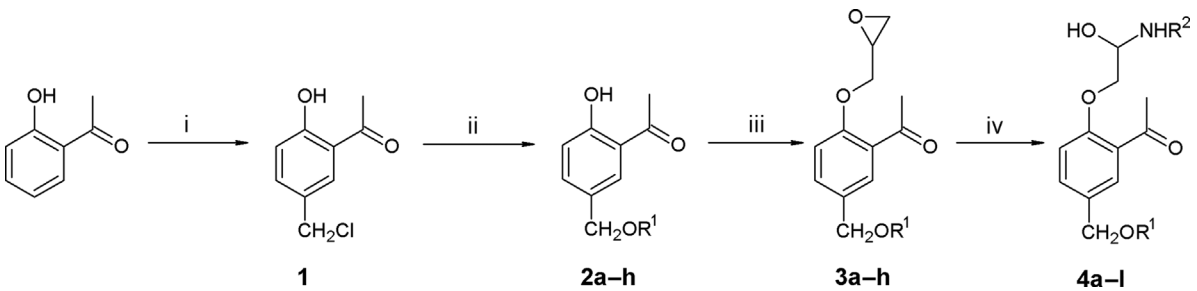
The compounds were prepared by a four-step synthesis using 2-hydroxyphenylethanone as the starting material (Scheme 1). In the first step (i), the chloromethyl derivative (**1**) was prepared by the reaction of the starting ketone with concentrated HCl and paraformaldehyde according to the published procedure [25]. In the second step (ii), alkoxymethylderivatives (**2a–h**) were obtained by reaction of the chloromethyl intermediate with the appropriate alcohol in the presence of $NaHCO_3$ [26]. The most difficult step, which is common in the syntheses of various β-blockers, is the reaction with chloromethyloxirane. This reaction can be performed in water [27] or in the excess of chloromethyloxirane [18]. In this case, the oxirane derivatives (**3a–h**) were prepared in the third step (iii) by reaction of the alkoxymethylderivatives with excess of chloromethyloxirane in the nitrogen atmosphere to obtain better yield (iii). The final bases (**4a–l**) were prepared in the final fourth step (iv) by the reaction of oxirane derivatives with *tert*-butylamine or isopropylamine. The compound **4** was prepared by the same synthetic route except for the first and second steps which were omitted because of absence of alkoxymethyl substituent. For the pharmacological investigation, fumarate and oxalate salts were prepared and used. The general synthetic route to the target compounds is shown in Scheme 1, the list of prepared structures in Table 1.

Pharmacological activity

The structure–activity relationship of prepared compounds was evaluated on the basis of basic pharmacological evaluation *in vitro*. The basic pharmacological property of all β-blockers is negative chronotropic effect or antiisoprenaline activity. The evaluated compounds at a concentration 10^{-7} mol/dm^3 decreased heart frequency of spontaneous beating isolated guinea pigs atria and this effect was increasing during next 20 min after application (Tables 2 and 3). The derivatives with *tert*-butylamino group were compared with celiprolol, since it also contains *tert*-butylamino group, and the derivatives with isopropyl group were compared with acebutolol.

The capacity of evaluated compounds to influence β_2-adrenergic receptors of tracheal smooth muscle was tested on histamine-contracted isolated guinea pig trachea. The relaxation of tracheal smooth muscle was expressed as pA_2 for the β_2-adrenergic receptor (Tables 4 and 5).

To determine the selectivity of β-blockers, it was necessary to compare their effects in tissues bearing two basic subtypes of β-adrenoceptors. The most suitable way to examine β_1- and β_2-adrenergic effects was to study the inhibition of the positive chronotropic effect of isoprenaline on the isolated atria and on the relaxation of the tracheal smooth muscle of



1 **2a–h** **3a–h** **4a–l**

Scheme 1. Synthesis of the target compounds. Reagents and conditions: (i) 36% HCl, $(CH_2O)_n.nH_2O$, 30°C, 7 h; yield 69% (ii) appropriate alcohol, $NaHCO_3$, 4 h room temp., 30–40°C, 4 h; yield 60–83% (iii) chloromethyloxirane, 85% KOH, N_2, 50–55°C, 4 h; yield 64–84% (iv) EtOH $(CH_3)_3CNH_2$ or $(CH_3)_2CHNH_2$, reflux, 4–5 h, yield 40–84%.

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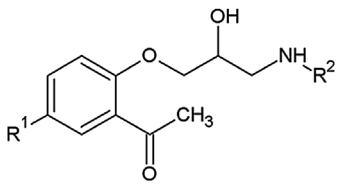
Table 1. The prepared compounds.



Compound	R^1	R^2
4	–	$-C(CH_3)_3$
4a	$-CH_2OCH_3$	$-C(CH_3)_3$
4b	$-CH_2OCH_3$	$-CH(CH_3)_2$
4c	$-CH_2OC_2H_5$	$-C(CH_3)_3$
4d	$-CH_2OC_2H_5$	$-CH(CH_3)_2$
4e	$-CH_2OC_3H_7$	$-C(CH_3)_3$
4f	$-CH_2OC_3H_7$	$-CH(CH_3)_2$
4g	$-CH_2Oi C_3H_7$	$-C(CH_3)_3$
4h	$-CH_2OC_4H_9$	$-C(CH_3)_3$
4i	$-CH_2OC_5H_{11}$	$-C(CH_3)_3$
4j	$-CH_2OC_5H_{11}$	$-CH(CH_3)_2$
4k	$-CH_2OC_2H_4OCH_3$	$-C(CH_3)_3$
4l	$-CH_2OCH_2CH=CH_2$	$-C(CH_3)_3$

the guinea pig [27]. Cardioselectivity is a very important pharmacological property of β-blockers in clinical use due to better therapeutic utilization and elimination of side effects.

From the point of view of structure–activity relationship, the substitution on the aromatic ring affects specific antiisoprenaline activity and cardioselectivity. Generally, the antiisoprenaline activity of compounds bearing *tert*-butyl moiety is higher than in the group of compounds bearing isopropyl moiety. In the group of compounds with *tert*-butyl moiety, antiisoprenaline activity depends on the type and length of alkoxymethyl chain as follows:

methoxyethoxymethyl < allyloxymethyl < pentyloxymethyl < isopropyloxymethyl < butyloxymethyl < propoxymethyl < ethoxymethyl < methoxymethyl

Higher number of carbon atoms in alkoxymethyl chain and isosteric replacement by oxygen decrease antiisoprenaline activity. The compounds with longer alkoxymethyl chain and *tert*-butyl moiety have lower antiisoprenaline activity but their cardioselectivity rises in comparison with standard celiprolol. The results show that the cardioselectivity of the *tert*-butyl derivatives decreases from the methoxymethyl (**4a**) to propoxymethyl (**4e**) substitution from 13.8 to 2.10. With increasing length or branching of the alkoxymethyl chain or replacement of the methylene group by oxygen, cardioselectivity increases up to 13.5. In the case of compound **4** without alkoxymethyl chain there was no cardioselectivity.

The antiisoprenaline activity of derivatives related to acebutolol with *N*-isopropyl moiety was nearly ten times lower than antiisoprenaline activity of *tert*-butyl derivatives but within the same group the activity was very similar for all compounds. Structure–activity relationship suggests that antiisoprenaline activity is determined particularly by the isopropyl moiety while cardioselectivity depends on the type and length of alkoxymethyl chain. The most cardioselective compound seems to be **4f** bearing propoxymethyl chain, the value expressed cardioselectivity was identical with acebutolol.

Conclusion

The aim of this work was the study of the relationship between structure and activity of 13 racemic analogs as potential β-blockers of the aryloxyaminopropanol type

Table 2. Negative chronotropic effect in spontaneously beating isolated guinea pig atria of the evaluated compounds bearing a *tert*-butyl moiety at a concentration of 10^{-7} mol/dm^3.

Compound	Heart rate (%)			
	5th min	10th min	15th min	20th min
4	94.2 ± 1.8	90.7 ± 2.6	85.8 ± 3.1	82.3 ± 3.3
4a	92.9 ± 1.7	85.8 ± 0.4	83.3 ± 3.1	82.2 ± 2.7
4c	88.8 ± 3.7	85.8 ± 2.0	84.4 ± 3.2	84.0 ± 2.7
4e	91.4 ± 3.2	86.3 ± 3.2	83.9 ± 2.7	80.6 ± 2.5
4g	92.6 ± 2.7	89.6 ± 2.9	86.4 ± 3.0	84.7 ± 4.1
4h	98.3 ± 0.7	94.9 ± 0.8	91.8 ± 1.1	90.1 ± 1.4
4i	96.9 ± 2.2	92.3 ± 1.8	87.8 ± 3.2	85.9 ± 2.5
4k	96.9 ± 1.0	92.2 ± 1.4	89.8 ± 1.4	88.0 ± 1.8
4l	88.6 ± 5.1	86.8 ± 5.3	81.5 ± 4.3	78.1 ± 4.2
Saline	101.8 ± 0.4	101.2 ± 0.5	100.8 ± 0.4	100.6 ± 0.4
Celiprolol	98.5 ± 10	95.4 ± 1.1	90.6 ± 1.3	86.6 ± 0.9

Each value represents the mean ± SEM from 5 to 7 experiments.

Table 3. Negative chronotropic effect in spontaneously beating isolated guinea pig atria of evaluated compounds bearing isopropylamine moiety at a concentration of 10^{-7} mol/dm^3.

Compound	Heart rate (%)			
	5th min	10th min	15th min	20th min
4b	96.4 ± 1.4	94.2 ± 1.1	91.6 ± 1.2	90.7 ± 1.3
4d	98.3 ± 1.9	95.2 ± 1.7	93.5 ± 1.6	92.2 ± 1.7
4f	97.1 ± 1.1	95.0 ± 2.8	92.8 ± 2.0	92.0 ± 1.7
4j	99.1 ± 0.9	97.0 ± 1.1	94.9 ± 1.3	94.9 ± 1.3
Saline	101.8 ± 0.4	101.2 ± 0.5	100.8 ± 0.4	100.6 ± 0.4
Acebutolol	96.8 ± 0.8	95.0 ± 1.1	88.3 ± 2.5	84.5 ± 1.8

Each value represents the mean ± SEM from 6 to 7 experiments.

derived from 2-hydroxyphenylethanone. The β-antiadrenergic activity of synthesized compounds was tested on isolated guinea pig atria (β_1) and trachea (β_2) and expressed as pA_2 values against tachycardia induced by isoprenaline. The cardioselectivity was expressed as β_1/β_2 ratio. Compounds containing *tert*-butylamino group in the hydrophilic part of molecule and with an alkoxy group (CH_3O, iC_3H_5O, $C_5H_{11}O$, $CH_2{=}CHCH_2O$, $CH_3OCH_2CH_2O$) on the aromatic ring show higher cardioselectivity than standard celiprolol. Compounds with isopropylamino group show lower activity than standard acebutolol.

Experimental

Chemistry

All reactions were carried out using commercial grade reagents and solvents. Diethylether was dried by refluxing over potassium hydroxide and sodium followed by distillation. The purity of the newly prepared compounds was assessed using TLC silica gel plates UV 254 (Merck) and the solvent system of ethyl acetate/diethylamine (9:1 v/v) was used. The melting point was determined using a Kofler hot stage microscope and was quoted uncorrected.

Elemental analysis was performed using a Flash 2000 Organic Elemental Analyzer (Thermo Scientific). Ultraviolet spectra were recorded on a Hewlett-Packard 8452 spectrophotometer. IR spectra were recorded using an IMPACT 400D (Nicolet) FTIR spectrophotometer in KBr. NMR spectra were recorded with Varian Gemini 2000 spectrometer, 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR, using $Si(CH_3)_4$ as the reference. Chemical shifts are reported in ppm (δ). In reporting the NMR multiplicities, we used the following abbreviations: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet.

The InChI codes of the investigated compounds are provided as Supporting Information.

*General procedure for the preparation of [5-(chloromethyl)-2-(hydroxy)phenyl]ethanone (**1**)*

To the mixture of (2-hydroxyphenyl)ethanone (24 g, 20 mmol), 150 mL of concentrated hydrochloric acid and paraformaldehyde (6 g, 20 mmol) were added during 30 min at 30°C. The mixture was kept at 30°C for 7 h under stirring. Subsequently, the mixture was poured into 2000 mL of cold water and filtered.

Table 4. pA_2 values in atria and trachea of guinea pigs and β_1/β_2 selectivity ratio calculated as the antilog (pA_2 β_1–pA_2 β_2) of derivatives bearing *tert*-butyl moiety at a concentration of 10^{-7} mol/dm^3.

Compound	pA_2 β_1 (atria)	pA_2 β_2 (trachea)	β_1/β_2
4	8.16 ± 0.21	8.72 ± 0.15	0.3
4a	9.01 ± 0.11	7.01 ± 0.13	13.8
4c	8.82 ± 0.34	8.04 ± 0.21	6.0
4e	8.47 ± 0.22	8.15 ± 0.11	2.1
4g	8.22 ± 0.10	7.41 ± 0.07	10.2
4h	8.36 ± 0.17	7.79 ± 0.44	3.7
4i	8.21 ± 0.21	7.20 ± 0.12	10.2
4k	7.94 ± 0.19	6.81 ± 0.26	13.5
4l	8.04 ± 0.28	7.01 ± 0.17	11.2
Celiprolol	8.62 ± 0.11	7.84 ± 0.10	6.00

Table 5. pA_2 values in atria and trachea of guinea pigs and β_1/β_2 selectivity ratios calculated as antilog (pA_2 β_1–pA_2 β_2) of derivatives bearing isopropylamine moiety at a concentration of 10^{-7} mol/dm^3.

Compound	pA_2 β_1 (atria)	pA_2 β_2 (trachea)	β_1/β_2
4b	7.73 ± 0.17	7.05 ± 1.63	4.8
4d	7.99 ± 0.07	7.15 ± 0.10	6.9
4f	7.96 ± 0.38	7.01 ± 0.10	9.3
4j	7.73 ± 0.23	7.97 ± 0.30	0.6
Acebutolol	7.55 ± 0.66	6.58 ± 0.24	9.3

The remaining substance was crystallized from methanol to afford yellow solid. $C_9H_9ClO_2$, yield 16.6 g (69%). Mp 90–92°C. ^{1}H NMR ($CDCl_3$): δ 2.65 (s, 3H, COCH_3); 4.57 (s, 2H, ClCH_2); 6.99–7.02 (d $J = 9$ Hz, 1H, Ar^3*H*); 7.47–7.50 (d $J = 9$ Hz, 1H, Ar^4*H*); 7.73 (s,1H, Ar^6*H*); 12.31 (s, 1H, O*H*). ^{13}C NMR ($CDCl_3$): δ 29.7, 45.7, 128.1, 130.8, 131.1, 136.8, 137.0, 162.4, 204.1.

General procedure for the preparation of [(5-(alkoxymethyl)-2-(hydroxyphenyl)]ethanones ***2a–h***

$NaHCO_3$ (24 g, 290 mmol) was added continually to the mixture of the chloromethyl derivative (26.7 g, 145 mmol) and 150 mL of appropriate alcohol. The mixture was stirred for 4 h at room temperature and then for an additional 4 h at 30–40°C. After filtration of the mixture, the remaining alcohol was removed under reduced pressure. Distilled water (50 mL) was added and the residue was extracted three times with diethylether (3 × 100 mL). The combined organic layers were dried with anhydrous $MgSO_4$ and evaporated to dryness. The product was used in the next reaction step without any further purification. Yields 60–83%, **2a**: ^{1}H NMR ($CDCl_3$): δ 2.55 (s, 3H, COCH_3); 3.50 (s, 3H, OCH_3); 4.30 (s, 2H, Ar-CH_2-O-); 6.90–6.93 (d $J = 9$ Hz, 1H, Ar^3*H*); 7.11–7.15 (d $J = 12$ Hz, 1H, Ar^4*H*); 7.50 (s, 1H, Ar^6*H*); 12.25 (s, 1H, ArO*H*).

General procedure for the preparation of [(5-(alkoxymethyl)-2-(oxirane-2-ylmethoxy)phenyl]ethanones ***3a–h***

To a stirred solution of the appropriate substituted phenol (150 mmol) in 3 mol of (±)-chloromethyloxirane 170 mmol of 85% KOH was added. The mixture was stirred at 50–55°C under nitrogen for 4 h. The inorganic salts were filtered off and the (±)-chloromethyloxirane was distilled off under reduced pressure. Distilled water (50 mL) was added and the residue was extracted three times with diethylether (3 × 100 mL). The organic phase was separated and dried with anhydrous Na_2SO_4. The filtered solution was concentrated under reduced pressure and the remaining oil was used without previous purification in the next reaction step. Yields: 64–84%. **3a**: ^{1}H NMR ($CDCl_3$): δ 2.54 (s, 3H, COCH_3); 2.76–2.79 (d $J = 9$ Hz, 1H, C$H_2^{oxirane}$); 2.94–2.98 (t, 1H, C$H_2^{oxirane}$); 3.03–3.08 (m, 1H, C$H^{oxirane}$); 3.32 (s, 3H, OCH_3); 3.98–4.01 (d $J = 9$ Hz, 1H, CH_2CH); 4.07–4.10 (d $J = 9$ Hz, 1H, CH_2CH); 4.51 (s, 2H, ArCH_2); 6.96–6.99 (d $J = 9$ Hz, 1H, Ar^3*H*); 7.42–7.47 (t, 1H, Ar^4*H*); 7.63–7.66 (d $J = 9$ Hz, 1H, Ar^6*H*).

General procedure for the preparation of [(5-(alkoxymethyl)-2-(2-hydroxy-3-alkylaminopropoxy)phenyl]ethanones ***4a–l***

The solution of the oxirane derivative (80 mmol) and *tert*-butylamine (180 mmol) or isopropylamine (180 mmol) in ethanol was kept at 30°C for 2 h and then was heated for additional 5 h at reflux temperature. The solvent was distilled off. To the residue distilled water (50 mL) was added and the residue was washed three times with diethylether (3 × 100 mL). The combined organic layers were washed with brine (100 mL), then with water, separated and dried with K_2CO_3. The filtered solution was concentrated under reduced pressure and the remaining oil was crystallized from *n*-hexane to afford the final base. The salts were prepared by quantitative reaction of anhydrous ether solution of base and anhydrous ether solution of fumaric acid. The crystals were collected by filtration and recrystallized from ethyl acetate to give fumarate salt as a white solid.

*[2-(3-tert-Butylamino)-2-hydroxypropoxy-5-(methoxymethyl)phenyl]ethanone (****4a****)*

Yield: 70%, mp: 83–85°C. IR (cm^{-1}): 3200 (ν, OH, NH), 1661 (ν, C=O), 1594 (ν, C=Car.), 1296 (ν, C-O-Car.), 763 (δ, Ar). ^{1}H NMR ($CDCl_3$) δ: 1.12 (s, 9H, C(CH_3)$_3$); 2.63 (s, 3H, COCH_3); 2.70–2.90 (m, 2H, CH_2N); 3.36 (s, 3H, OCH_3); 3.96–4.01 (m, 2H, ArOCH_2); 4.08–4.11 (m, 1H, C*H*OH); 4.44 (s, 2H, ArCH_2); 6.98–7,01 (d $J = 9$ Hz, 1H, Ar^3*H*); 7.44–7.49 (d $J = 15$ Hz, 1H, Ar^4*H*); 7.71 (s, 1H, Ar^6*H*). ^{13}C NMR ($CDCl_3$) δ: 22.9, 26.7, 49.3, 52.7, 59.0, 69.2, 73.0, 73.3, 117.2, 128.4, 129.9, 130.2, 133.7, 162.0, 199.6. UV (MeOH) λ_{max} 306 (ε 363), 246 (ε 812), 214 (ε 2398). Anal. calcd. for $C_{34}H_{54}N_2O_8.C_4H_4O_4$, Mr 734.9; % C 62.11, % H 7.96, % N 3.81. Found % C 61.91, % H 7.86, % N 3.61.

*[2-(2-Hydroxy-3-(isopropylamino)propoxy)-5-(methoxymethyl)phenyl]ethanone (****4b****), oxalate salt*

Yield: 77%, mp: 94–97°C. IR (cm^{-1}): 3308 (ν, OH, NH), 1675 (ν, C=O), 1574 (ν, C=Car.), 1295 (ν, C-O-Car.), 793 (δ, Ar). ^{1}H NMR (CD_3OD) δ 1.37–1.40 (m, 6H, (CH_3)$_2$), 2.64 (s, 3H, COCH_3), 3.19–3.26 (m, 2H, CH_2N), 3.42 (s, 3H, OCH_3), 3.47–3.52 (m, 1H, C*H*(CH_3)$_2$), 4.16–4.25 (m, 2H, ArOCH_2), 4.31–4.35 (m, 1H, C*H*OH), 4.57 (s, 2H, ArCH_2), 7.12–7.15 (m, 1H, Ar^3*H*), 7.50–7.54 (m, 1H, Ar^4*H*), 7.73 (m, 1H, Ar^6*H*). ^{13}C NMR (CD_3OD): δ 17.36, 29.97, 50.73, 56.87, 57.12, 68.92, 73.21, 77.14, 112.83, 124.10, 127.07, 130.51, 134.22, 157.19, 200.35. UV (MeOH) λ_{max} 306 (ε 524), 246 (ε 1412), 212 (ε 4897). Anal. calcd. for $C_{34}H_{54}N_2O_8.C_2H_2O_4$, Mr 708.9; % C 61.00, % H 7.96, % N 3.96. Found % C 61.21, % H 7.96, % N 3.71.

*[2-(3-tert-Butylamino)-2-hydroxypropoxy)-5-(ethoxymethyl)-phenyl]ethanone (****4c****)*

Yield: 64%, mp: 97–100°C. IR (cm^{-1}): 3400 (ν, OH, NH), 1660 (ν, C=O), 1594 (ν, C=Car.), 1298 (ν, C-O-Car.), 875 (δ, Ar). ^{1}H NMR ($CDCl_3$) δ 1.12–1.17 (t, 3H, CH_2CH_3); 1.25 (s, 9H, (CH_3)$_3$); 2.64 (s, 3H, COCH_3); 2.70–2.90 (m, 2H, CH_2N); 3.57–3.66 (q, 2H, CH_2CH$_3$); 3.97–4.01 (m, 2H, CH_2CH); 4.09–4.12 (m, 1H, C*H*OH); 4.45 (s, 2H, ArCH_2); 6.99–7.03 (d $J = 12$ Hz, 1H, Ar^3*H*); 7.46–7,48 (d $J = 6$ Hz, 1H, Ar^4*H*); 7.73 (s, 1H, Ar^6*H*). UV (MeOH) λ_{max} 306 (ε 363), 246 (ε 1000), 214 (ε 2884). Anal. calcd. for $C_{36}H_{58}N_2O_8$. $C_4H_4O_4$, Mr 762.9; % C 62.99, % H 8.14, % N 3.69. Found % C 63.11, % H 7.96, % N 3.81.

*[(5-(Ethoxymethyl)-2-(2-hydroxy-3-isopropylaminopropoxy)-phenyl]ethanone (****4d****)*

Yield: 74%, mp: 80–82°C. IR (cm^{-1}): 3414 (ν, OH, NH), 1674 (ν, C=O), 1578 (ν, C=Car.), 1357 (ν, C-O-Car.), 720 (δ, Ar). ^{1}H NMR (fumarate) (CD_3OD): δ 1.15–1.24 (m, 3H,CH_2CH_3), 1.34–1.37 (d $J = 9$ Hz, 6H, (CH_3)$_2$), 2.64 (s, 3H, COCH_3), 3.13–3.20 (m, 2H, CH_2N), 3.24–3.26 (m, 1H, C*H*(CH$_3$)$_2$), 3.52–3.59 (m, 2H, CH_2CH$_3$), 4.17–4.21 (m, 2H, CH_2CH), 4.24–4.30 (m, 1H, C*H*OH), 4.47 (s, 2H,

Ar-CH_2), 6.65 (s, 2H, fum), 7.12–7.15 (d $J = 9$ Hz, 1H, Ar^3H), 7.51–5.54 (d $J = 9$ Hz, 1H, Ar^4H), 7.73 (s, 1H, Ar^6H). ^{13}C NMR (fumarate) (CD_3OD): δ 14.00, 17.57, 18.17, 29.95, 50.47, 65.33, 65.39, 71.09, 71.25, 112.82, 129.95, 131.29, 133.57, 135.66, 157.15, 172.89. UV (MeOH) λ_{max} 306 (ε 309), 246 (ε 741), 214 (ε 2290). Anal. calcd. for $C_{34}H_{54}N_2O_8.C_4H_4O_4$, Mr 734.9; % C 62.11, % H 7.96, % N 3.81. Found % C 62.31, % H 8.15, % N 3.95.

*[2-(2-Hydroxy-3-tert-butylamino)propoxy)-5-(propoxymethyl)phenyl]ethanone (**4e**)*
Yield: 52%, mp: 148–150°C. IR (cm^{-1}): 3300 (ν, OH), 1670 (ν, C=O), 1567 (ν, C=Car.), 1235 (ν, C-O-Car.), 728 (δ, Ar). ^{1}H NMR (CD_3OD): δ 0.92–0.97 (t, 3H, CH_2CH_3), 1.41 (s, 9H, $C(CH_3)_3$), 1.59–1.66 (m, 2H, CH_2CH_3), 2.64 (s, 3H, $COCH_3$), 3.14–3.18 (m, 2H, $CH_2CH_2CH_3$), 3.47–3.50 (m, 2H, CH_2NH), 4.16–4.25 (m, 3H, CH_2CHOH), 4.47 (s, 2H, Ar-CH_2), 6.66 (s, 2H, fum), 7.12–7.15 (d $J = 9$ Hz, 1H, Ar^3H), 7.50–7.54 (d $J = 12$ Hz, 1H, Ar^4H), 7.72 (s, 1H, Ar^6H). ^{13}C NMR (CD_3OD): δ 9.52, 22.48, 24.59, 30.01, 44.58, 56.29, 65.64, 70.93, 71.40, 71.74, 109.99, 112.87, 129.78, 131.39, 133.45, 157.13. UV (MeOH) 308 (ε 566), 246 (ε 1595), 215 (ε 7302). Anal. calcd. for $C_{38}H_{62}N_2O_{12}.C_4H_4O_4$, Mr 791.0; % C 63.78, % H 8.41, % N 3.54. Found % C 63.65, % H 8.20, % N 3.75.

*[2-(2-Hydroxy-3-(isopropylamino)propoxy)-5-(propoxymethyl)phenyl]ethanone (**4f**)*
Yield: 60%, mp: 84–86°C. IR (cm^{-1}): 3426 (ν, OH,NH), 1664 (ν, C=O), 1495 (ν, C=Car.), 1274 (ν, C-O-Car.), 814 (δ, Ar). ^{1}H NMR ($CDCl_3$): δ 0.94–1.03 (t, 3H, CH_2CH_3), 1.10–1.13 (d $J = 9$ Hz, 6H $(CH_3)_2$), 1.62–1.69 (m, 2H, CH_2CH_3), 2.56 (s, 3H, $COCH_3$), 2.86–2.93 (m, 4H, CH_2N, $CH(CH_3)_2$), 3.96–4.04 (m, 2H, OCH_2CH_2), 4.07–4.12 (m, 3H, CH_2CHOH), 4.80 (s, 2H, ArCH_2), 6.99–7.02 (d $J = 9$ Hz, 1H, Ar^3H), 7.46–7.50 (d $J = 12$ Hz, 1H, Ar^4H), 7.73 (s, 1H, Ar^6H). UV (MeOH) λ_{max} 310 (ε 756), 248 (ε 1034), 216 (ε 2334). Anal. calcd. for $C_{34}H_{54}N_2O_8.C_4H_4O_4$, Mr 734.9; % C 62.11, % H 7.96, % N 3.81. Found % C 62.31, % H 8.15, % N 3.95.

*[2-(3-tert-Butylamino)-2-hydroxypropoxy)-5-(isopropoxymethyl)phenyl]ethanone (**4g**)*
Yield: 62%, mp: 72–75°C. IR (cm^{-1}): 3424 (ν, OH, NH), 1662 (ν, C=O), 1594 (ν, C=Car.), 1247 (ν, C-O-Car.), 812 (δ, Ar). ^{1}H NMR ($CDCl_3$): δ 1.12–1.17 (d $J = 15$ Hz, 6H, $(CH_3)_2$), 1.21 (s, 9H $(CH_3)_3$), 2.64 (s, 3H, $COCH_3$), 2.60–2.71 (m, 2H, CH_2N), 3.67–3.71 (m, 1H, $CH(CH_3)_2$), 3.95–3.99 (m, 2H, CH_2CH); 4.07 (m, 1H, $CHOH$); 4.80 (s, 2H, ArCH_2), 6.95–6.99 (d $J = 12$ Hz, 1H, Ar^3H), 7.45–7.48 (d $J = 9$ Hz, 1H, Ar^4H), 7.67 (s, 1H, Ar^6H). UV (MeOH) λ_{max} 310 (ε 758), 250 (ε 1023), 216 (ε 2344). Anal. calcd. for $C_{38}H_{62}N_2O_8.C_4H_4O_4$, Mr 791.00; % C 63.78, % H 8.41, % N 3.54. Found % C 63.31, % H 7.94, % N 3.95.

*[(5-(Butoxymethyl)-2-(3-tert-butylamino)-2-hydroxypropoxy)phenyl]ethanone (**4h**)*
Yield: 76%, mp: 57–59°C. IR (cm^{-1}): 3367 (ν, OH,NH), 1672 (ν, C=O), 1599 (ν, C=Car.), 1237 (ν, C-O-Car.), 824 (δ, Ar). ^{1}H NMR (CD_3OD): δ 0.90–0.95 (t, 3H, CH_2CH_3), 1.36–1.43 (m, 11H, CH_2CH_3, $C(CH_3)_3$), 1.56–1.60 (m, 2H, $CH_2CH_2CH_3$), 2.64 (s, 3H, $COCH_3$), 3.15–3.18 (m, 2H, CH_2NH), 3.46–3.51 (m, 2H, OCH_2CH_2), 4.17–4.28 (m, 3H, CH_2CHOH), 4.46 (s, 2H, Ar-CH_2), 6.66 (s, 2H, fum), 7.12–7.15 (d $J = 9$ Hz, 1H, Ar^3H), 7.50–7.53 (d $J = 9$ Hz, 1H, Ar^4H), 7.71 (s, 1H, Ar^6H). ^{13}C NMR (CD_3OD): δ 12.78, 18.97, 24.54, 30.09, 31.45, 44.56, 56.42, 65.56, 69.77, 70.91, 71.44, 112.88, 127.28, 129.76, 131.37, 133.47, 135.77, 157.16, 173.17, 200.26. UV (MeOH) λ_{max} 308 (ε 616), 246 (ε 1698), 216 (ε 6760). Anal. calcd. for $C_{40}H_{66}N_2O_8.C_4H_4O_4$, Mr 819.06; % C 64.52, % H 8.61, % N 3.42. Found % C 64.40, % H 8.78, % N 3.65.

*[2-(3-tert-Butylamino)-2-hydroxypropoxy)-5-(pentyloxymethyl)phenyl]ethanone (**4i**), fumarate salt*
Yield: 63%, mp: 127–128°C. IR (cm^{-1}): 3432 (ν, OH), 1672 (ν, C=O), 1574 (ν, C=Car.), 1580 (ν_{as}, COO^-), 1236 (ν, C-O-Car.), 824 (δ, Ar). ^{1}H NMR (DMSO-d_6): δ 0.88–0.93 (t, 3H, CH_2CH_3), 1.29–1.61 (m, 17H $(CH_3)_3$, $CH_2CH_2CH_2CH_3$, CH_2N), 2.62 (s, 1H, $COCH_3$), 3.42–3.51 (m, 2H, OCH_2CH_2), 4.05–4.16 (m, 3H, CH_2CHOH), 4.41 (s, 2H, ArCH_2), 6.66 (s, 2H, fum), 6.92–6.95 (d $J = 9$ Hz, 1H, Ar^3H), 7.40–7.43 (d $J = 9$ Hz, 1H, Ar^4H), 7.63 (s, 1H, Ar^6H). UV (MeOH) λ_{max} 308 (ε 380), 246 (ε 1071), 214 (ε 5248). Anal. calcd. for $C_{42}H_{70}N_2O_8.C_4H_4O_4$, Mr 847,10; % C 65.22, % H 8.81, % N 3.31. Found % C 64.91, % H 8.61, % N 3.39.

*[2-(2-Hydroxy-3-(isopropylamino)propoxy)-5-(pentyloxymethyl)phenyl]ethanone (**4j**), oxalate salt*
Yield: 84%, mp: yellow oil. IR (cm^{-1}): 3415 (ν, OH, NH), 1676 (ν, C=O), 1610 (ν, C=Car.), 1248 (ν, C-O-Car.), 816 (δ, Ar). ^{1}H NMR ($CDCl_3$) 0.88–0.93 (t, 3H, CH_2CH_3), 1.27–1.58 (m, 14H, $(CH_3)_2$, $CH_2CH_2CH_2CH_3$, CH_2N), 2.62 (s, 1H, $COCH_3$), 3.42–3.51 (m, 2H, OCH_2CH_2), 4.05–4.16 (m, 3H, CH_2CHOH), 4.41 (s, 2H, ArCH_2), 6.66 (s, 2H, fum), 6.92–6.95 (d $J = 9$ Hz, 1H, Ar^3H), 7.40–7.43 (d $J = 9$ Hz, 1H, Ar^4H), 7.63 (s, 1H, Ar^6H). UV (MeOH) λ_{max} 320 (ε 52), 252 (ε 182), 218 (ε 173). Anal. calcd. for $C_{40}H_{66}N_2O_8.C_2H_2O_4$, Mr 793,02; % C 63.55, % H 8.32, % N 3.53. Found % C 60.31, % H 8.24, % N 3.90.

*[2-(3-tert-Butylamino)-2-hydroxypropoxy)-5-((2-methoxyethoxy)methyl)phenyl]ethanone (**4k**), fumarate salt*
Yield: 69%, mp: 121–124°C. IR (cm^{-1}): 3425 (ν, OH), 1671 (ν, C=O), 1497 (ν, C=Car.), 1578 (ν_{as}, COO^-), 1241 (ν, C-O-Car.), 810 (δ, Ar). ^{1}H NMR (CD_3OD): δ 1.40 s (s, 9H, $C(CH_3)_3$), 2.63 (s, 3H, $COCH_3$), 3.15–3.18 (m, 2H, CH_2NH), 3.35 (s, 3H, CH_2OCH_3), 3.54–3.58 (m, 2H, $CH_3OCH_2CH_2$), 3.61–3.64 (m, 2H, $CH_3OCH_2CH_2$), 4.16–4.28 (m, 3H, CH_2CHOH), 4.51 (s, 2H, ArCH_2), 6.65 (s, 2H, fum), 7.11–7.14 (d $J = 9$ Hz, 1H, Ar^3H), 7.51–7.54 (d $J = 9$ Hz, 1H, Ar^4H), 7.73 (s, 1H, Ar^6H). ^{13}C NMR (CD_3OD): δ 24.56, 30.04, 44.57, 57.69, 62.83, 65.57, 69.02, 70.93, 71.54, 71.73, 112.88, 127.28, 129.00, 129.9, 131.06, 133.54, 135.78, 157.19, 173.19, 200.26. UV (MeOH) λ_{max} 308 (ε 616), 248 (ε 1445), 214 (ε 724). Anal. calcd. for $C_{38}H_{62}N_2O_8.C_4H_4O_4$, Mr 791.00; % C 63.78, % H 8.41, % N 3.54. Found % C 63.41, % H 8.15, % N 3.71.

*[(5-(Allyloxymethyl)-2-(3-tert-butylamino)-2-hydroxypropoxy)phenyl]ethanone (**4l**)*
Yield: 50%, mp: yellow oil. IR (cm^{-1}): 3422 (ν, OH,NH), 1670 (ν, C=O), 1605 (ν, C=Car.), 1247 (ν, C-O-Car.), 810 (δ, Ar). ^{1}H NMR (CD_3OD): δ 1.40 (s, 9H, $C(CH_3)_3$), 2.64 (s, 3H, $COCH_3$), 3.10–3.16 (m, 2H, CH_2NH), 4.02–4.04 (d $J = 6$ Hz, 2H, $CH_2CH{=}CH_2$), 4.04–4.26 (m, 3H, CH_2CHOH), 4.28 (s, 2H, Ar-CH_2), 5.17–5.33 (m, 2H, $CH_2CH{=}CH_2$), 5.88–5.99 (m, 1H, $CH_2CH{=}CH_2$), 6.66 (s, 2H, fum), 7.12–7.15 (d $J = 9$ Hz, 1H, Ar^3H), 7.51–7.55 (d $J = 12$, 1H, Ar^4H), 7.72–7.73 (s, 1H, Ar^6H). ^{13}C NMR (CD_3OD): δ 24.64, 44.58, 56.21, 63.07, 65.70, 70.75, 70.96, 112.91, 116.06, 129.92, 131.12, 133.57, 134.52, 135.67, 172.92. UV (MeOH) λ_{max} 308 (ε 489), 248 (ε 549), 218 (ε 2290). Anal. calcd. for $C_{38}H_{58}N_2O_8 \cdot C_4H_4O_4$, Mr 786.96; % C 64.10, % H 7,94, % N 3.56. Found % C 64.31, % H 8.15, % N 3.65.

Pharmacology

Experiments involving isolated tissues were performed in accordance with the European Guidelines for the use of animals. The isolated guinea pig atria and trachea preparations were used, known to preferentially express β_1- and β_2-adrenoreceptor mediated responses, respectively.

Antiisoprenaline activity estimation on isolated guinea pig atria
Guinea pigs were killed by cervical dislocation after light anesthesia with ether. The heart was quickly removed and placed in Krebs–Henseleit solution. The right atria were excised with an intact sinus mode, the auricles ligated and suspended in an organ chamber suitable for measuring the spontaneous beating activity. The right atria of guinea pig heart were connected to an isometric transducer in Tyrode solution at 30°C under a resting tension of 1 g and gassed with pneumoxide ($O_2 + 5\%$ CO_2). The preparations were allowed to stabilize for at least 30 min and isoprenaline. HCl (IPN) was added cumulatively (10^{-11} to 10^{-5} mol/dm^3) and concentration–response curves (CRC) were then plotted. Then, the atria were washed and allowed to reequilibrate. The evaluated compound in the form of racemate was added to the bath 20 min before the second CRC was obtained. The affinity for IPN was expressed as EC_{50} (agonist concentration producing 50% of maximal response). The antagonist potency of the compound was calculated from the shift in CRC of IPN and expressed as dissociation constants (pA_2 values) according to the modified method of Van Rossum [28].

Antiisoprenaline activity estimation on isolated guinea pig tracheal strip
Tracheal strip from guinea pig atria was isolated and placed under a resting tension of 2 g in a bath filled with Krebs–Henseleit solution at 37°C and aerated with pneumoxide. Reactibility to IPN was recorded isometrically. After 1 h rest, the preparation was preconcentrated with histamine (6.7×10^{-7} mol/dm^3) and then relaxation caused by cumulative application of IPN (10^{-9} to 10^{-5} mol/dm^3) and CRC was obtained. The studied compound in the form of racemate was applied and allowed to equilibrate for 30 min before the IPN curve was re-established. The EC_{50} and pA_2 values from the shift in CRC of IPN were then calculated according to the method described above.

Data analysis
Values presented are the means ± SEM of 5–7 experiments. Comparison between the two data was made by Student's *t*-test for paired or unpaired data. In this test, $p < 0.05$ was considered indicative of a significant difference.

Experiments involving isolated tissues were performed in accordance with the European Guidelines for the use of animals in research.

This study was supported by the Slovak Research and Development Agency under contract No. APVV-0516-12.

The authors have declared no conflicts of interest.

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