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Synthesis, pharmacological activity and chromatographic separation of some novel potential β-blockers of the aryloxyaminopropanol type

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Received April 2, 2002, accepted August 7, 2002

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Pharmazie 58: 237–241 (2003)

Following our previous structure-activity relationship studies, some novel compounds of the aryloxyaminopropanol type, derived from 2- or 4-hydroxyphenylalkanones, with phenethyl or 3,4-dimethoxyphenethyl groups in the hydrophilic part of the molecule were synthesized and pharmacologically evaluated. The compounds were prepared by means of two methods and their structures were confirmed by the interpretation of their IR, UV and ^{1}H NMR spectra. The enantiomers were separated by HPLC on vancomycin (Chirobiotic V) and teicoplanin (Chirobiotic T) chiral stationary phases. The affinity of the prepared racemic compounds to β_1- and β_2-adrenergic receptors was pre-determined on isolated guinea pig atria and trachea. The assumed cardioselectivity was expressed as the β_1/β_2 ratio. Reciprocal changes in the position of the phenoxysubstituents did not influence the antiisoprenaline activity of the compounds. On the other hand, the increase of the N-substituent size in the hydrophilic part of molecule (3,4-dimethoxyphenethyl moiety led to a substantially higher affinity for cardiac (β_1) than for tracheal (β_2) tissue.

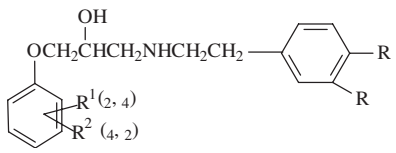
1. Introduction

In our previous studies, newly synthesized structures of the aryloxyaminopropanol type with isopropyl and *tert*-butyl groups on the basic nitrogen were described. Some of them showed β-adrenoceptor blocking and antiarrhythmic activities [1, 2]. In view of the fact that the pharmacological activity could be influenced by a large variety of substitutions in both moieties (hydrophobic and hydrophilic of the basic aryloxyaminopropanol structure, many other pharmacological activities, such as Ca^{2+}-antagonistic [3], α_1-adrenolytic [4], antihypertensive [5], vasodilating [6] and anticonvulsive [7], have been described.

In continuation of these studies some other aryloxyaminopropanol structures having different substitutions on the 2- or 4-position of the phenyl ring as well as with phenethyl and 3,4-dimethoxyphenethyl groups in the basic part of the molecule have been synthesized.

In order to complete the existing evidence together with an initial explanation of the structural requirements for selectivity to cardiac tissue, the synthesis of some potential β-adrenoceptor blockers and their basic pharmacological investigation are described here.

Those compounds which possess a stereogenic centre exist as stereoisomers which often show different pharmacological activities. It thus seems advisable to resolve the racemic mixture and show the express some chromatographic characteristics of each enantiomer. The most widely used technique for the separation and quantification of enantiomers seems to be HPLC.



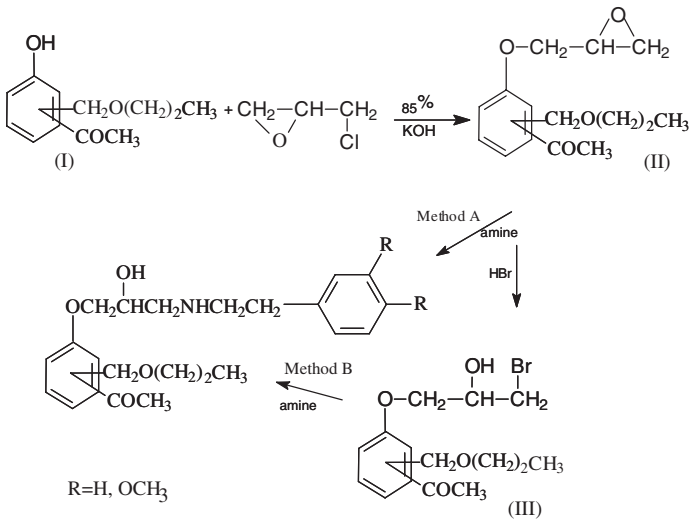
2. Investigations, results and discussion

2.1. Chemistry

The compounds described in Table 1 were prepared by a multi-step synthesis from hydroxyphenylalkanones as starting materials as shown in the Scheme.

Chlomethylderivatives prepared by chloromethylation of hydroxyphenylalkanones were reacted with propan-1-ol in presence of sodium hydrogencarbonate to yield 4-hydroxy-3-propoxymethylphenylalkanones or 2-hydroxy-5-propoxymethylphenylethanone (I). Oxirane intermediates (II) prepared by the subsequent reaction of these ketones with chloromethyloxirane were used without further purification. In method B the next step of the procedure was opening the of oxirane intermediate with hydrobromic acid. The oxirane intermediate (II) (method A) or the bromoderivative (III) (method B) formed final products by reaction with phenethyl- and 3,4-dimethoxyphenethylamine. These were isolated in the form of free bases or salts with organic acids, especially with fumaric acid. Yields of final products were higher with method B and ranged from 42 to 62% (Table 1). The purity of the final products was checked by TLC (Table 1) and their structures were confirmed by IR, UV and ^{1}H NMR spectra (Table 2, 3).

Scheme



The stretching vibrations of the characteristic groups in the IR spectra were νOH 3000–3300 cm^{-1}, $\nu_{(C=C)}$ = 1558–1604 cm^{-1}, $\nu_{(C=O)}$ = 1668–1675 cm^{-1}, $\nu_{(CAlOCAr)}$ = 1258–1262 cm^{-1}, and for the base $\nu_{(OH, NH)}$ = 3260–3436 cm^{-1} (Table 2).

The UV spectra of bases and salts display bands corresponding to $\pi - \pi^*$ transitions at λ_{max} = 218–224 nm, 272–278 nm, $\varepsilon = 2.70–3.0\ m^2 \cdot mol^{-1}$ (Table 2) and the ^1H NMR spectra of some free bases showed proton signals of the aminopropanol chain (Table 3).

2.2. Separation of enantiomers by HPLC

HPLC separation of a number of β-blockers using protein-based chiral stationary phases such as bovine serum albumin [8], ovomucoid [9], α_1-glycoprotein [10] and cellobiohydrolase [11] appears to be the preferred method in many cases.

In the present study, enantiomeric separation of some compounds was performed using macrocyclic bonded stationary phases. In our previous work [12] teicoplanin and vancomycin chiral stationary phases were used for separation of 62 aryloxyaminopropanol derivatives by HPLC techniques in the polar-organic mode. The results obtained for the separated enantiomers show that these structural modifications influenced the retention factor values only slightly. However, the effect on the resolution of the enantiomers was noticeable.

The HPLC method used in this study employed a vancomycin or teicoplanin chiral stationary phase and a mixture of methanol : acetonitrile : acetic acid : triethylamine (45 : 55 : 0.3 : 0.2 v/v/v/v) as the mobile phase. The separation of all compounds under study on both chiral stationary phases was performed using this mobile phase. The results of enantioseparation are summarised in Ta-

Table 1: Physico-chemical parameters of prepared compounds

Compd.	R^1	R^2	R	Empirical formula	M.p. (°C)	Yield (%)	
						Method A	Method B
Form of compds				M_r	Solvent	R_F	
1a	2-$CH_2O(CH_2)_2CH_3$	4-$COCH_3$	H	$C_{46}H_{63}O_8N_2 \cdot C_4H_4O_4$	118–120	40	62
fumarate				887.08	a	0.56	
2	2-$CH_2O(CH_2)_2CH_3$	4-$COCH_3$	OCH_3	$C_{25}H_{35}O_6N$	72–3	38	52
base				445.61	b	0.58	
2a				$C_{50}H_{70}O_{12}N_2 \cdot C_4H_4O_4$	115 –7	39	60
fumarate				1007.15	a	0.58	
3	2-$CH_2O(CH_2)_2CH_3$	4-COC_2H_5	H	$C_{25}H_{33}O_6N$	78–80	3	54
base				339.53	b	0.60	
3a				$C_{48}H_{66}O_8N_2 \cdot C_4H_4O_4$	85–87	35	62
fumarate				1035.20	b	0.60	
3b				$C_{24}H_{33}O_8N \cdot HCl$	113–5	32	57
hydrochloride				436.07	a	0.60	
4	2-$CH_2O(CH_2)_2CH_3$	4-COC_2H_5	OCH_3	$C_{26}H_{37}O_6N$	79– 80	33	54
base				459.56	b	0.62	
4a				$C_{52}H_{74}O_{12}N_2 \cdot C_4H_4O_4$	97–8	32	54
fumarate				1035.20	a	0.62	
5a	2-$COCH_3$	4-$CH_2O(CH_2)_2CH_3$	OCH_3	$C_{50}H_{70}O_{12}N_2 \cdot C_4H_4O_4$	135–7	32	42
fumarate				1007.15	a	0.45	

a – ethyl acetate, b – hexane, heptane

Table 2: Values of λ_{max} and log ε in UV spectra and values of stretching vibration in IR spectra of prepared compounds

Compd.	λ_1 log ε_1	λ_2 log ε_2	ν_{OH} (cm^{-1})	$\nu_{OH,NH}$ (cm^{-1})	$\nu_{C=C}$ $\nu_{C=O}$	$\nu_{CalOCar}$
1a	224 3.60	272 3.57	3200–3300		1601 1668	1261
2	222 3.90	272 3.87		3402	1601 1675	1262
2a	218 3.33	272 3.13	3200–3300		1602 1668	1261
3	220 3.62	272 3.70		3260	1602 1670	1253
3b	shoulder	274 3.12	3317		1603 1671	1271
4	shoulder	270 2.24		3436	1604 1685	1262
4a	221 2.70	274 2.56	3379		1603 1678	1260
5a	218 2.93	278 2.35	3000–3200		1558 1671	1258

ε in ($mol^{-1} \cdot m^2$)

ble 4. It is evident that there is no significant difference in the separation of the enantiomers on the two chiral stationary phases with the mobile phase tested (the values of resolution factors are very similar). Comparing of the character of substituents on the aromatic rings more effective separation (the highest value of resolution factor) was obtained for derivatives possessing smaller substituents (acyl group) in the 2-position on the aromatic ring (compound 5a and celiprolol). From the previous studies the highest resolution was shown for enantiomers with any substitution on the 2-position. It is plausible, that the 2-position substituents crowd the environment near the adjacent stereogenic centre and decrease interaction with the stationary phase [12, 13].

The type of the nitrogen substitution, which is not so near the stereogenic centre, had only a small influence on enantioseparation (compounds **1a**–**4a**). It can be observed, that the retention factors values were only higher for derivatives with a 3,4-dimethoxyphenethyl group (compounds **2a**, **4a** and **5a**).

The interaction of enantiomers with the stationary phase leads to chiral discrimination and it can be expressed as the difference of the free energy $-\Delta_{1,2}\,\Delta G^0$. The results show that very small energy differences were needed

Table 3: ^{1}H NMR spectral data of some free bases

Groups	Compd.								
	3			**4** δ (ppm)			**2**		
$\underline{CH_3}CH_2CH_2$	1.0	t	3 H	1.1	t	3 H	1.1	t	3 H
$\underline{CH_3}CH_2CO$	1.3	t	3 H	1.3	t	3 H			
$CH_3\underline{CH_2}CH_2$	1.5–1.8	m	2 H	1.5–1.8	m	2 H	1.5–1.8	m	2 H
$\underline{CH_3}CO$							2.5	s	3 H
$CH_2NHCH_2CH_2 + CH_2CO + CH_2O$	2.6–3.1	m	11 H	2.6–3.1	m	11 H	2.6–3.1	m	9 H
CH_3O				3.9	s	6 H	3.9	s	6 H
$ArCH_2O$	4.1	s	2 H	4.1	s	2 H	4.1	s	2 H
$ArCH_2CH(OH)$	4.6	m	4 H	4.6	m	4 H	4.6	m	4 H
H_{Arom}	6.9–7.8	m	8 H	6.9–7.8	m	6 H	6.9–7.8	m	6 H

Table 4: Chromatographic data for the enantioseparation of prepared compounds on bonded chiral stationary phases

Compd.	Vancomycin				Teicoplanin			
	k_1	α	Rs	$-\Delta_{1,2}\,\Delta G^0$	k_1	α	Rs	$-\Delta_{1,2}\,\Delta G^0$
1a	2.16	1.04	0.93	96.57	2.62	1.04	0.83	96.57
2a	2.39	1.04	1.03	96.57	2.75	1.05	0.93	120.13
3a	2.01	1.04	0.82	96.57	2.44	1.06	0.96	143.47
4a	2.22	1.04	0.94	96.57	2.56	1.05	0.92	120.13
5a	2.41	1.05	1.22	120.13	2.73	1.05	1.05	120.13
Celiprolol	2.40	1.10	1.24	234.67	3.27	1.12	1.23	279.04

ΔG^0 in ($J \cdot mol^{-1}$)

Table 5: Negative chronotropic activity in spontaneously beating isolated guinea pig atria of evaluated compounds at 10^{-7} mol · l^{-1}, their pA_2 values determined in atria and tracheal muscle of guinea pigs and β_1/β_2 selectivity ratios calculated as antilog ($pA_2\beta_1 - pA_2\beta_2$)

Compd.	Heart rate (%)	pA_2 values		β_1/β_2 ratio
		atria (β_1)	trachea (β_2)	
1a	94.2 ± 2.4*	6.89 ± 0.12	5.79 ± 0.13	13
2a	88.3 ± 2.2*	8.07 ± 0.13	5.59 ± 0.20	13
4a	85.6 ± 1.4*	7.80 ± 0.28	4.81 ± 0.19	977
5a	89.9 ± 3.1*	7.85 ± 0.28	5.45 ± 0.18	251
Celiprolol	86.6 ± 0.9*	8.62 ± 0.11	7.84 ± 0.10	6 [16]

Each value represents the mean ± SEM from 5–7 experiments. $p < 0{,}05$ against saline group

for the HPLC enantioseparation of the analytes studied (Table 4).

2.3. Pharmacology

The potential β-adrenoceptor blocking potency of the compounds evaluated was measured by means of positive chronotropic responses to the β-adrenergic agonist isoprenaline (IPN) on isolated atria ($\beta 1$) and relaxation of smooth muscle of trachea (β_2) and the compounds ability to inhibit the effects. The specific antiisoprenaline activity was expressed as pA_2 values which correspond to dissociation constants of the receptor-antagonist complex [14].

As can be seen from Table 5 all the compounds evaluated are effective as IPN antagonists on both tissues studied. A comparison of the pA_2 values indicates that the most effective compound seems to be **2a** ($pA_2 = 8.07 \pm 0.13$) with 3,4-dimethoxyphenetyl substitution on the amino-nitrogen.

Replacement of the 4-acetyl substituent in the phenyl ring by a 4-propionyl group (**4a**) did not significantly influence the antiisoprenaline activity in the atria.

However, this structural change led to a drop in the tracheal (β_2) antiisoprenaline activity and hence to an increase of more than three times in the cardiac (β_1) selectivity when calculated as the β_1/β_2 activity ratio.

Enlargement of the substituent in the 4-position increases affinity to myocardial adrenoreceptors and decreases affinity to tracheal tissue (**2a** vs **5a**), which correlates well with our previous results [15]. Other replacements of the substituents on the phenyl ring in the 2- and 4-positions (**2a** and **5a**) did not significantly influence either the cardioselectivity or the potency of the two compounds evaluated.

When compounds **1a** (N-phenethyl substitution) and **4a** (N-dimethoxyphenethyl substitution) were compared, the more bulky substitution was found to favour atrial inhibiting efficacy but not tracheal. Thus, the cardioselectivity of **4a** was found to be more than 75 times higher (Table 5) and all cardioselectivity values were higher than those of standard celiprolol and compounds with a *tert*-butyl group on the basic nitrogen [16].

The influence of the compounds on heart rate was also measured under *in vitro* conditions before estimation of antiisoprenaline activity. All compounds studied at a conc. of 1×10^{-7} mol · l^{-1} significantly (compared with a saline pre-treated group) influenced the spontaneous rate of isolated guinea pig atria (Table 5). The heart rate, measured 20 min after giving the compound, was decreased by 5.8 to 14.4%.

In summary, all the compounds investigated possess the basic properties typical of β-adrenergic blockers. The observed synergism of two suitable 2- and 4-positioned substituents on the phenyl ring and also enlargement of the N-substitution have a positive influence on the interaction of the compounds with the presumed cardiac (β_1) adrenoreceptors. However, other pharmacodynamic and physicochemical approaches are needed.

3. Experimental

3.1. Chemistry

3.1.1. Equipment

Melting points were determined using a Kofler Micro Hot Stage and are quoted uncorrected. Elemental analysis, performed using a Model 1108 elemental analyser (Carlo Erba), showed good correlation with theoretical values. The purity of the formed compounds was assessed on TLC SILUFOL® UV-254 (Kavalier) plates and using a solvent system of (ethyl acetate : diethylamine 95 : 5 v/v). UV spectra were run on a Hewlett-Packard 8452 spectrophotometer. IR spectra were recorded using an IMPACT 400 D (Nicolet) FTIR spectrophotometer in CCl_4. ^{1}H NMR spectra of the prepared compounds dissolved in deuterochloroform were recorded on a Varian VXR – 3000 spectrometer at 300 MHz using TMS as internal standard.

3.1.2. Starting materials

3-Chloromethyl-4-hydroxyphenylalkanones were prepared according to [17] and 5-chloromethyl-2- hydroxyphenylethanone according to [18]. 4-Hydroxy-3-propoxymethylphenylalkanones were prepared according to [19].

3.1.3. 4-(Alkylamino-2-hydroxypropoxy)-3-(propoxymethyl)phenylalkanones

Method A: according to [1].

Method B: 0.15 Mol of 4-hydroxy-3-propoxymethylphenylalkanones or 2-hydroxy-5-propoxymethylphenylethanone, 3 mol (235 ml) of chloromethyloxirane and 11 g 85% KOH were reacted for 4 h with stirring at a temperature of 50–55 °C. The KCl produced was sucked off and residual chloromethyloxirane was distilled off in vacuum. The residue was diluted in chloroform (200 ml) and shaken with NaOH solution (2 mol · l^{-1}) and with saturated NaCl solution. After distilling off chloroform the oxirane derivative produced was diluted in acetone (170 ml) and 150 ml of water and 21 ml 48% hydrobromic acid at a temperature of 15–25 °C were slowly added. After 3 h the acetone was distilled off and the rest was shaken out into ether. After distilling off ether 1.3 mol (101 ml) of the amine and an equal amount of water were added to 0.1 mol of the intermediate product obtained and the mixture was reacted for 24 h at a temperature of 20–25 °C. Unreacted amine was distilled off and the base was shaken out into ether and washed with water several times. The etheric solution of the base was dried with K_2CO_3 and after distilling off ether the oily base was crystallized from hexane or heptane. From the pure base in etheric solution corresponding salts (with fumaric or hydrochloric acid) were prepared by adding fumaric acid solution or hydrogen chloride in ether and crystallized from ethyl acetate.

3.2. HPLC analysis

3.2.1. Chromatographic conditions

Studies were performed with a Hewlett Packard (series 1100) HPLC system consisting of a quaternary pump equipped with a injection valve (Rheodyne) and diode array detector. The macrocyclic chiral stationary phases were Chirobiotic T and Chirobiotic V (250 × 4 mm I.D. particle

size 5 µm Advanced Separation Technologies. Inc. USA). The mobile phase was a mixture of methanol and acetonitrile to which acetic acid and triethylamine were added (methanol : acetonitrile : acetic acid : diethylamine 45 : 55 : 0.3 : 0.2 v/v/v/v). The separation was carried out at a flow rate of 1 ml/min and the column temperature was 23 °C. The chromatograms were scanned at Hewlett Packard (series 1100) 276 nm. The injection volume was 20 µl. The analytes were dissolved in methanol (concentration 1mg/ml)

3.2.2. Chromatographic characteristics

The separation factor was expressed as

$$\alpha = k_2/k_1$$

where k_1, k_2 are the retention factors for the first and second eluting enantiomers. The retention factor k′ was calculated as follows:

$$k_1 = (t_1 - t_0) / t_0 \quad \text{and} \quad k_2 = (t_2 - t_0)/t_0$$

where t_0, t_1 and t_2 are the dead elution time and elution time of enantiomers 1 and 2.
The stereochemical resolution factor (R_s) of the first and second eluting enantiomers was calculated by the ratio of the difference between the retention times t_1 and t_2 to the arithmetic mean of the two peak widths w_1 and w_2.

$$R_s = 2\,(t_2 - t_1) / (w_1 + w_2)$$

The free energy difference $-\Delta_{1,2}\,\Delta G^0$ was calculated from the separation factor α according to the equations

$$-\Delta_{1,2}\,\Delta G^0 = RT\ \ln\ k_2/\ k_1 = RT\ \ln\ \alpha$$

3.2.3. Chemicals

All HPLC grade solvents (methanol, acetonitrile) were obtained from Merck (Germany). Triethylamine and acetic acid were obtained from Lachema (Czech Republic). Celiprolol · HCl was gift from Rhône-Poulenc Rorer, UK.

3.3. Pharmacology

3.3.1. Antiisoprenaline activity estimation on isolated guinea pig atria

The right atria of a guinea pig heart were connected to an isometric transducer in Tyrode solution at 30 °C under a resting tension 1 g and gassed with pneumoxide (5% CO_2). The preparations were allowed to stabilize for at least 30 min and then isoprenaline. HCl (IPN) was added cumulatively (10^{-11}–10^{-5} mol · l^{-1}) and concentration-response curves (CRC) were plotted. After the atria were washed and allowed to reequilibrate, the studied compounds were 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 compounds 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 [14].

3.3.2. Antiisoprenaline activity estimation on isolated guinea pig tracheal strip

Tracheal strip from guinea pigs 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 precontracted with histamine (6.7×10^{-7} mol · l^{-1}) and then relaxation caused by cumulative application of IPN (10^{-9}–10^{-5} mol · l^{-1}) and CRC were obtained. The studied compounds were then given 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.

Acknowledgements: This work was supported by the Slovak Grant Agency for Science (Grants No. 1/7277/20, 1/7369/20 and 1/9127/02)

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