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ORIGINAL PAPER

Synthesis, pharmacological activity, and chromatographic enantioseparation of new heterocyclic compounds of the aryloxyaminopropanol type derived from 4-hydroxyphenylalkanones

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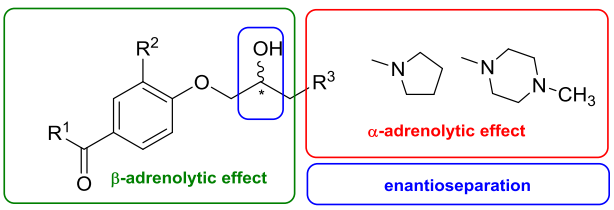
Abstract

In the paper, a series of six pharmacologically active compounds (β-adrenolytics) derived from 4-hydroxyphenylethanone and 4-hydroxyphenylpropan-1-one are reported. The compounds incorporate pyrrolidin-1-yl and 4-methylpiperazin-1-yl substituents in the hydrophilic part of the molecule and ethoxymethyl and methoxyethoxymethyl side chains on the aromatic ring in the lipophilic moiety. They were prepared by a four-step synthesis from 4-hydroxyalkanones via chloromethyl, alkoxymethyl, and oxirane intermediates. The purity of the target compounds was checked by TLC and their structures were confirmed by the interpretation of the IR, UV, ^{1}H NMR, and ^{13}C NMR spectra. The pharmacological evaluation of the obtained compounds confirmed their vasodilatory and specific antiisoprenaline activities. All evaluated compounds at conc. 10^{-6} mol dm^{-3} inhibited vasoconstrictory effect of phenylephrine (8.22–33.7%) on isolated rat aorta. The ability to inhibit positive chronotropic effect of isoprenaline was observed on isolated spontaneously beating rat's atria after pre-treatment with the evaluated compounds at conc. 10^{-7} and 10^{-6} mol dm^{-3}. The calculated pA_2 values of specific antagonistic effect against isoprenaline, related to their apparent β-adrenolytic activity, ranged between 6.54 and 7.57. The value for the standard compound carvedilol was 8.15 ± 0.22. The majority of the evaluated compounds at conc. 10^{-6}–10^{-7} mol dm^{-3} also showed negative chronotropic effect on the basic heart rate of atria. Enantioseparation of the prepared compounds was performed by chiral HPLC on an amylose tris(3,5-dimethylphenylcarbamate) column (Chiralpak AD) and a native teicoplanin column (Chirobiotic T). The chromatographic characteristics as retention, separation, and resolution factors were reported.

Graphical abstract



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Introduction

In the group of various drugs acting on the cardiovascular system, compounds with a built-in heterocycle moiety in the lipophilic and/or in the hydrophilic part of the molecule exhibit significant biological activity. One of the most famous β-adrenolytics with indole in the lipophilic part of the molecule is pindolol [1, 2] and its prodrug form bopindolol [3]. Carvedilol with incorporated carbazole is a clinically used drug with combined α- and β-adrenolytic efficacy that exhibits antihypertensive activity and a high-antioxidant potency [4–6].

Derivatives with phenylpiperazine moiety in the hydrophilic part of the molecule are under consideration as potential antihypertensive agents [7, 8]. Their activity is based on their interaction with the binding site of the α_1- and β-adrenergic receptors [9, 10]. Some piperazine derivatives show antidepressant-like- [11] and H_1-antihistaminic effects [12].

The structure of naftopidil was obtained by replacing the propan-2-amino (isopropylamino) group in the molecule of propranolol with the 2-methoxyphenylpiperazine-1-yl substituent. The antagonistic effect was shifted towards selective α_1-receptor blockade, while the affinity for α_2-and β-adrenoceptors was very weak [13, 14]. Naftopidil also blocks Ca^{2+} channels, inhibits serotonin-induced platelet aggregation, and reduces serotonin uptake by thrombocytes [15]. Other results demonstrated its use in benign prostatic hyperplasia [16].

Since compounds of the aryloxyaminopropanol type possess a stereogenic center in their structure, chiral HPLC technique was used for the separation of individual enantiomers. Chiral stationary phases used for enantioseparation of aryloxyaminopropanols are based on teicoplanin and vancomycin [17, 18], cyclodextrin [19], ovomucoid [20], and on derivatives of cellulose and amylose [21, 22].

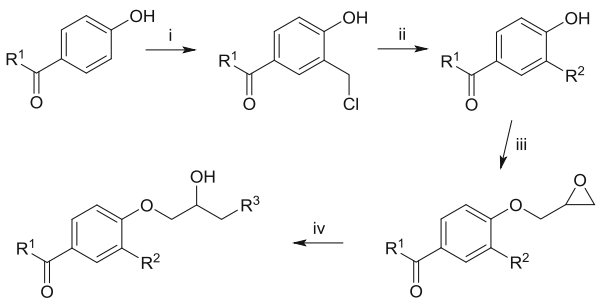
The aim of this work was the preparation, basic pharmacological evaluation and the study of HPLC enantioseparation of new aryloxyaminopropanol derivatives with pyrrolidin-1-yl and 4-methylpiperazin-1-yl substituent in the hydrophilic part of molecule. The pharmacological in vitro evaluation of the prepared compounds was orientated towards their vasodilatory and specific antiisoprenaline properties, which are inherent to all clinically used β-blockers. Chiral columns, based on amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) and native teicoplanin (Chirobiotic T), were used to separate the individual enantiomers.

Results and discussion

Previously, we reported several aryloxyaminopropanol derivatives with isopropyl and *tert*-butyl groups [23] and with the 3,4-dimethoxyphenethyl moiety, respectively [17]. These newly prepared β-blockers exhibited higher affinity for β_1-receptors in comparison with celiprolol and acebutolol used as standard compounds.

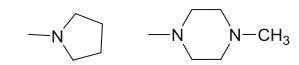
In the present work, we prepared a series of compounds with pyrrolidine or *N*-methylpiperazine moieties in the hydrophilic part of the molecule and an acyl substituent on the aromatic ring, in anticipation of an additive vasodilatory effect. The target compounds **1–5** were prepared by an established four-step synthetic procedure (Scheme 1, Table 1). In the first step, (4-hydroxyphenyl)alkanones reacted with paraformaldehyde and 36% HCl to give (3-chloromethyl-4-hydroxyphenyl)alkanones in 70–75% yield [24]. The isolated chloromethyl derivatives were treated with dry ethanol (compounds **1–4**) or 2-methoxyethanol (compound **5**) and solid $NaHCO_3$, yielding (3-alkoxymethyl-4-hydroxyphenyl)alkanones in 50–60% yield. These intermediates then produced 1-[3-alkoxymethyl-4-(oxirane-2-yl)phenyl]alkanones by reaction with oxirane (54–66% yield) [25]. In the fourth step, oxirane intermediates in ethanol reacted with pyrrolidine and *N*-methylpiperazine to attach the basic heterocyclic moiety. An exception was the compound **6**, which could be

Scheme 1



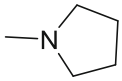
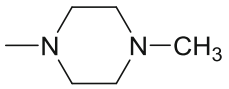
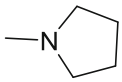
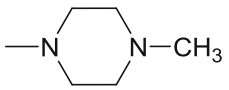
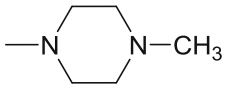
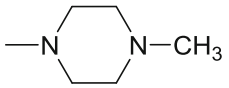
R^1 = -CH_3, , -CH_2CH_3

R^2 = -H, -$CH_2OCH_2CH_3$, -$CH_2OCH_2CH_2OCH_3$

R^3 = 

i. paraformaldehyde, 36% HCl, 4-5 h, 45-50 °C; ii. ethanol, 2-methoxyethanol, $NaHCO_3$; iii. chloromethyloxirane, KOH, 4 h, 50-55 °C; iv. pyrrolidine, *N*-methylpiperazine / diethylether, fumaric acid.

Table 1 The prepared compounds

Compound	R^1	R^2	R^3
1	$-CH_3$	$-CH_2OCH_2CH_3$	
2	$-CH_3$	$CH_2OCH_2CH_3$	
3	$-CH_2CH_3$	$-CH_2OCH_2CH_3$	
4	$-CH_2CH_3$	$CH_2OCH_2CH_3$	
5	$-CH_3$	$-CH_2OCH_2CH_2OCH_3$	
6	$-CH_3$	-H	

prepared, because of absence of the alkoxymethyl group, by a simplified two-step procedure. The basic intermediates were finally transformed into salts with fumaric acid in a 2:1 ratio (base:acid) and crystallized from ethyl acetate or propan-2-ol [23]. The yields in the final synthetic step ranged between 34 and 65%. The prepared aryloxyaminopropanols in the form of their fumarates were white solids. General procedures for each of the four synthetic steps are given in the experimental part.

The purity of the products was tested by TLC and their structures were investigated by spectral methods (UV, IR, ^{1}H and ^{13}C NMR). In the IR spectra, the stretching vibrations of the OH (3402–3460 cm^{-1}), C=O (1668–1675 cm^{-1}), C=C (1600–1602 cm^{-1}), and ArOalk (1259–1268 cm^{-1}) groups could be observed. The UV spectra display three bands corresponding to $\pi \rightarrow \pi^*$ transitions. The signals in ^{1}H and ^{13}C NMR spectra correspond to the expected structure of the compounds, showing clearly the successful attachment of the heterocyclic moiety. The NMR spectra are available online in the supplementary material for this article.

The effect of the target compounds on α-adrenergic receptors was studied in isolated, phenylephrine reconstituted aorta of Wistar rats. The described vasodilatory effect of the investigated compounds was comparable to or stronger than that of carvedilol (EC_{50} = 4.97 ± 1.36 × 10^{-7} mol dm^{-3}). Experimental results (Table 2) show that all compounds at conc. 10^{-6} mol dm^{-3} on isolated aorta inhibited the vasoconstrictor effect of phenylephrine (8.2–33.7%). The inhibitory effect seems to be non-competitive, involving several potential mechanisms. Besides β-antagonism, the heterocyclic moiety (pyrrolidine or *N*-methylpiperazine) in the basic part of aryloxyaminopropanols may have additional vasodilatory (apparently α-adrenolytic) effect.

The ability of the compounds to inhibit positive chronotropic effect of the β-adrenergic agent isoprenaline at conc. 10^{-7} and 10^{-6} mol dm^{-3} was evaluated on spontaneously beating rat atria. The specific antiisoprenaline activity was expressed as pA_2 values, corresponding to the dissociation constants of the receptor–antagonist complex.

A comparison of the pA_2 values indicated that the most effective compound was carvedilol (8.15 ± 0.22), while the generally inferior pA_2 values of the evaluated compounds were in the range from 6.54 to 7.57.

All the evaluated compounds, at used concentrations, decreased the spontaneous heart rate of isolated rat's atria (except compd. **2** at conc. 10^{-7} mol dm^{-3}) with maximum effect at 20th min after their administration (Table 3).

Enantioseparation of the studied compounds was performed on a Chiralpak AD column based on derivatized amylose (amylose tris(3,5-dimethylcarbamate) chiral stationary phase) and on Chirobiotic T column based on native teicoplanin. As shown in Table 4, compounds **2** and **4** were separated to the baseline on the Chiralpak-AD column with α = 1.17–1.24 and R_S = 0.82–3.64, whereas on the Chirobiotic T column no separation was obtained. Using the mobile phase methanol/acetonitrile/acetic acid/triethylamine 45/55/0.3/0.2) v/v/v/v on a Chirobiotic T column, compounds **1** and **3** with the pyrrolidine moiety were separated with α = 1.05–1.06 and R_S = 1.41–1.55.

These results show that the Chirobiotic T column was most suitable for compounds **1** and **3** with the pyrrolidine moiety, while for compounds **2**, **4**, **5**, and **6** the Chiralpak-AD-H column showed the best efficiency (Table 4, Fig. 1).

Table 2 Inhibitory effect of the evaluated compounds and carvedilol on isolated aorta of rats (% inhibition ± SEM) and mean effective concentration of phenylephrine (EC_{50} ± SEM) in the presence of the compounds

Compound	Conc./mol dm^{-3}	% Inhibition	EC_{50}/mol dm^{-3}
1	10^{-6}	8.20 ± 3.10	2.82 ± 1.65 × 10^{-10}
2	10^{-6}	8.4 ± 3.20	9.18 ± 0.39 × 10^{-8}
3	10^{-6}	21.6 ± 6.36	5.98 ± 0.66 × 10^{-8}
4	10^{-6}	33.7 ± 11.35	1.01 ± 0.39 × 10^{-7}
5	10^{-6}	16.1 ± 2.24	5.05 ± 1.72 × 10^{-8}
6	10^{-6}	10.44 ± 7.50	4.86 ± 1.42 × 10^{-8}
Carvedilol	10^{-7}	73.14 ± 9.58	4.97 ± 1.36 × 10^{-7}

Table 3 Influence of the evaluated compounds on heart rate of spontaneously beating rat's atria and calculated pA_2 values expressing their apparent β-adrenolytic potency

Compound	Conc./mol dm^{-3}	5 min	10 min	15 min	20 min	pA_2
1	10^{-7}	100.0 ± 2.81	98.0 ± 2.91	96.0 ± 2.40	92.0 ± 1.95	7.53 ± 0.21
2	10^{-7}	115.0 ± 3.29	110.0 ± 3.10	108.0 ± 3.62	100.0 ± 3.71	7.57 ± 0.50
3	10^{-6}	101.3 ± 0.33	100.3 ± 0.33	98.4 ± 0.95	97.4 ± 0.70	6.57 ± 0.13
4	10^{-6}	99.3 ± 0.42	98.9 ± 1.13	97.4 ± 0.96	96.8 ± 0.76	6.60 ± 0.19
5	10^{-6}	101.0 ± 1.38	99.6 ± 1.59	98.4 ± 1.42	96.8 ± 1.66	6.54 ± 0.14
6	10^{-6}	96.7 ± 1.23	97.8 ± 0.78	98.2 ± 0.98	98.5 ± 0.87	6.99 ± 0.23
Carvedilol	10^{-7}	98.8 ± 0.79	96.3 ± 1.35	95.4 ± 1.05	94.6 ± 1.52	8.15 ± 0.22

Table 4 HPLC enantioseparation on Chirobiotic T (T) and Chiralpak AD (AD-H) columns

Compound	k_1	k_2	α	R_S	Columns
1	9.50	10.01	1.05	1.41	T
1	10.7	11.14	1.04	0.82	AD-H
2	5.49	5.49	1.0	0	T
2	2.10	2.47	1.17	2.30	AD-H
3	4.99	5.28	1.06	1.55	T
3	1.64	1.64	1.00	0	AD-H
4	27.90	27.90	1.00	0	T
4	2.05	2.57	1.22	3.33	AD-H
5	5.85	6.85	1.17	1.94	AD-H
6	8.25	10.22	1.24	3.64	AD -H

Conclusion

The aim of this paper was the study of the relationship between structure and activity of six newly prepared compounds of the aryloxyaminopropanol type. The compounds were derived from 4-hydroxyphenylethanone and 4-hydroxyphenylpropan-1-one, with pyrrolidin-1-yl and 4-methylpiperazin-1-yl in the hydrophilic part of molecule and with ethoxymethyl or methoxyethoxymethyl side chain on the aromatic ring in the lipophilic moiety. The substances were successfully synthesized in a four-step synthesis and in satisfactory yield. The structure and purity of the compounds was established by appropriate analytical techniques, first and foremost by ^{1}H and ^{13}C NMR. The compounds were then subjected to biological testing. It could be shown that all evaluated compounds at the conc. 10^{-6} mol dm^{-3} exhibited vasoconstrictor effect of phenylephrine on isolated rat aorta. The specific antiisoprenaline activity was expressed as pA_2 values, which were in the range from 6.54 to 7.57.

The compounds prepared as racemates could be enantioseparated using chiral HPLC. The results of the enantioseparation were dependent on the chiral column used, thus the compounds with the pyrrolidin-1-yl moiety were most efficiently separated on a Chirobiotic T column, whereas for compounds with the 4-methylpiperazin-1-yl substituent the most effective column was the Chiralpak AD.

Experimental

All HPLC grade solvents were obtained from Merck (Germany). All reactions were carried out using commercial grade reagents and solvents. Diethyl ether was dried by refluxing over potassium hydroxide and sodium followed by distillation.

The melting points were determined using a Kofler Micro Hot Stage instrument. The purity of prepared compounds was assessed using Silufol® UV 254 (Merck) sheets

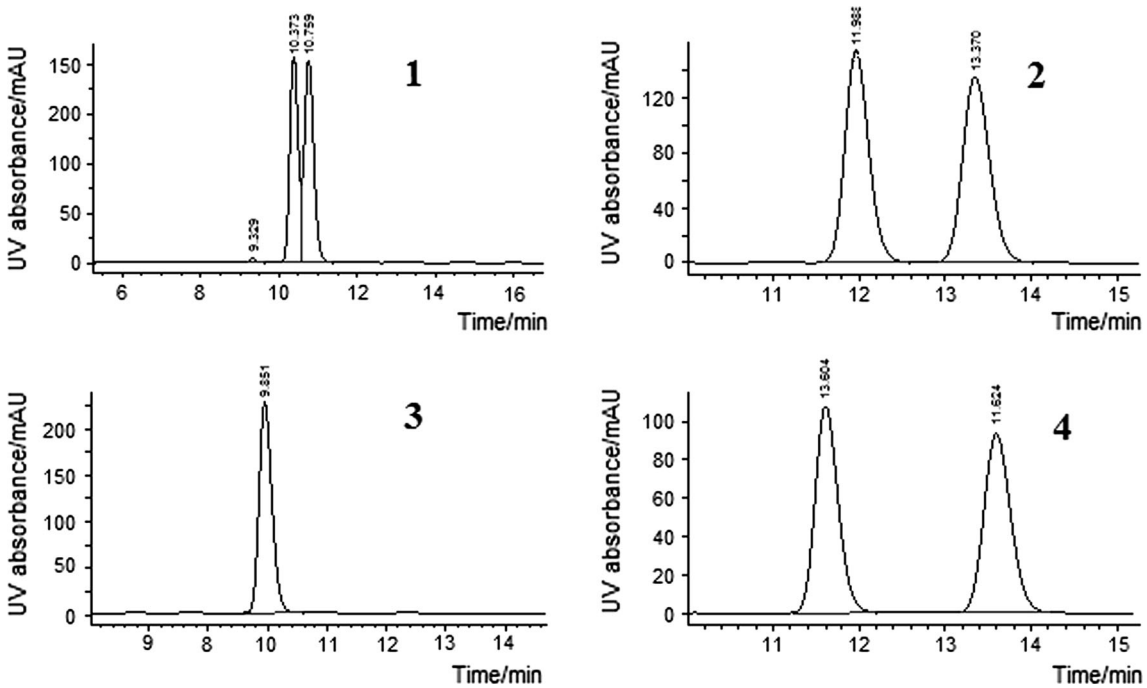


Fig. 1 Representative chromatograms illustrating the enantiomeric resolution of the compounds **1**, **2**, and **4** on the Chiralpak AD CSP (compound **3** shows no resolution on this column). Mobile phase: hexane/ethanol/methanol/ethylethanamine (87/11/11/0.0.1, v/v/v/v), flow rate 0.8 cm^3/min

in the solvent system ethyl acetate/diethylamine (9.5/0.5 v/v). UV spectra were measured on the spectrophotometer GENESYS 10 s UV–Vis in methanol. IR spectra were recorded using Nicolet 6700 (Thermo Scientific). ^{1}H NMR and ^{13}C NMR were recorded on the Varian Gemini 2000 Spectrometer operating at 300 MHz for protons and 75 MHz for carbons. Elemental analysis was carried out on a FLESH 2000 (Thermo Scientific) analyzer and the results were within 0.3% of the theoretical values.

General procedures for (3-chloromethyl-4-hydroxyphenyl)alkanones

To a sulfonation flask set up with mechanical stirrer, contact thermometer, and powder funnel, 0.15 mol of 4-hydroxyphenylalkanone and 90 cm^3 of concentrated HCl were added. The temperature was raised to 45–50 °C and 7.5 g of paraformaldehyde was gradually added. The mixture was subsequently stirred at the same temperature and the reaction was allowed to proceed for 4.5 h. Following the precipitation, the solid product was collected, washed with water, and crystallized from benzene or ethyl acetate.

General procedures for (3-ethoxymethyl-4-hydroxyphenyl)alkanones

To a sulfonation flask equipped with mechanical stirrer, reflux condenser, and thermometer, 0.12 mol of (3-chloromethyl-4-hydroxyphenyl)alkanone and 100 cm^3 of dried ethanol were added. The temperature was raised to 40–50 °C and 19.2 g of solid sodium hydrogen carbonate (0.23 mol) was added. The mixture was stirred for 6 h. Then, $NaHCO_3$ was filtered off and ethanol was removed from the filtrate by distillation. The residue was crystallized from hexane or cyclohexane.

General procedures for [3-alkoxymethyl-4-(3-heterocyclo-2-hydroxypropoxy)phenyl]alkanones

(3-Alkoxymethyl-4-hydroxyphenyl)alkanone (0.15 mol) with 3 mol of chloromethyloxirane and 0.17 mol of 85% KOH were heated at 50–55 °C for 4 h. The unreacted chloromethyloxirane was removed in vacuum and [4-(oxiran-2-ylmethoxy)phenyl]ethanone or 1-[3-(alkoxymethyl)-4-(oxirane-2-ylmethoxy)phenyl]alkanones were extracted into diethylether and isolated as oils.

General procedures for final products

In the last step, 0.08 mol of the oxirane intermediate and 0.16 mol pyrrolidine or *N*-methylpiperazine in 150 cm^3 ethanol were heated for 4 h under reflux. The solvent and the unreacted amine were distilled off, the residue was diluted with 50 cm^3 water and the basic product was then taken into diethyl ether. The final products as salts were prepared by adding fumaric acid into an ether solution of the base.

(2*RS*)-Bis[1-[3-[4-acetyl-2-(ethoxymethyl)phenoxy]-2-hydroxypropyl]pyrrolidinium] fumarate (1, $C_{40}H_{58}O_{12}N_2$) Yield: 43%; m.p.: 128–131 °C (ethyl acetate); R_f = 0.83; IR (solid): $\bar{\nu}$ = 3426 (OH), 1675 (C=O), 1601 (C=C), 1268 (ArOalk) cm^{-1}; UV–Vis (methanol): λ_{max} (log ε) = 218 (3.28), 270 (3.18) nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.22–1.27 (t, J = 7.5 Hz, 3H, OCH_2CH_3), 2.05–2.10 (m, 4H, $CH_2^{pyr3,4}$), 2.54 (s, 3H, $COCH_3$), 3.29–3.41 (m, 6H, NCH_2, $CH_2^{pyr2,5}$), 3.57–3.64 (q, J = 7 Hz, 2H, OCH_2CH_3), 4.12–4.14 (d, 2H, OCH_2CH), 4.35–4.37 (m, 1H, OCH_2CH), 4.59 (s, 2H, $ArCH_2$), 6.66 (s, 2H, CH^{fum}), 7.05–7.07 (d, J = 9 Hz, 1H, CH^{Ar6}), 7.94–8.00 (m, 2H, $CH^{Ar3,5}$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 15.7 (OCH_2CH_3), 24.1 ($C^{pyr3,4}$), 26.6 ($COCH_3$), 55.8 ($CHCH_2N$), 58.9 ($C^{pyr2,5}$), 67.0 (OCH_2CH_3), 67.3 ($CHCH_2N$), 68.4 ($ArCH_2$), 71.8 ($ArOCH_2$), 112.3 (C^{Ar6}), 128.6 (C^{Ar5}), 130.6 (C^{Ar3}), 131.5 (C^{Ar2}), 131.7 (C^{Ar4}), 137.3 (CH^{fum}), 161.7 (C^{Ar1}), 174.6 (COO^-), 199.5 (CO) ppm.

(2*RS*)-Bis[1-[3-[4-acetyl-2-(ethoxymethyl)phenoxy]-2-hydroxypropyl]-4-methylpiperazinium] fumarate (2, $C_{42}H_{64}O_{12}N_4$) Yield: 53%; m.p.: 145–148 °C (ethyl acetate); R_f = 0.46; IR (solid): $\bar{\nu}$ = 3435 (OH), 1670 (C=O), 1602 (C=C), 1259 (ArOalk) cm^{-1}; UV–Vis (methanol): λ_{max} (log ε) = 218 (3.36), 270 (3.02) nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.22–1.27 (m, 3H, OCH_2CH_3), 2.55 (s, 3H, $COCH_3$), 2.68–2.72 (m, 9H, $CH_2^{pip2,6}$, $CHCH_2N$, NCH_3), 3.03–3.07 (m, 4H, $CH_2^{pip3,5}$), 3.57–3.64 (q, J = 7 Hz, 2H, $COCH_2CH_3$), 4.07–4.17 (m, 3H, OCH_2CH), 4.59 (s, 2H, $ArCH_2$), 6.67 (s, 2H, CH^{fum}), 7.05–7.07 (d, J = 9 Hz, 1H, CH^{Ar6}), 7.94–8.00 (m, 2H, $CH^{Ar3,5}$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 15.7 (OCH_2CH_3), 26.6 ($COCH_3$), 44.3 (NCH_3), 52.6 ($CH_2^{pip2,6}$), 54.9 ($CH_2^{pip3,5}$), 61.1 ($CHCH_2N$), 67.4 (CH), 68.4 (OCH_2CH_3), 68.4 ($ArCH_2$), 72.3 ($ArOCH_2$), 112.3 (C^{Ar6}), 128.6 (C^{Ar5}), 130.7 (C^{Ar3}), 131.3 (C^{Ar2}), 131.6 (C^{Ar4}), 136.9 (CH^{fum}), 162.1 (C^{Ar1}), 173.4 (COO^-), 199.5 (CO) ppm.

(2*RS*)-Bis[1-[3-[2-(ethoxymethyl)-4-propanoylphenoxy]-2-hydroxypropyl]pyrrolidinium] fumarate (3, $C_{42}H_{62}O_{12}N_2$) Yield: 51%; m.p.: 130–133 °C (ethyl acetate); R_f = 0.70; IR (solid): $\bar{\nu}$ = 3460 (OH), 1668 (C=O), 1602 (C=C), 1268 (ArOalk) cm^{-1}; UV–Vis (methanol): λ_{max} (log ε) = 218 (3.38), 268 (3.27) nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.14–1.19 (t, J = 7 Hz, 3H, $COCH_2CH_3$), 1.22–1.27 (t, J = 7 Hz, 3H, OCH_2CH_3), 2.05–2.09 (m, 4H, $CH_2^{pyr3,4}$), 2.97–3.04 (q, J = 7 Hz, 2H, $COCH_2CH_3$), 3.29–3.11 (m, 6H, NCH_2, $CH_2^{pyr2,5}$), 3.57–3.64 (q, J = 7 Hz, 2H, OCH_2CH_3), 4.11–4.13 (d, 2H, OCH_2CH), 4.33–4.35 (m, 1H, OCH_2CH), 4.60 (s, 2H, $ArCH_2$), 6.66 (s, 2H, CH^{fum}), 7.08–7.05 (d, J = 9 Hz, 1H, CH^{Ar6}), 7.95–8.01 (m, 2H, $CH^{Ar3,5}$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 8.9 ($COCH_2CH_3$), 15.7 (OCH_2CH_3), 24.1 ($C^{pyr3,4}$), 32.5 ($COCH_2CH_3$), 55.8 ($CHCH_2N$), 58.9 ($C^{pyr2,5}$), 67.0 (OCH_2CH_3), 67.2 ($CHCH_2N$), 68.5 ($ArCH_2$), 71.8 ($ArOCH_2$), 112.4 (C^{Ar6}), 128.6 (C^{Ar5}), 130.5 (C^{Ar3}), 131.2 (C^{Ar4}), 131.3 (C^{Ar2}), 137.2 (CH^{fum}), 161.6 (C^{Ar1}), 174.3 (COO^-), 202.1 (CO) ppm.

(2*RS*)-Bis[1-[3-[2-(ethoxymethyl)-4-propanoylphenoxy]-2-hydroxypropyl]-4-methylpiperazinium] fumarate (4, $C_{44}H_{68}O_{12}N_4$) Yield: 51%; m.p.: 173–175 °C (ethyl acetate); R_f = 0.51; IR (solid): $\bar{\nu}$ = 3432 (OH), 1670 (C=O), 1602 (C=C), 1259 (ArOalk) cm^{-1}; UV–Vis (methanol): λ_{max} (log ε) = 218 (3.38), 270 (3.20) nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.15–1.19 (t, J = 6 Hz, 3H, $COCH_2CH_3$), 1.22–1.27 (t, J = 7.5 Hz, 3H, OCH_2CH_3), 2.72–2.92 (m, 9H, $CH_2^{pip2,6}$, $CHCH_2N$, NCH_3), 2.97–3.02 (q, J = 5 Hz, 2H, $COCH_2CH_3$), 3.18–3.22 (m, 4H, $CH_2^{pip3,5}$), 3.57–3.64 (q, J = 7 Hz, 2H, OCH_2CH_3), 4.12–4.17 (m, 3H, OCH_2CH), 4.59 (s, 2H, $ArCH_2$), 6.71 (s, 2H, CH^{fum}), 7.06–7.08 (d, J = 6 Hz, 1H, CH^{Ar6}), 7.96–8.01 (m, 2H, $CH^{Ar3,5}$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 9.5 ($COCH_2CH_3$), 15.6 (OCH_2CH_3), 32.3 ($COCH_2CH_3$), 43.8 (NCH_3), 52.1 ($CH_2^{pip2,6}$), 54.7 ($CH_2^{pip3,5}$), 60.7 ($CHCH_2N$), 67.2 (CH), 68.2 (OCH_2CH_3), 68.4 ($ArCH_2$), 72.0 ($ArOCH_2$), 112.2 (C^{Ar6}), 128.4 (C^{Ar5}), 130.4 (C^{Ar3}), 131.0 (C^{Ar4}), 131.1 (C^{Ar2}), 135.8 (CH^{fum}), 161.9 (C^{Ar1}), 170.0 (COO^-), 202.0 (CO) ppm.

(2*RS*)-Bis[1-[3-[2-[(2-methoxyethoxy)methyl]-4-propanoylphenoxy]-2-hydroxypropyl]-4-methylpiperazinium] fumarate (5, $C_{44}H_{68}O_{12}N_4$) Yield: 34%; m.p.: 173–175 °C (ethyl acetate); R_f = 0.24; IR (solid): $\bar{\nu}$ = 3402 (OH), 1675 (C=O), 1601 (C=C), 1261 (ArOalk) cm^{-1}; UV–Vis (methanol): λ_{max} (log ε) = 218 (3.38), 270 (3.20) nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.37 (s, 3H, $COCH_3$), 2.51–2.56 (m, 9H, $CH_2^{pip2,6}$, $CHCH_2N$, NCH_3), 3.24–3.32 (m, 4H, $CH_2^{pip3,5}$), 3.39 (s, 3H, OCH_3), 3.61–3.65 (m, 4H, CH_2CH_2O), 3.98–4.07 (m, 3H, OCH_2CH), 4.54 (s, 2H, $ArCH_2$), 6.61 (s, 2H, CH^{fum}), 7.0–7.1 (d, J = 7 Hz, 1H, CH^{Ar5}), 7.90–7.93 (m, 2H, $CH^{Ar2,6}$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 26.8 ($COCH_3$), 43.4 (NCH_3), 51.3 ($CH_2^{pip2,6}$), 53.1 ($CH_2^{pip3,5}$), 58.6 (OCH_3), 60.2 ($CHCH_2N$), 66.7 (CH), 67.2 (CH_2OCH_2), 69.9 ($ArOCH_2$), 71.4 ($ArCH_2$), 71.8 (CH_2OCH_3), 111.5 (C^{Ar5}), 127.4 (C^{Ar6}), 128.7 (C^{Ar2}), 129.9 (C^{Ar3}), 130.3 (C^{Ar1}), 134.9 (CH^{fum}), 160.2 (C^{Ar4}), 173.4 (COO^-), 196.8 (CO) ppm.

(2*RS*)-Bis[1-[3-(4-acetylphenoxy)-2-hydroxypropyl]-4-methylpiperazinium] fumarate (6, $C_{36}H_{52}O_{10}N_4$) Yield: 65%; m.p.: 197–200 °C (ethyl acetate); R_f = 0.52; IR (solid): $\bar{\nu}$ = 3326 (OH), 1671 (C=O), 1600 (C=C), 1261 (ArOalk) cm^{-1}; UV–Vis (methanol): λ_{max} (log ε) = 218 (3.48), 272 (3.49) nm; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.40 (s, 3H, $COCH_3$), 2.49–2.67 (m, 13H, CH_2N, $CH_2^{pip2,3,5,6}$, NCH_3), 3.95–4.08 (m, 3H, OCH_2CH), 6.60 (s, 2H, CH^{fum}), 7.02–7.05 (d, J = 9 Hz, 2H, $CH^{Ar2,6}$), 7.91–7.94 (d, J = 9 Hz, 2H, $CH^{Ar3,5}$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ = 26.9 ($COCH_3$), 44.6 (NCH_3),

52.4 ($\underline{C}H_2^{pip2,6}$), 54.1 ($\underline{C}H_2^{pip3,5}$), 60.6 (CH$\underline{C}H_2$N), 66.8 ($\underline{C}$H), 71.6 (ArO$\underline{C}H_2$), 110.0 ($C^{Ar2,6}$), 114.8 ($C^{Ar4}$), 131.0 ($C^{Ar3,5}$), 136.4 ($\underline{C}H^{fum}$), 163.0 ($C^{Ar1}$), 173.6 ($\underline{C}OO^-$), 199.6 ($\underline{C}$O) ppm.

Pharmacological evaluation

β-Adrenolytic activity of the synthesized compounds was evaluated on isolated rat's atria and expressed as pA_2 values against tachycardia, induced by isoprenaline, according to [26]. The vasodilatory activity was evaluated on phenylephrine-induced contraction of rat aortal strips. The inhibitory effect on phenylephrine-induced contraction of isolated aorta was expressed as % of inhibition ± SEM and mean effective concentration of phenylephrine in the presence of tested compounds at previously determined concentration (EC_{50} ± SEM) [27]. Each value of pharmacological evaluation represents the mean ± SEM from 5 to 7 experiments.

All animal care and experimental procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the local Committee for Animals.

HPLC analysis

HPLC studies were carried out on the HPLC system AGILENT 1200 with a quaternary pump and a diode detector, using the chiral stationary phases (Chiralpak AD) based on amylase tris(3,5-dimethylphenylcarbamate) (0.46 × 25). The mobile phases consisted of hexane/ethanol/methanol/diethylamine 87/11/11/0.1, v/v/v/v. Samples for analyses were prepared as approximately 1 mg/cm^3 solution in methanol. Compounds were diluted in the ratio 1:4 (1–4) and 1:10 (5–6). Separations were carried out at the flow rate of 0.8 cm^3/min and the column temperature was maintained at 25 °C. Chromatograms were scanned at the wavelength 267 ± 8 nm.

Second HPLC studies were performed with a Hewlett-Packard (series 1100) HPLC system consisting of a quaternary pump equipped with an injection valve (Rheodyne) and diode array detector. The macrocyclic chiral stationary phase was Chirobiotic T (250 × 4 mm LD-particle size 5 µm Advanced Separation technologies. Inc. USA). The mobile phase was a mixture of methanol/acetonitrile/acetic acid/triethylamine 45/55/0.3/0.2) v/v/v/v. The separation was carried out at the flow rate of 1 cm^3/min and the column temperature was 23 °C. The chromatograms were scanned at 270 nm. The injection volume was 20 mm^3. The analyte was dissolved in methanol (concentration 1 mg/cm^3).

Chromatographic characteristics

The separation factor was expressed as $\alpha = k_1/k_2$, where k_1, k_2 are retention factors for the first and second eluting enantiomers. The retention factors k' were calculated as follows: $k_1 = (t_1 - t_0)/t_0$ and $k_2 = (t_2 - t_0)/t_0$, where t_0, t_1, and t_2 are the dead elution time and elution times of enantiomers 1 and 2. The stereochemical resolution factor (R_S) of the first and second eluting enantiomer was calculated as the ratio of the difference between the retention times t_1 and t_2 to the arithmetic sum of the two peaks' widths w_1 and w_2: $R_S = 2(t_2 - t_1)/(w_1 + w_2)$.

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