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Eur. Pharm. J. 2020, 67(2), 34-44.
ISSN 1338-6786 (online) and ISSN 2453-6725 (print version),
DOI: 10.2478/afpuc-2020-0012

EUROPEAN PHARMACEUTICAL JOURNAL

Synthesis, antimicrobial and antiradical activity of (3-alkoxymethyl-4-hydroxyphenyl)propan-1-ones, intermediates of biologically active compounds and activity comparison with 3-(alkoxymethyl)-4-(alkylamino-2-hydroxypropoxyphenyl)alkanones type of beta blockers

Original Paper

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Received 4 April, 2020, accepted 23 January, 2021

Abstract A homologous series of (3-alkoxymethyl-4-hydroxyphenyl)propan-1-ones was prepared by the reaction of (3-chloromethyl-4-hydroxyphenyl)propan-1-ones with the corresponding alcohols (methanol – decan-1-ol, propan-2-ol, 2-methylpropan-1-ol, 3-methylbutan-1-ol, cyclopentanol, benzylalcohol) in the presence of sodium hydrogen carbonate. The composition of the synthesised compounds was elucidated by IR, UV and ^{1}H-NMR and ^{13}C-NMR spectra. Selected compounds were tested against human pathogens: gram-positive bacterium *Staphylococcus aureus* (CNCTC Mau 29/58), gram-negative bacterium *Escherichia coli* (CNCTC 377/79) and yeast *Candida albicans* (CCM 8186). Their antimicrobial activities were expressed as minimum inhibitory concentrations. Antioxidant activity was determined using DPPH and $ABTS^{\cdot+}$ methods. It could be shown that both biological activities, antimicrobial and antioxidant, were lower in comparison with the (2*RS*)-bis [3-(4-acetyl-2-propoxymethyl)phenoxy-2-hydroxypropyl]isopropylammonium fumarate type of beta blockers.

Keywords *substituted phenols – antimicrobial activity – antioxidant activity – antiradical activity*

INTRODUCTION

The introduction of a phenolic group into drug molecules confers upon them a reactive functionality with acidic nature. Phenols can form chelates with metal ions (Hider et al., 2001; Fernandez et al., 2002) and are able to bind to basic functional groups of proteins, hence their broad spectrum of biological activities, such as antimicrobial (Taguri et al., 2004; Taguri et al., 2006; Cueva et al., 2010; Park et al., 2001) and antioxidative activity (Sroka & Cisowski, 2003; Bendary et al., 2013).

The bioactivity of phenols and their toxicity are both influenced by the number of phenolic groups and their relative position (Calliste et al., 2001; Amouar et al., 2009; Kadoma et al., 2010).

Fujisawa (Fujisawa et al., 2004) studied antioxidative activity of 2-methoxy- and 2-*tert*-butylphenols having up to three substituents on the aromatic ring. It could be shown that antioxidative activity decreases as a result of exchange of the methoxymethyl group against a methyl group. Derivatives with a *tert*-butyl group exhibited marked increase in antioxidative activity.

Kadoma et al. (2008) and Kadoma et al. (2009) investigated, besides antioxidative properties, also the cytotoxicity of 2- or 2,6-*tert*-butylphenols and 2-methoxyphenols in several cancer cell lines. Both antioxidative and cytotoxic activities followed similar structure–activity relationship. The introduction of a *tert*-butyl group as well as dimerisation led to a distinct increase in cytotoxicity.

The structurally similar eugenol (2-methoxy-4-(prop-2-en-1-yl)phenol) exhibits antibacterial (Devi et al., 2010), antifungal (Morcia et al., 2012; Abbaszadeh et al., 2014), antioxidant

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(Fujisawa et al., 2002), local anaesthetic (Park et al., 2001) and anti-depressant activities (Irie et al., 2004).

Herein, we report a two-stage synthesis of the target compounds starting from 4-hydroxyphenylpropan-1-one (Table 1 and Figure 1). In the first step, the electrophilic substitution reaction of 4-hydroxyphenylpropan-1-one with paraformaldehyde and hydrochloric acid yields 1-[3-(chloromethyl)-4-hydroxy-phenyl]propan-1-one. Nucleophilic substitution of this intermediate by corresponding alcohols leads to 1-[-3-(alkoxymethyl)-4-hydroxyphenyl]propan-1-ones, where the substituent can be an aliphatic alkyl with chain length C_1–C_{10}, cyclopentyl and phenylmethyl (benzyl).

Many of these substances find application as intermediates in the synthesis of biologically active compounds of the aryloxyaminopropanol type with beta adrenoceptor blocking, antiarrhythmic and anticonvulsive activity (Čižmáriková et al., 1985; Čižmáriková et al., 1986; Čižmáriková et al., 2003).

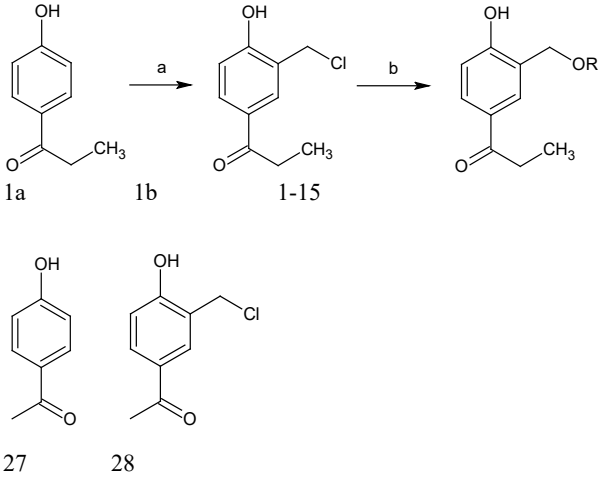


Figure 1. Synthetic route for derivatives of propiophenone and chemical structures of derivatives of acetophenone.

EXPERIMENTAL

The melting points were determined using a Kofler micro hot stage and were quoted uncorrected. Elemental analysis was carried out on a FLASH 2000 (Thermo Scientific) analyser, and the results were within 0.3% of the theoretical values.

The purity of newly prepared compounds was assessed by TLC using Silufol® UV 254 (Merck) sheets with the mobile phase cyclohexane/ethyl acetate (8:2 v/v). UV spectra were recorded on the spectrometer Hewlett-Packard 8452 in methanol. IR spectra were measured using FTIR IMPACT 400 D (Nicolet) 6700. ^{1}H-NMR were recorded on Varian Gemini 2000 spectrometer operating at 3,000 MHz for protons.

Synthesis

(3-Chloromethyl-4-hydroxyphenyl)propan-1-one (1b) (Čižmáriková et al., 1991)

To a sulfonation flask setup with mechanical stirring contact thermometer and powder funnel, 0.15 mol of 4-hydroxyphenylpropan-1-one (1a) and 90 cm^3 of concentrated HCl were added. The temperature was subsequently maintained, the mixture was stirred and the reaction was allowed to proceed for 4.5 h. Following the precipitation, the solid product was collected using suction filtration, washed with water and crystallised from benzene or ethyl acetate. M.p. 132-5°C, yield 75% (da Re & Verlichi, 1956) m.p. 133-6°C, yield 57%).

(3-Alkoxymethyl-4-hydroxyphenyl)propan-1-one (1-15) (Čižmáriková et al., 1991)

To a sulfonation flask setup with mechanical stirring, reflux condenser and contact thermometer, 0.12 mol (chloro-4-hydroxyphenyl)propan-1-one and 100 cm^3 of dried corresponding alcohol were added. The temperature was raised to 40–50°C, and 19.2 g (0.23 mol) of sodium hydrogen carbonate was added gradually during 1 h. The products were crystallised from heptane.

(2RS)- bis[3-(4-acetyl-2-propoxymethyl)phenoxy-2-hydroxypropyl]isopropylammonium fumarate

0.57 mol chloromethyloxirane was gradually added to a solution of 0.55 mol (3-alkoxymethyl-4-hydroxyphenyl) ethanone in 0.59 mol potassium hydroxide dissolved in 50 cm^3 water. The stirred mixture was left to react at room temperature for 24 h, the product was extracted with diethyl ether or chloroform, the extract was washed with 5% sodium hydroxide and water. The organic layer was dried with magnesium sulphate, and the solvent was evaporated. The residue formed by 4-(2,3-epoxypropoxy)-3-(alkoxymethyl) ethanone (cca. 60% yield) was dissolved without previous purification in ethanol or propan-1-ol (50 cm^3) and reacted with isopropylamine (10 cm^3). The mixture was kept at 30°C for 3 h and then under reflux for 4 h. The solvent and unreacted isopropylamine was removed under reduced pressure, the residue was diluted with water (25 cm^3) and the base was extracted to diethyl ether. The extract was dried with potassium carbonate. Addition of an ethereal solution of fumaric acid resulted in separation of the salt, which was crystallised from an appropriate solvent.

EP1 1-(3-methoxymethyl-4-hydroxyphenyl)propan-1-one (1)

$C_{11}H_{14}O_3$ M_r 194.23, Anal. calcd. %C 68.02, %H 7.27 found %C 68.30, %H 7.20. Yield: 63%, R_F 0.53, Mp. 78–80°C (heptane), IR (cm^{-1}) 3,236 (νOH$_{asoc.}$), 1,653 (νC=O), 1,596 (νC=C), 1,274 (νCOC), UV (CH_3OH, λ in nm, ε in m^2.mol^{-1}) λ_{max} 222 nm (log ε_1 3.24), 273 (log ε_2 3.25)

^{1}H-NMR ($CDCl_3$): 1.18–1.23 (t, 3H, $COCH_2CH_3$), 2.90–2.99 (q, 2H, $COCH_2CH_3$), 3.47 (s, 3H, CH_2OCH_3), 4.72 (s, 2H, Ar-CH_2O), 6.89–6.92 (d, 1H, CH_{AR}^6), 7.70–7.71 (d, 1H, CH_{AR}^3), 7.83–7.87 (dd, 1H, CH_{AR}^5), 8.16 (s, 1H, ArOH)

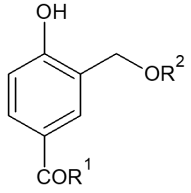


Table 1. Overview of the studied 1-[3-(alkoxymethyl)-4-hydroxyphenyl]alkanones.

Substance Number	Description	R1	R2
1	EP1	CH_2CH_3	CH_3
2	EP2	CH_2CH_3	CH_2CH_3
3	EP3	CH_2CH_3	$(CH_2)_2CH_3$
4	EP3i	CH_2CH_3	$CH(CH_3)_2$
5	EP4n	CH_2CH_3	$(CH_2)_3CH_3$
6	EP4i	CH_2CH_3	$CH_2CH(CH_3)_2$
7	EP5n	CH_2CH_3	$(CH_2)_4CH_3$
8	EP5i	CH_2CH_3	$(CH_2)_2CH(CH_3)_2$
9	EP6n	CH_2CH_3	$(CH_2)_5CH_3$
10	EP7n	CH_2CH_3	$(CH_2)_6CH_3$
11	EP8n	CH_2CH_3	$(CH_2)_7CH_3$
12	EP9n	CH_2CH_3	$(CH_2)_8CH_3$
13	EP10n	CH_2CH_3	$(CH_2)_9CH_3$
14	EP5c	CH_2CH_3	Cyclopentyl
15	EPbenz	CH_2CH_3	CH_2phenyl
16	EA1	CH_3	CH_3
17	EA2	CH_3	CH_2CH_3
18	EA3n	CH_3	$CH_2CH_2CH_3$
19	EA3i	CH_3	$CH(CH_3)_2$
20	EA4n	CH_3	$(CH_2)_3CH_3$
21	EA5n	CH_3	$(CH_2)_4CH_3$
22	EA7n	CH_3	$(CH_2)_6CH_3$
23	EA8n	CH_3	$(CH_2)_7CH_3$
24	EA9n	CH_3	$(CH_2)_8CH_3$
25	EA5c	CH_3	Cyclopentyl
26	EAbenz	CH_3	CH_2phenyl
27	4-OHacet		
28	EAch		

^{13}C-NMR ($CDCl_3$): 8.45 ($COCH_2CH_3$), 31.34 ($COCH_2CH_3$), 58.49 (CH_2OCH_3), 73.97 (Ar-CH_2O), 116.39 (C_{AR}^6), 121.90 (C_{AR}^3), 128.53 (C_{AR}^2), 129.32 (C_{AR}^5), 130.19 (C_{AR}^4), 160.60 (C_{AR}^1), 199.43 (CO)

EP2 1-(3-ethoxymethyl-4-hydroxyphenyl)propan-1-one (2) $C_{12}H_{16}O_3$ M_r 208.26, Anal. calcd. %C 69.21, %H 7.74 found % C69.01, %H 7.93. Yield: 57%, R_F 0.52, Mp. 65–67°C (heptane), IR (cm^{-1}) 3,236 ($\nu OH_{asoc.}$), 1,653 (νC=O), 1,596 (νC=C), 1,274 (νCOC), UV (CH_3OH, λ in nm, ε in $m^2.mol^{-1}$); λ_{max} 201 nm (log ε_1 3.29), 216 (log ε_2 3.26), 264 (log ε_3 3.22)

^{1}H-NMR ($CDCl_3$): 1.17–1.21 (t, 3H, $COCH_2CH_3$), 1.22–1.26 (t, 3H, CH_{3alk}^2), 2.92–2.95 (q, 2H, $COCH_2CH_3$), 3.59–3.67 (q, 2H, $CH_{2\,alk}^1$), 4.76 (s, 2H, Ar-CH_2O), 6.89–6.92 (d, 1H, CH_{AR}^6), 7.69–7.70 (d, 1H, CH_{AR}^3), 7.82–7.85 (dd, 1H, CH_{AR}^5), 8.39 (s,1H, ArOH)

^{13}C-NMR ($CDCl_3$): 9.08 ($COCH_2CH_3$), 15.60 (C_{alk}^2), 32.28 ($COCH_2CH_3$), 67.25 (Ar-CH_2O), 68.60 (C_{alk}^1), 115.89 (C_{AR}^6), 126.33

(C_{AR}^{2}), 129.89 (C_{AR}^{5}), 130.91 (C_{AR}^{4}), 131.12 (C_{AR}^{3}), 161.53 (C_{AR}^{1}), 202.23 (CO)

EP3n 1-(4-hydroxy-3-propoxymethylphenyl)propan-1-one (3)

$C_{13}H_{18}O_3$ M_R=222.29 Anal. calcd. %C 70.24 %H 8.16 found %C 70.54, %H 8.32. Yield: 65%, R_F 0.55 (Mp. 61–63°C (heptane), IR (cm^{-1}), 3,248 ($\nu OH_{asoc.}$), 1,686 ($\nu C=O$), 1,602 ($\nu C=C$), 1,272 (νCOC), UV (CH_3OH, λ in nm ε in $m^2.mol^{-1}$); λ_{max} 227 nm (log ε_1 3.01), 264 (log ε_2 3.132), 277 (log ε_3 3.10)

^{1}H-NMR ($CDCl_3$): 0.93–0.98 (t, 3H, $CH_{3\ alk}^{3}$), 1.13–1.18 (t, 3H, $COCH_2CH_3$), 1.61–1.68 (m, 2H, $CH_{3\ alk}^{2}$), 2.92–3.00 (q, 2H, $COCH_2CH_3$), 3.51–3.55 (t, 2H, $CH_{3\ alk}^{1}$), 4.75 (s, 2H, Ar-CH_2O), 6.82–6.85 (d, 1H, CH_{AR}^{6}), 7.69–7.70 (d, 1H, CH_{AR}^{3}), 7.82–7.91 (dd, 1H, CH_{AR}^{5}), 8.43 (s,1H, ArOH)

^{13}C-NMR ($CDCl_3$): 9.08 ($COCH_2CH_3$), 11.12 (C_{alk}^{3}), 24.05 (C_{alk}^{2}), 32.28 ($COCH_2CH_3$), 68.74 (Ar-CH_2O), 73.62 (C_{alk}^{1}), 115.87 (C_{AR}^{6}), 126.41 (C_{AR}^{2}), 129.86 (C_{AR}^{5}), 130.85 (C_{AR}^{4}), 131.02 (C_{AR}^{3}), 161.74 (C_{AR}^{1}), 199.81(CO)

EP3i 1-(4-hydroxy-3-isopropoxymethylphenyl)propan-1-one (4)

$C_{13}H_{18}O_3$ M_r 222.29 Anal. calcd. % C 70.24% H 8.16 found % C 70.54, % H 8.32. Yield: 65%, R_F 0.55, Mp. 61-63°C (heptane), IR (cm^{-1}), 3,248 ($\nu OH_{asoc.}$), 1,686 ($\nu C=O$), 1,602 ($\nu C=C$), 1,272 (νCOC), UV (CH_3OH, λ in nm, ε in $m^2.mol^{-1}$); λ_{max} 226 nm (log ε_1 3.14), 263 (log ε_2 3.16), 276 (log ε_3 3.12)

^{1}H-NMR: 1.17–1.23 (t, 3H, $COCH_2CH_3$), 1.25–1.27 (d, 6H, $CH(CH_3)_2$), 2.89–2.97 (q, 2H, $COCH_2CH_3$), 3.74–3.82 (m, 1H, CH), 4.76 (s, 2H, Ar-CH_2O), 6.88–6.91 (d, 1H, CH_{AR}^{6}), 7.68–7.69 (d, 1H, CH_{AR}^{3}), 7.81–7.84 (dd, 1H, CH_{AR}^{5}), 8.57 (s,1H, ArOH)

^{13}C-NMR: 9.07 (COCH2CH3), 22.55 ($CH(CH_3)_2$), 32.23 ($COCH_2CH_3$), 66.25 (Ar-CH_2O), 73.04 (CH), 115.80 (C_{AR}^{6}), 126.70 (C_{AR}^{3}), 129.82 (C_{AR}^{4}), 130,72 (C_{AR}^{5}), 130.90 (C_{AR}^{2}), 161.39 (C_{AR}^{1}), 202.11 (CO)

EP4n 1-(3-butyloxymethyl-4-hydroxyphenyl)propan-1-one (5)

$C_{14}H_{20}O_3$ M_r=236.36, Anal. calcd. %C 71.16 %H 8.53 found %C 71.40, %H 8.52. Yield: 69%, R_F 0.57, Mp. 68–70°C (heptane), IR (cm^{-1}), 3,340 ($\nu OH_{asoc.}$), 1,684 ($\nu C=O$), 1,600 ($\nu C=C$), 1,270 (νCOC), UV (CH_3OH, λ in nm, ε in $m^2.mol^{-1}$); λ_{max} 201 (log ε_1 3.23), 217 (log ε_2 3.22), 264 (log ε_3 3.13); 277 (log ε_4 3.20)

^{1}H-NMR ($CDCl_3$): 0.91–0.96 (t, 3H, $CH_{3\ alk}^{4}$), 1.13–1.18 (t, 3H, $COCH_2CH_3$), 1.41–1.46 (m, 2H, $CH_{2\ alk}^{3}$), 1.56–1.64 (m, 2H, $CH_{2\ alk}^{2}$), 2.92–3.00 (m, 2H, $COCH_2CH_3$), 3.52–3.57 (t, 2H, CH_{2alk}^{1}), 4.54 (s, 2H, Ar-CH_2O), 6.81–6.86 (d, 1H, CH_{AR}^{6}), 7.80–7.86 (d, 1H, CH_{AR}^{3}), 7.95–7.98 (dd, 1H, CH_{AR}^{5}), 8.39 (s,1H, ArOH)

^{13}C-NMR ($CDCl_3$): 9.10 ($COCH_2CH_3$), 14.39 (C^{alk4}), 20.55 (C_{alk}^{3}), 32.25 (C_{alk}^{2}), 33.02 ($COCH_2CH_3$), 68.78 (Ar-CH_2O), 71.67 (C_{alk}^{1}), 115.88 (C_{AR}^{6}), 126.41 (C_{AR}^{2}), 129.87 (C_{AR}^{5}), 130.85 (C_{AR}^{4}), 131.04 (C_{AR}^{3}), 161.51 (C_{AR}^{1}), 202.21 (CO)

EP4i 1-(4-hydroxy-3-isobutyloxymethylphenyl)propan-1-one (6)

$C_{14}H_{20}O_3$ M_r 236.36 Anal. calcd. %C 71.97 %H 8.86 found %C 72.30, %H 8.70. Yield: 62%, R_F 0.62, Mp. 52–54°C (heptane), IR (cm^{-1}) 3344 ($\nu OH_{asoc.}$), 1656 ($\nu C=O$), 1592 ($\nu C=C$), 1277 (νCOC), UV (CH_3OH, λ in nm, ε in $m^2.mol^{-1}$); λ_{max} 203 (log ε_1 3.26), 222 (log ε_2 3.28), 274 (log ε_3 3.20)

^{1}H-NMR ($CDCl_3$): 0.94–0.96 (d, 6H, $CH(CH_3)_2$), 1.19–1.21 (t, 3H, $COCH_2CH_3$), 1.90–1.99 (m, 1H, $CH(CH_3)_2$), 2.92–2.67 (q, 2H, $COCH_2CH_3$), 4.75 (s, 2H, Ar-CH_2O), 6.89–6.90 (d, 1H, CH_{AR}^{6}), 7.68–7.69 (d, 1H, CH_{AR}^{3}), 7.82–7.92 (dd, 1H, CH_{AR}^{3}), 8.38 (s,1H, ArOH)

^{13}C-NMR ($CDCl_3$): 9.11 ($COCH_2CH_3$), 19.91 ($CH(CH_3)_2$), 29.80 ($CH(CH_3)_2$), 32.29 ($COCH_2CH_3$), 68.91 (Ar-CH_2O), 78.84 (CH_2-CH), 115.86 (C_{AR}^{6}), 126.52 (C_{AR}^{2}), 129.87 (C_{AR}^{5}), 130.79 (C_{AR}^{4}), 130.92 (C_{AR}^{3}), 161.47 (C_{AR}^{1}), 202.25 (CO)

EP5n 1-(4-hydroxy-3-pentyloxymethylphenyl)propan-1-one (7)

$C_{15}H_{22}O_3$ M_r 250.34, Anal. calcd. % C 71.97 %H 8.86 found %C 72.20, %H 8.60. Yield: 68%, R_F 0.60, Mp. 30–32°C (heptane), IR (cm^{-1}) 3340 ($\nu OH_{asoc.}$), 1684 ($\nu C=O$), 1604 ($\nu C=C$), 1272 (νCOC). UV (CH_3OH, λ in nm, ε in $m^2.mol^{-1}$); λ_{max} 202 (log ε_1 2.90), 222 nm (log ε_2 3.25), 274 (log ε_3 3.21)

^{1}H-NMR ($CDCl_3$): 0.88–0.93 (t, 3H, $CH_{3\ alk}^{5}$), 1.18–1.23 (t, 3H, $COCH_2CH_3$), 1.32–1.35 (m, 4H, $CH_{2\ alk}^{3,4}$), 1.61–1.70 (m, 2H, $CH_{2\ alk}^{2}$), 2.90–2.97 (q, 2H, $COCH_2CH_3$), 3.54–3.58 (t, 2H, $CH_{2\ alk}^{1}$), 4.75 (s, 2H, Ar-CH_2O), 6.89–6.92 (d, 1H, CH_{AR}^{6}), 7.69–7.70 (d, 1H, CH_{AR}^{3}), 7.82–7.90 (dd, 1H, CH_{AR}^{5}), 8.41(s,1H, ArOH)

^{13}C-NMR ($CDCl_3$): 9.08 ($COCH_2CH_3$), 14.57 ($C_{al}k^{5}$), 23.70 (C_{alk}^{4}), 29.65 (C_{alk}^{3}), 30.57 (C_{alk}^{2}), 32.26 ($COCH_2CH_3$), 68.76 (Ar-CH_2O), 71.95 (Calk^1), 115.85 (C_{AR}^{6}), 126.37 (C_{AR}^{2}), 129.82 (C_{AR}^{5}), 130.80 (C_{AR}^{4}), 130.98 (C_{AR}^{3}), 161.46 (C_{AR}^{1}), 202.11 (CO)

EP5i 1-(4-hydroxy-3-isopentyloxymethylphenyl)propan-1-one (8)

$C_{15}H_{22}O_3$ M_r 250.34, Anal. calcd. % C 71.97 %H 8.86 found %C 72.30, %H 8.70. Yield: 63%, R_F 0.58, Mp. 68–70°C (heptane), IR (cm^{-1}), 3,342 ($\nu OH_{asoc.}$), 1,683 ($\nu C=O$), 1,604 ($\nu C=C$), 1,273 (νCOC), UV (CH_3OH, λ in nm, ε in $m^2.mol^{-1}$); λ_{max} 204 (log ε_1 3.18), 223 nm (log ε_2 3.15), 274 (log ε_3 3.23)

^{1}H-NMR ($CDCl_3$): 0.88–0.90 (d, 6H, $CH(CH_3)_2$), 1.18–1.20 (t, 3H, $COCH_2CH_3$), 1.51–1.56 (m, 1H, $CH(CH_3)_2$), 2.92–2.95 (q, 2H, $COCH_2CH_3$), 4.75 (s, 2H, Ar-CH_2O), 6.89–6.92 (d, 1H, CH_{AR}^{6}), 7.69–7.70 (d, 1H, CH_{AR}^{3}), 7.82–7.86(dd, 1H, CH_{AR}^{5}), 8.40 (s,1H, ArOH)

^{13}C-NMR ($CDCl_3$): 9.27 ($COCH_2CH_3$), 19.91 ($CH(CH_3)_2$), 29.80 ($CH(CH_3)_2$), 32.13 ($COCH_2CH_3$), 70.49 (Ar-CH_2O), 77.82 (CH_2-CH), 117.15 (C_{AR}^{6}), 123.03 (C_{AR}^{2}), 129.11 (C_{AR}^{5}), 129.97 (C_{AR}^{4}), 130.86 (C_{AR}^{3}), 161.50 (C_{AR}^{1}), 200.35 (CO)

EP6n 1-(3-hexyloxymethyl-4-hydroxyphenyl)propan-1-one (9)

$C_{16}H_{24}O_3$ M_r 264.36, Anal. calcd. %C 72.69 %H 9.15 found %C 72.77, %H 8.90. Yield: 60%, R_F 0.61, Mp. 51–53°C (heptane), IR (cm^{-1}), 3,340 ($\nu OH_{asoc.}$), 1,684 ($\nu C=O$), 1,600 ($\nu C=C$), 1,272 (νCOC). UV (CH_3OH, λ in nm, ε in $m^2.mol^{-1}$) λ_{max} 202 (3.26), 216 nm (log ε_1 3.25), 264 (log ε_2 3.24)

^{1}H-NMR ($CDCl_3$): 0.89–0.93 (m, 3H, $CH_{3\ alk}^{6}$), 1.13–1.17 (t, 3H, $COCH_2CH_3$), 1.28–1.40 (m, 8H, $CH_{2\ alk}^{3,4,5,}$), 1,58–1,62 (m, 2H, $CH_{2\ alk}^{2}$), 2.94–3.01 (q, 2H, $COCH_2CH_3$), 3.50–3.53 (t, 2H, $CH_{2\ alk}^{1}$), 4.73 (s, 2H, Ar-CH_2O), 6.82–6.84 (d, 1H, CH_{AR}^{6}), 7.78–7.81 (d, 1H, CH_{AR}^{3}), 7.85–7.88 (dd, 1H, CH_{AR}^{5}), 8.39 (s,1H, ArOH)

^{13}C-NMR ($CDCl_3$): 9.13 ($COCH_2CH_3$), 14.56 (C_{alk}^{6}), 23.82 (C_{alk}^{5}), 27.40 (C_{alk}^{4}), 30.40 (Calk^3), 30.90 (C_{alk}^{5}), 32.32 (C_{alk}^{2}), 33.15 ($COCH_2CH_3$), 68.75 (Ar-CH_2O), 71.93 (C_{alk}^{1}), 115.90 (C_{AR}^{6}), 126.42 (C_{AR}^{2}), 129.83 (C_{AR}^{5}), 130.85 (C_{AR}^{4}), 131.08 (C_{AR}^{3}), 161.60 (C_{AR}^{1}), 202.20 (CO)

EP7n 1-(3-heptyloxymetyl-4-hydroxyphenyl)propan-1-one (10)

$C_{17}H_{26}O_3$ M_r 278.19, Anal. calcd. %C 73.35 %H 9.41 found %C 73.10, %H 9.20. Yield: 57%, R_F 0.56, Mp. 47-49°C (heptane), IR (cm^{-1}), 3,352 ($\nu OH_{asoc.}$), 1,686 ($\nu C=O$), 1,602 ($\nu C=C$), 1,272 (νCOC), UV (CH_3OH, λ in nm, ε in $m^2.mol^{-1}$); λ_{max} 202 (3.30), 222 nm (log ε_1 3.31), 264 (log ε_2 3.24)

^{1}H-NMR ($CDCl_3$): 0.86–0.90 (m, 3H, CH_3 alk^7), 1.14–1.19 (t, 3H, $COCH_2CH_3$), 1.29–1.41 (m, 8H, $CH_{2\ alk}^{3,4,5,6}$), 1.59–1.64 (m, 2H, $CH_{2\ alk}^{2}$), 2.90–2.97 (q, 2H, $COCH_2CH_3$), 3.54–3.58 (t, 2H, $CH_{2\ alk}^{1}$), 4.76 (s, 2H, Ar-CH_2O), 6.89–6.93 (d, 1H, CH_{AR}^{6}), 7.69–7.70 (d, 1H, CH_{AR}^{5}), 7.83–7.86 (dd, 1H, CH_{AR}^{5}), 8.38 (s,1H, ArOH)

^{13}C-NMR ($CDCl_3$): 9.11 ($COCH_2CH_3$), 14.59 (C_{alk}7), 23.84 (C_{alk}^{6}), 27.42 (C_{alk}^{4}), 30.41 (Calk^3), 30.91 (C_{alk}^{5}), 32.30 (C_{alk}^{2}), 33.17 ($COCH_2CH_3$), 68.75 (Ar-CH_2O), 71.93 (C_{alk}^{1}), 115.92 (C_{AR}^{6}), 126.44 (C_{AR}^{2}), 129.85 (C_{AR}^{5}), 130.87 (C_{AR}^{4}), 131.09 (C_{AR}^{3}), 161.61 (C_{AR}^{1}), 202.23 (CO)

EP8n 1-(4-hydroxy-3-octyloxymetylphenyl)propan-1-one (11)

$C_{18}H_{28}O_3$ M_r 292.45, Anal. calcd. %C 73.93 %H 9.65 found %C 73.7 0, %H 9.45. Yield: 56%, R_F 0.57, Mp. 54-57°C (heptane), IR (cm^{-1}), 3,352 ($\nu OH_{asoc.}$), 1,686 ($\nu C=O$), 1,600 ($\nu C=C$), 1,272 (νCOC). UV (CH_3OH λ in nm, ε in $m^2.mol^{-1}$); λ_{max} 206 (3.26), 222 nm (log ε_1 3.29), 274 (log ε_2 3.25)

^{1}H-NMR ($CDCl_3$): 0.86–0.90 (t, 3H, $CH_{3\ alk}^{8}$), 1.18–1.206 (t, 3H, $COCH_2CH_3$), 1.21–1.28 (m, 10H, $CH_{2\ alk}^{3-7}$), 1.60–1.65 (m, 2H, CH_{2alk}^{2}), 2.90–2.98 (m, 2H, $COCH_2CH_3$), 3.54–3.58 (t, 2H, CH_{2alk}^{1}), 4.75 (s, 2H, Ar-CH_2O), 6.89–6.92 (d, 1H, CH_{AR}^{6}), 7.69–7.92 (d, 1H, CH_{AR}^{3}), 7.83–7.91 (dd, 1H, CH_{AR}^{5}), 8.40 (s,1H, ArOH)

^{13}C-NMR ($CDCl_3$): 9.09 ($COCH_2CH_3$), 14.57 (C^{alk8}), 23.86 (C_{alk}^{7}), 27.45 (C_{alk}^{6}), 30.57 (C_{alk}^{5}), 30.67 (C_{alk}^{4}), 30.88 (C_{alk}^{3}), 32.29 (C_{alk}^{2}) 33.14 ($COCH_2CH_3$), 68.73 (Ar-CH_2O), 71.91 (C_{alk}^{1}), 115.88 (C_{AR}^{6}), 126.40 (C_{AR}^{2}), 129.86 (C_{AR}^{5}), 130.82 (C_{AR}^{4}), 131.07 (C_{AR}^{3}), 161.51 (C_{AR}^{1}), 202.17 (CO)

EP9n 1-(4-hydroxy-3-nonyloxymethylphenyl)propan-1-one (12)

$C_{19}H_{30}O_3$ M_r 306.45, Anal. calcd. %C 74.47 %H 9.87 found %C 74.10 %H 9.50. Yield: 67%, R_F 0.60, Mp. 59-61°C (heptane), IR (cm^{-1}), 3,343 ($\nu OH_{asoc.}$), 1,671 ($\nu C=O$), 1,600 ($\nu C=C$), 1,275 (νCOC), UV (CH_3OH, λ in nm, ε in $m^2.mol^{-1}$); λ_{max} 206 (3.23), 222 nm (log ε_1 3.26), 275 (log ε_2 3.23)

^{1}H-NMR ($CDCl_3$): 0.86–0.92 (t, 3H, $CH_{3\ alk}^{9}$), 1.18–1.21 (t, 3H, $COCH_2CH_3$), 1.24–1.27 (m, 12H, $CH_{2\ alk}^{3-8}$), 1.57–1.64 (m, 2H, $CH_{2\ alk}^{2}$), 2.92–3.00 (m, 2H, $COCH_2CH_3$), 3.51–3.55 (t, 2H, $CH_{2\ alk}^{1}$), 4.74 (s, 2H, Ar-CH_2O), 6.81–6.86 (d, 1H, CH_{AR}^{6}), 7.79–7.83 (d, 1H, CH_{AR}^{5}), 7.84–7.86 (dd, 1H, CH_{AR}^{5}), 8.38 (s,1H, ArOH)

^{13}C-NMR ($CDCl_3$): 9.10 ($COCH_2CH_3$), 14.61 (C_{alk}^{9}), 23.88 (C_{alk}^{8}), 27.45 (C_{alk}^{7}), 30.57 (C_{alk}^{6}), 30.72 (C_{alk}^{5}), 30.87 (C_{alk}^{4}), 30.89 (C_{alk}^{3}), 32.29 (C_{alk}^{2}), 33.20 ($COCH_2CH_3$), 68.75 (Ar-CH_2O), 71.93 (C_{alk}^{1}), 115.89 (C_{AR}^{6}), 126.40 (C_{AR}^{2}), 129.85 (C_{AR}^{5}), 130.81 (C_{AR}^{4}), 131.03 (C_{AR}^{3}), 161.50 (C_{AR}^{1}), 202.12 (CO)

EP10n 1-(3-decyloxymethyl-4-hydroxyphenyl)propan-1-one (13)

$C_{20}H_{32}O_3$ M_r 320.48, Anal. calcd. %C 74.96 %H 10.06 found %C 74.80 %H 9.90. Yield: 62%, R_F 0.62, Mp. 52-54°C (heptane), IR (cm^{-1}), 3,343 ($\nu OH_{asoc.}$), 1,670 ($\nu C=O$), 1,602 ($\nu C=C$), 1,272 (νCOC), UV (CH_3OH, λ in nm, ε in $m^2.mol^{-1}$); λ_{max} 222 (log ε_1 3.26), 275 (log ε_2 3.23)

^{1}H-NMR ($CDCl_3$): 0.86–0.91 (t, 3H, $CH_{3\ alk}^{10}$), 1.13–1.18 (t, 3H, $COCH_2CH_3$), 1.26–1.40 (m, 12H, $CH_{2\ alk}^{3-9}$), 1.57–1.64 (m, 2H, CH_{2alk}^{2}), 2.88–3.00 (m, 2H, $COCH_2CH_3$), 3.51–3.55 (t, 2H, $CH_{2\ alk}^{1}$), 4.54 (s, 2H, Ar-CH_2O), 6.83–6.87 (d, 1H, CH_{AR}^{6}), 7.69–7.70 (d, 1H, CH_{AR}^{3}), 7.74–7.86 (dd, 1H, CH_{AR}^{5}), 8.40 (s,1H, ArOH)

^{13}C-NMR ($CDCl_3$): 9.10 ($COCH_2CH_3$), 14.62 (C_{alk}^{10}) 23.89 (C_{alk}^{9}), 27.46 (C_{alk}^{8}), 30.62 (C_{alk}^{7}, 30.67 (C_{alk}^{6}), 30.81 (C_{alk}^{5}), 30.86 (C_{alk}^{4}), 30.89 (C_{alk}^{3}), 32.29 (C_{alk}^{2}), 33.22 ($COCH_2CH_3$), 68.74 (Ar-CH_2O), 71.93 (C_{alk}^{1}), 115.88 (C_{AR}^{6}), 126.40 (C_{AR}^{2}), 129.84 (C_{AR}^{5}), 130.80 (C_{AR}^{4}), 131.02 (C_{AR}^{3}), 161.49 (C_{AR}^{1}), 202.17 (CO)

EP5c 1-(4-hydroxy-3-cyclopentyloxymethylphenyl) propan-1-one (14)

$C_{15}H_{20}O_3$ M_r 248.32, Anal. calcd. %C 72.55 %H 8.12 found %C 72.30, %H 8.30, Yield: 62%, R_F 0.62 Mp. 68-69°C (heptane), IR (cm^{-1}), 3,255 ($\nu OH_{asoc.}$), 1,657 ($\nu C=O$), 1,601 ($\nu C=C$), 1,250 (νCOC). UV (CH_3OH, λ in nm, ε in $m^2.mol^{-1}$); λ_{max} 203 (log ε_1 3.17), λ_{max} 222 (log ε_1 3.20), 274 (log ε_3 3.19)

^{1}H-NMR ($CDCl_3$): 1.19–1.22 (t, 3H, $COCH_2CH_3$), 1.59; 1.74–1.79 (m, m, 2H, 6H, $CH_2^{alk2,3,4,5}$), 2.91–2.96 (q, 2H, $COCH_2CH_3$), 4.08 (m, 1H, CH^{alk1}), 4.73 (s, 2H, Ar-CH_2O), 6.89–6.91 (d, 1H, CH_{AR}^{6}), 7.69–7.70 (d, 1H, CH_{AR}^{3}), 7.82–7.85 (dd, 1H, CH_{AR}^{5}), 8.54 (s, 1H, Ar-OH)

^{13}C-NMR ($CDCl_3$): 9.09 ($COCH_2CH_3$), 24.66 ($C_{alk}^{3,4}$), 32.28 ($COCH_2CH_3$), 33.37 (Calk^{2,5}), 66.82 (Ar-CH_2O), 82.96 (C_{alk}^{1}), 115.82 (C_{AR}^{6}), 126.70 (C_{AR}2), 129.87 (C_{AR}^{5}), 130.77 (C_{AR}^{4}), 131.03 (CAR^3), 161.47 (C_{AR}^{1}), 202.23 (CO)

EPbenzyl 1-(3-phenylmethoxymethyl-4-hydroxyphenyl) propan-1-one (15)

$C_{17}H_{18}O_3$ M_r 270, Anal. calcd. %C 75.53 %H 6.71 found %C 75.40, %H 6.50. Yield: 62%, R_F 0.62, Mp. 52-54°C (heptane), IR (cm^{-1}), 3,361 ($\nu OH_{asoc.}$), 1,667 ($\nu C=O$), 1,593 ($\nu C=C$), 1,278 (νCOC). UV (CH_3OH, λ in nm, ε in $m^2.mol^{-1}$); λ_{max} 206 (log ε_1 3.39), λ_{max} 219 (log ε_2 3.29), 274 (log ε_3 3.19)

^{1}H-NMR **(**$CDCl_3$**):** 1.19–1.22 (t, 3H, $COCH_2CH_3$), 2.90–2.96 (q, 2H, $COCH_2CH_3$), 4.62 (s, 2H, CH_2-phenyl), 4.79 (s, 2H, Ar-CH_2O), 6.92–6.94 (d, 1H, $CH_{AR}{}^6$), 7.33–7.40 (m, 5H, phenyl), 7.70–7.71 (d, 1H, $CH_{AR}{}^3$), 7.85–7.87 (dd, 1H, $CH_{AR}{}^5$), 8.16 (s, 1H, Ar-OH)

^{13}C-NMR ($CDCl_3$): 8.94 ($COCH_2CH_3$), 32.13 ($COCH_2CH_3$), 68.11 (Ar-CH_2O), 73.60 (CH_2-phenyl), 115.75 ($C_{AR}{}^6$), 126.07 ($C_{phenyl}{}^4$), 128.72 ($C_{AR}{}^2$), 128.96 ($C_{phenyl}{}^{2,6}$), 129.39 ($C_{phenyl}{}^{3,5}$), 129.73 ($C_{AR}{}^5$), 130.78 ($C_{AR}{}^4$), 131.01 ($C_{AR}{}^3$), 139.55 ($C_{phenyl}{}^1$), 161.36 ($C_{AR}{}^1$), 202.06 (CO)

ANTIMICROBIAL ACTIVITY

Antimicrobial activity of prepared (3-alkoxymethyl-4-hydroxyphenyl)propan-1-ones was evaluated *in vitro* and expressed as the minimum inhibitory concentration (MIC). It was determined using the standard broth dilution method (Valentová et al., 2018). The following strains of gram-positive, gram-negative bacteria and a yeast pathogen were selected for the experiments: *Staphylococcus aureus* CNCTC Mau 29/58, *Escherichia coli* CNCTC 377/79 and *Candida albicans* CCM 8186, respectively. Tested bacterial strains were purchased from Czech National Collection of Type Cultures (Prague, Czech Republic); yeast was obtained from Czech Collection of Microorganisms (Brno, Czech Republic). For the sake of comparison, antibacterial activity of the antibiotic ciprofloxacin **(**1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid) was also evaluated.

ANTIOXIDATIVE ACTIVITY

DPPH assay (Brand-Williams et al., 1995)

The evaluation of antioxidative capacity by this method is based on the redox reaction of the tested compounds with the stable radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH). The solution of this radical is purple coloured with maximum absorption at 517 nm. In the course of the reduction of DPPH, the solution changes its colour from purple to yellow, resulting in corresponding shift in UV-VIS spectrum. The lower the measured absorption, the higher the antioxidative capacity of the tested compound.

A solution of DPPH in methanol was prepared, in the concentration 44 μg/ml (112 μM). Subsequently, solution of the tested sample in methanol in the concentration 10^{-2} mol. dm^{-3} or 10^{-3} mol. dm^{-3} was prepared. For the spectrophotometric assay, 270 mL of the DPPH solution and 30 mL of tested compound solution or standard were mixed, and the absorbance using a microplate reader was determined at 517 nm at 5 min after mixing. The absorbance at each time point was corrected for the absorbance of a DPPH blank. Three parallel measurements were made for each sample. Trolox was used as a standard for measured antioxidant activity of the target compounds.

ABTS assay (Re et al., 1999)

Antiradical activity was measured as % inhibition of $ABTS^{+}$. Aqueous solutions of ABTS (7.7 μg/ml, 14 mM) and $K_2S_2O_8$ (1.32 mg/ml, 4.9 mM) were prepared. These two solutions were mixed in a 1:1 vol. ratio and allowed to stand for 24 h in the refrigerator. The spectrophotometric measurement was carried out using a 96-well plate reader. Each well on the microplate was filled with 60 μl of sample solution (10^{-2} or 10^{-3} $mol.dm^{-3}$, respectively), and 240 μl of ABTS solution. Absorbance was assessed spectrophotometrically at 734 nm at 5 min after mixing. For each sample, three parallel measurements were made. During the reaction, the colourless 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid is oxidised by potassium peroxydisulfate, yielding the stable blue-green $ABTS^{\bullet+}$ radical. The addition of antioxidants leads to reduction of the $ABTS^{\bullet+}$ radical and discolouration of the solution.

DISCUSSION

The aim of the presented research was the synthesis of (3-alkoxymethyl-4-hydroxyphenyl)propan-1-ones (Table 1), screening of antimicrobial and antioxidant activities of selected products, as well as comparison of the results with the previously prepared (3-alkoxymethyl-4-hydroxyphenyl) ethanones and (2*RS*)-bis [3-(4-acetyl-2-propoxymethyl) phenoxy-2-hydroxypropyl]isopropylammonium fumarate with beta-blocking activity.

The compounds 1–15 (Table 1) were synthesised by an established two-step procedure from 4-hydroxyphenylpropan-1-one (Čižmáriková et al., 1991). During the first stage, (3-chloromethyl-4-hydroxyphenyl)propan-1-one was prepared in 57% yield via electrophilic substitution reaction. This intermediate reacts in the second step of the synthesis with the respective alcohol in the presence of $NaHCO_3$ to give (3-alkoxymethyl-4-hydroxyphenyl)propan-1-ones in 60% yield. The products are white solids with mp between 30 and 70°C. Their purity was checked by TLC on silica, the mobile phase consisting of cyclohexane/ethyl acetate in 8:2 v/v ratio. The structure of the final (3-alkoxymethyl-4-hydroxyphenyl) propan-1-ones 1-15 was confirmed by spectral analysis. The following bands could be assigned in the infrared spectra: 3,236–3,352 cm^{-1} ($\nu OH_{asoc.}$), 1,653–1,686 cm^{-1} ($\nu C=O$), 1,596–1,604 cm^{-1} ($\nu C=C$) and 1,270–1,275 cm^{-1} (νCOC). In the ^{1}H and ^{13}C-NMR spectra, the signals of the aromatic ring, the propanoyl and the alkoxymethyl groups were identified. Two or four bands can be seen in the UV spectra, corresponding to π-π* transitions at 202–206, 216–227, 264 and 274–277 nm.

Table 2. Antimicrobial activity of 1-[3-(alkoxymethyl)-4-hydroxyphenyl]alkanones.

Substance Number	Description	MIC (mmol/L) E.coli	MIC (mmol/L) S. aureus
2	EP2	5.01	5.01
3	EP3n	2.46	n
6	EP4i	1.06	n
9	EP6n	0.38	n
12	EP9n	1.43	1.43
13	EP10n	0.77	0.13
14	EP5c	1.29	0.65
15	EPbenz	1.25	0.31
1a	4OHPr	n	n
18	EA4n	0.31	n
20	EA7n	0.94	n
21	EA8n	0.09	n
22	EA5c	4.11	2.05
28	EAch	0.53	n

n = non-measurable values, compounds not active against Candida albicans

Table 3. Antioxidant activities of 1-[3-(alkoxymethyl)-4-hydroxyphenyl]alkanones.

Substance Number	Description	Inhibition DPPH [%]±SD	Inhibition ABTS [%]±SD
1	EP1	n	3.9 ±0.04
2	EP2	2.5±0.1	8.5±0.4
3	EP3n	0.6±0.01	11.9±0.4
4	EP3i	3.7±0.02	28.1±0.3
6	EP4i	2.4±0.7	10.2±0.2
7	EP5n	1.8±1.3	1.3 ±0.4*
8	EP5i	12.6±0.6	5.8±0.7*
11	EP8n	6.2±0.7	n
13	EP10n	6.3±2.1	n
14	EP5c	3.3±0.03	25.0±1.6
15	EPbenzyl	3.3±0.9	1.5 ±0.3*
16	EA1	n	6.1±0.2
17	EA2	n	1.4±1.0
18	EA3n	n	6.1±0.7
19	EA3i	n	7.5±0.2
20	EA4n	n	1.5± 1.0
21	EA5n	3.9±0.8	24.8±0.8
22	EA7n	n	0.9±0.2*
23	EA8n	n	n
24	EA9n	3.9±0.7	n
25	EA5c	n	8.4±0.7
26	EAbenzyl	1.59±1.4	16.7±0.7
27	4-OHacet	n	0.9±0.01
28	EAch	4.32±2.5	8.2±0.4*

**concentration 10^{-3} mol.dm^{-3} n = non-measurable values*

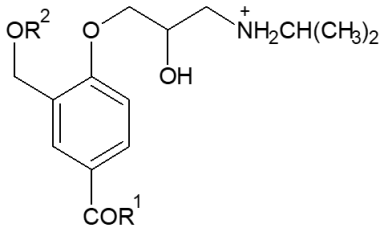
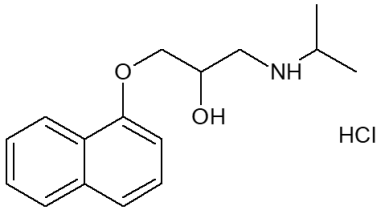


Table 4. Antioxidative activity of (2RS)-bis [3-(4-acetyl-2-alkoxymethyl)phenoxy-2-hydroxypropyl]isopropylammonium fumarate exhibiting beta-blocking activity.

Description	R1	R2	Inhibition DPPH [%]±SD	Inhibition ABTS [%]±SD
FA23i	CH_3	CH_2CH_3	N	99.0±1.3
FA5n3i	CH_3	$(CH_2)_4CH_3$	N	48.5±0.8
FA5i3i	CH_3	$CH_2CH_2CH(CH_3)_2$	N	67.2±1.4
FA7n3i	CH_3	$(CH_2)_6CH_3$	0.4±0.04	52.2±1.3
FA8n3i	CH_3	$(CH_2)_7CH_3$	N	89.8±3.7
FA9n3i	CH_3	$(CH_2)_8CH_3$	N	86.7±5.3
FAB3i	CH_3	CH_2phenyl	1.6±0.04	n
Propranolol			4.6±1.9	97.5±2.3

n = non-measurable values



propranolol

Table 5. Antimicrobial activity of (2RS)- bis [3-(4-propionyl-2-alkoxymethyl)phenoxy-2-hydroxypropyl]isopropylammonium fumarate.

Description	R1	R2	MIC(mmol/L) *E. coli*	MIC(mmol/L) *S. aureus*	MIC(mmol/L) *C. albicans*
FpP4n3i	CH_3CH_2	$(CH_2)_3CH_3$	N	0.61	n
FpP5n3i	CH_3CH_2	$(CH_2)_4CH_3$	N	0.35	n
FpP6n3i	CH_3CH_2	$(CH_2)_5CH_3$	0.34	0.08	0.23
FpP7n3i	CH_3CH_2	$(CH_2)_6CH_3$	0.22	0.03	0.07
FpP9n3i	CH_3CH_2	$(CH_2)_8CH_3$	0.20	0.01	0.01
FpA5n3i	CH_3	$(CH_2)_4CH_3$	N	0.61	n
FpA6n3i	CH_3	$(CH_2)_5CH_3$	0.35	0.08	0.24
FpA7n3i	CH_3	$(CH_2)_7CH_3$	0.23	0.07	0.23
FpA8n3i	CH_3	$(CH_2)_7CH_3$	0.22	0.03	0.08
FpA9n3i	CH_3	$(CH_2)_8CH_3$	0.21	0.01	0.02
ciprofloxacin			3.10^{-4}	$6.89.10^{-4}$	n

n = non-measurable values

The compounds 16–26 (Table 1) derived from 4-hydroxyphenylethanones are described in (Čižmáriková et al., 2002).

Antimicrobial activity of selected final products (Table 2) was tested against gram-negative bacterium (*Escherichia coli*), gram-positive bacterium (*Staphylococcus aureus*) and human fungal pathogen (*Candida albicans*).

The comparison between the tested compounds showed that maximum activity against *E. coli* can be found in the propanone derivative with the hexyloxymethyl-side chain (EP6n, MIC 0.38 mmol/L). Ethanone derivative with the butoxymethyl group (EA4n, MIC 0.31 mmol/L) showed similar activity. Comparison of activities of ethanone (EA5c) and propanone (EP5c) derivatives with the cyclopentyloxymethyl moiety revealed that the activity of the propanone derivative was higher (MIC 1.29 mmol/L) than the activity of the ethanone derivative (MIC 4.11 mmol/L). Prolongation of the alkyl chain caused an increase in activity from ethoxymethyl up to nonyloxymethyl (EP2 through EP9n).

Similar observation was made also with *S. aureus*. The highest effect was shown by the compound with decyloxymethyl substituent (EP10n, MIC 0.13 mmol/L). The compounds EP2 and EP10n exerted comparable activity both in *E. coli* and *S. aureus*. None of the tested compounds exhibited substantial activity against *C. albicans*.

Published data suggest that compounds with one or several phenolic hydroxyls act as radical scavengers and exert antioxidative activity. Hence, they impede oxidative stress, a condition that can be the main cause of numerous diseases, especially those of the cardiovascular system. The antioxidative activities of the prepared compounds were evaluated using methods based on DPPH (1,1-diphenyl-2-picrylhydrazyl) and $ABTS^{\cdot+}$ (2,2´-azinobis(3-ethylbenzothiazoline-6-sulfonic acid). The DPPH method involves the reaction between the antioxidant and the stable radical DPPH•, which acts as acceptor of hydrogen. The solution of this radical has intensely purple hue, caused by an unpaired electron of the hydrazyl group. Its reaction with the antioxidant yields the reduced form DPPH-H, and the solution discolours in the course of this reaction. The degree of antioxidant activity is determined from the decrease of absorbency of the solution at 517 nm wavelength. The ABTS method employs oxidation of the colourless 2,2´ azinobis(3-ethylbenzothiazoline-6-sulfonic acid) by potassium peroxydisulfate, yielding the stable blue-green radical-cation $ABTS^{\bullet+}$. The addition of antioxidants to such a solution leads to reduction of the $ABTS^{\bullet+}$ radical and discolouration of the solution.

The antioxidant activity of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) used as standard was determined along with the activities of investigated products. The values of antioxidative activities (Table 3) determined by the DPPH method were generally lower than those provided by the ABTS methods, in some cases, even below the detection threshold. The antioxidative activities of the products were in the range 0.6–4.3%, with the exception of the substance EP5i, in which case, it was 12.6%. The ABTS method provided activities in the range 0.9–28.1%, the highest activity being found in the substance EP3i with isopropoxymethyl substituent (28.1%). Neither of the methods provides a clear dependency between the activity and the length of the alkyl substituent.

The majority of the compounds in the group of 3-alkoxymethyl-4-hydroxyphenylethanones (16–26) did not show any activity detectable by the DPPH method. The highest activity provided by the ABTS method was found in the compound with pentyloxymethyl substituent (EA5n, 24.8%).

Low ABTS value was shown also by the parent structure 4-hydroxyphenylethanone. 3-Chloromethyl-4-hydroxyphenylethanones exerted higher activity both by the DPPH (4.4%) and ABTS (8.2%) methods (Table 3).

The activities correspond to the values for antioxidative activities of phenols reported in the literature. Compounds with only one hydroxy group in the molecule exhibit only minor antioxidant effects, markedly higher values being shown by compounds with two or more hydroxy groups (Sroka & Cisowski, 2003).

Several previously reported compounds with beta-adrenolytic effect (Čižmáriková et al., 1985; 1986; 2003) were tested for comparison. The compounds exerted marked anti-isoprenaline activity with negative chronotropic, dromotropic and inotropic effects. Optimum antiarrhythmic and anticonvulsive activity was found in derivatives with methoxymethyl and propoxymethyl groups. To assess the antioxidant activity, (2*RS*)-bis[3-(4-acetyl-2-propoxymethyl) phenoxy-2-hydroxypropyl]isopropylammonium fumarate with varying length of the alkoxymethyl chain was selected. The ABTS method was found to be more convenient compared to the DPPH assay. The antioxidative (Table 4) activities acquired by the ABTS method were in the range 52.2–99.0%, the highest effect being observed in ethoxymethyl (FA23i, 99.0%) and octyloxymethyl (FA8n3i, 89.8%) derivatives (Table 4). Similarly to the investigated substances, the antioxidative activity of the standard compound propranolol appraised by the ABTS method (4.6%) was higher in comparison with DPPH.

In some cases (EP5, EPbenzyl, EA7, EAchlormet), the antioxidant activities were assessed at a lower concentration (10^{-3} mol.dm^{-3}). (2*RS*)-bis[3-(4-acetyl-2-propoxymethyl) phenoxy-2-hydroxypropyl]isopropylammonium fumarate was more active than the phenolic derivatives, suggesting potentiation of antioxidative activity as a result of introduction of isopropylaminopropanol moiety into the molecule. The published results implicate correlation between the antioxidant activity, connected to reduction in oxidative stress and therapeutic effect in cardiovascular disease.

The antimicrobial activity (Table 5) of selected (2*RS*)-bis[3-(4-acetyl-2-propoxymethyl)phenoxy-2-hydroxypropyl] isopropylammonium fumarate in tested microbial strains was higher in comparison with the intermediate etanones and

propanones. This increase in activity stems from the elevated lipophilicity connected to prolongation of the alkoxymethyl chain. In both series, the highest effect on all tested strains was observed in compounds with nonyloxymethyl substituent in the side chain. In comparison with the standard ciprofloxacin, the antimicrobial activities were inferior in both groups.
Considering both types of bioactivity, the antimicrobial effects (in *S. aureus* and *E. coli*) and the antioxidant activity (by ABTS assay), were both higher in comparison with the (2*RS*)-bis [3-(4-acetyl-2-propoxymethyl)phenoxy-2-hydroxypropyl] isopropylammonium fumarate with beta-blocking activity.

ACKNOWLEDGEMENTS

This work has been supported by the Scientific Grants Agency of the Slovak Republic (VEGA 1/0145/20).

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