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Synthesis and antimicrobial activity of a series of optically active quaternary ammonium salts derived from phenylalanine

Research Article

Miloš Lukáč[1]*, Ivan Lacko[1], Marián Bukovský[2], Zuzana Kyselová[1], Janka Karlovská[3], Branislav Horváth[4], Ferdinand Devínsky[1]

[1]*Department of Chemical Theory of Drugs, Faculty of Pharmacy, Comenius University, Bratislava 832 32, Slovakia*
[2]*Department of Cellular and Molecular Biology of Drugs, Faculty of Pharmacy, Comenius University, Bratislava 832 32, Slovakia*
[3]*Department of Physical Chemistry of Drugs, Faculty of Pharmacy, Comenius University, Bratislava 832 32, Slovakia*
[4]*Institute of Chemistry, Faculty of Natural Science, Comenius University, Bratislava 842 15, Slovakia*

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Abstract: We synthesized nine quaternary ammonium compounds (QUATs) starting from phenylalanine, *N*-alkyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromides, which were prepared as optically pure substances. Five compounds were prepared as *S*-enantiomers and four compounds as *R*-enantiomers. These compounds were evaluated by their activities against bacteria and fungi. Three microbial strains were used in the study: the gram-negative bacteria *Escherichia coli*, the gram-positive bacteria *Staphylococcus aureus* and the fungi *Candida albicans*. The activities were expressed as minimum bactericidal or fungicidal concentrations (MBC). The most active compounds were (*2S*)-*N*-tetradecyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromide and (*2R*)-N-tetradecyl-N,N-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromide, with MBC values exceeding those of commercial benzalkoniumbromide (BAB) used as standard. The relationships between structure and biological activity of the tested QUATs were quantified by the bilinear model (QSAR) and are discussed.

Keywords: *Microbicidal activity • Enantiomers • Phenylalanine • Quaternary ammonium compounds • Quantitative structure activity relationships (QSAR)*

1. Introduction

For a long time, positively charged compounds such as quaternary ammonium salt derivatives have been widely used as disinfectants in medicine, agriculture and industry. As more and more resistant organisms continue to emerge in the environment, identification of additional antimicrobial agents is becoming increasingly important [1,2]. It is expected that ideal antimicrobial agents for wide use should fulfill some of the following conditions. They have to possess strong antibacterial activity, be safe in relation to humans, and should not persist in the environment for a long time. Some of the quaternary ammonium compounds (QUATs) fulfill these conditions [3]. The mode of the antimicrobial action of QUATs lies in their interaction with cytoplasmic membrane of bacteria or fungi. QUATs show a generalized damage of cytoplasmic membrane so that the positively charged "head" of the molecule interacts with the negatively charged membrane components followed by penetration of the non-polar tenside constituent into its hydrophobic part. The crucial first step of membrane destruction is the reduction of its electrical potential by Coulombic interactions with the ammonium head

* *E-mail: lukac@fpharm.uniba.sk*

[4]. The "neutralization" of the negative charge of the membrane and interactions with the lipidic constituents with the hydrophobic tail of the molecule causes changes in the architecture and consequently in the fluidity of the membrane. Finally, the membrane loses its permeability, leakage follows and the intracellular constituents are released from the cell [4-8]. Other types of surfactants like nonionic or anionic detergents can increase susceptibility to QUATs via increasing membrane permeability (*Escherichia coli*) or they can reduce susceptibility to QUATs by lowering cell surface hydrophobicity (*Listeria monocytogenes*) [9]. Bjergbæk *et al*. investigated the effect of oxygen limitation, glucose-starvation and temperature on the susceptibility of *Escherichia coli* towards the quaternary ammonium biocide benzalkonium chloride. They discovered that increasing temperatures, the absence of oxygen, and energy substrates increased the antimicrobial effect of benzalkonium chloride towards *E. coli* [10]. Marcotte *et al.* investigated the influence of the QUATs head group and alkyl chain with different length on their permeability-perturbing power and on their affinity for lipidic membranes. Three series of QUATs were used: benzalkonium, alkylpyridinium and tetraalkylammonium derivates. They found that QUATs bearing C_{16} long chain were more efficient in decreasing the membrane permeability than their C_{12} analogues. In contrast, the chemical nature of the ammonium head group had practically no influence on the permeability perturbations caused by QUATs bearing C_{16} chains [11]. The antimicrobial activity of QUATs is closely related to their surfactant properties [12-15]. The common structure of QUATs is a long alkyl chain attached to a polar "head-group" that is formed by the ammonium cation portion of the molecule. Structure-activity relationship studies on a variety of synthetic QUATs showed that the long alkyl chain and ammonium cation may represent the minimal structural requirements for sufficient antimicrobial effects. Optimal antimicrobial activity is achieved at lengths of the alkyl chain ranging from 10 to 18 carbons [16]. The correlation between critical micelle concentration (cmc) and minimum inhibitory concentration (MIC) as a function of alkyl chain length show that compounds of the QUAT type with cmc in range $1 \cdot 10^{-2}$ to $1 \cdot 10^{-4}$ mol.dm^{-3} exhibit the best antimicrobial activity [17]. In a homologous series of QUATs, the antimicrobial activity increases progressively with increasing chain length up to a critical point beyond which the activity decreases. This behaviour is called the cut-off effect. It is a general feature of many of biologically active compounds, especially those with a repeating structural motive such as a long hydrophobic alkyl chain (*e.g.* alcohols, QUATs). There are many reasons for the existence of a cut-off in the biological activity of a given surfactant. These include limited aqueous solubility, kinetic effects, interaction with proteins or lipid bilayers [18,19]. QUATs are not only active against diseases caused by bacteria or fungi, but also exhibit antiprotozoal [20,21], antineoplastic [22-24] and immunomodulatory activity [25]. They act as neuromuscular relaxants [26], as selective inhibitors of Aβ fibril formation [27], or as antagonists to neuronal nicotinic acetylcholine receptors mediating dopamine release [28,29]. Recently QUATs and polycationic compounds have been intensively investigated as possible non-viral vectors for gene transfection [30-33].

QUATs derived from phenylalanine possess interesting physico-chemical properties. Diego-Castro *et al.* used (*2S*)-*N*-hexadecyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium chloride and (*2S*)-*N*-hexadecyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromide to study the enantioselective Diels-Alder cycloaddition reaction with cyclopentadiene and nonyl acrylate [34]. Our laboratory has also synthesized some QUATs derivatives of phenylalanine. It was found that racemic (3-dodecanoyloxy-1-phenylpropane-2-yl)trimethyl-ammonium bromide exhibits lower critical micelle concentration than its S-enantiomer [35]. In the last few years homochiral QUATs derived from other amino acids have also been synthesized [36-39].

Although the mode of action of QUATs is by destruction of the bacteria cell membrane(s), some fine structural features *e.g.* optical activity could have some influence on processes playing a crucial role during the inactivation of microorganism. If the QUATs are able to act via other mechanisms than nonspecific destruction of membrane, e.*g*. action via enzymes, they will have important roles in antimicrobial activity. Therefore we prepared and characterized a series of some novel optically pure active QUATs (*R* and *S* isomers) and tested their antimicrobial activity against different representatives of microorganisms (G^- and G^+ bacteria, fungi). The starting material for the synthesis of bioactive compounds was phenylalanine.

2. Experimental procedure

2.1. Materials and methods

All chemicals used for synthesis were purchased from commercial suppliers. BAB was obtained from Fluka, GmbH. Blood agar base No.2, Sabouraud agar, Nutrien broth No.2 and Sabouraud medium were purchased from Imuna Pharm a.s. Slovakia. The elemental analyses of C, H and N were performed using a Carlo Erba analyser. Melting points of the compounds synthesized were determined using a

Kofler Micro Hot Stage and were uncorrected. NMR spectra were recorded on a Varian Gemini 2000 spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C at 20°C with tetramethylsilane used as internal standard. Infrared spectra were recorded on a FT-IR Impact 400 D spectrophotometer as potassium bromide discs. Optical rotations of compounds were measured in ethanol solution c = 1.1 on a JASCO P1010 polarimeter. The purity of prepared compounds were examined by TLC plates technique (DC-Alufolien Kiesegel 60 F_{256}, Merck).

2.2. Synthesis of (2S)-N-alkyl-N,N-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromide and (2R)-N-alkyl-N,N-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromide

(*2S*)-2-Amino-3-phenyl-1-propanol **1** and (*2R*)-2-Amino-3-phenyl-1-propanol **2** were prepared as previously described [40].

(*2S*)-*N*,*N*-dimethyl-2-amino-3-phenylpropan-1-ol **3** and (*2R*)-*N*,*N*-dimethyl-2-amino-3-phenylpropan-1-ol **4** were prepared by a previously reported method [34].

(*2S*)-*N*-decyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromide **5S**. (*2S*)-*N*,*N*-Dimethyl-2-amino-3-phenyl-1-propanol (5.74 g, 32.0 mmol) and 1-bromodecane (8.36 g, 32.0 mmol) were added to 25 mL of acetonitrile, then heated under reflux for 16 h. The reaction mixture was cooled to room temperature and evaporated. Diethyl ether was added to precipitate the ammonium salt. The precipitate was filtered off and the crude product was recrystallized repeatedly from acetone-petrolether mixture.

(*2S*)-*N*-dodecyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromide **6S**, (*2S*)-*N*-tetradecyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromide **7S**, (*2S*)-*N*-hexadecyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromide **8S**, (*2S*)-*N*-octadecyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromide **9S**, (*2R*)-*N*-decyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromide **5R**, (*2R*)-*N*-dodecyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromide **6R**, (*2R*)-*N*-tetradecyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromide **7R**, (*2R*)-*N*-hexadecyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromide **8R** were prepared by the same procedure as **5S**, by alkylation of tertiary amine with the corresponding 1-bromoalkane.

2.3. Bactericidal and fungicidal studies

The microbicidal activity was tested against Gram-negative bacteria *Escherichia coli* CNCTC 377/79, Gram-positive bacteria *Staphylococcus aureus* ATCC 6538 and fungi *Candida albicans* CCM 8186. The minimum bactericidal or fungicidal concentration of compounds **5S-9S**, **5R-8R** and **rac-5–rac-8** was determined by the modified method described in [41].

Solutions of compounds studied, **5S-9S**, **5R-8R**, **rac-5–rac-8** and **BAB** were prepared in water. A suspension of the standard microorganism, prepared from 24 h cultures of bacteria in blood agar and from 24 h cultures in the Sabouraud agar for fungi had a concentration of 5×10^{7} cfu mL^{-1} of bacteria and 5×10^{5} cfu mL^{-1} of *Candida*. The suspensions were prepared in physiological solution (pH 7.2). Concentration of microorganisms were determined spectrophotometrically and the suspensions were adjusted to absorbance A = 0.35 at λ = 540 nm. The microorganism suspension (5 µL) was added to solutions containing the compound tested (100 µL) and to double concentrated peptone broth medium (8%) for bacteria or Sabouraud medium (12%) for *Candida* (100 µL). The stock solutions of the compounds tested had a concentration of 1000 µg mL^{-1}. For a testing assay the solutions were serially diluted by half (concentrations of the solutions were: 1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 1.95 µg mL^{-1}) and used. The cultures were done in 96-well microtiter plates. The microorganisms were incubated for 24 h at 37°C. Then from each well, 5 µL of suspension tested was taken and cultured on blood agar (bacteria) or on Sabouraud agar (fungi). The Petri dishes were incubated for 24 h at 37°C. The lowest concentration of QUATs that prevented colony formation was defined as the MBC.

2.4. QSAR study of (2S)-N-alkyl-N,N-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromides

The relationhips were calculated using Kubinyi's bilinear model. QSAR was performed on a series of compounds **5S – 9S**. Programe BILIN which is available at http://www.kubinyi.de/bilin-program.html was used for computation.

3. Results and discussion

QUATs derived from phenylalanine **5S-9S** and **5R-8R** (Table 1) were synthesized according to the procedure shown in Fig. 1. Five of the compounds were prepared as *S*-derivatives (**5S-9S**) and four as *R*-derivatives (**5R-8R**), in which the alkyl chains range from C_{10} (compound 5) to C_{18} (compound 9). The derivatives with different alkyl chain were prepared by reaction of the tertiary amine with the appropriate 1-bromoalkane. Quaternisation of

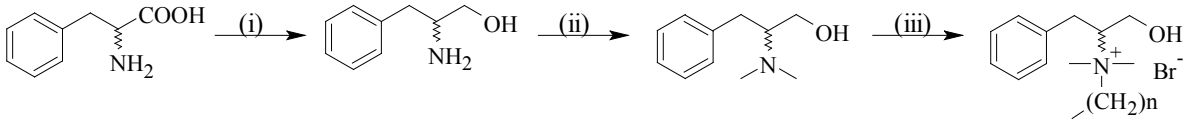

Figure 1. Synthesis of QUATs derived from phenylalanine. Reagents and conditions: (i) $Na[BH_4]$, I_2, THF, reflux, 70 – 94%; (ii) 35% HCHO, 85% HCOOH, reflux, 86 – 90%; 1-bromoalkane, CH_3CN, reflux, 57 – 86%

phenylalaninols was carried out with 1-bromodecane, 1-bromododecane, 1-bromotetradecane and 1-bromohexadecane; in the case of both enantiomers of *N*,*N*-dimethyl-2-amino-3-phenylpropan-1-ols. (*2S*)-*N*,*N*-dimethyl-2-amino-3-phenylpropan-1-ol was quaternised also with 1-bromooctadecane. The substances were obtained as white crystals after several crystallizations from acetone-methanol or acetone-petroleum ether. The yields ranged from 57 to 87%. Melting points were well defined and their values slightly increased based on dependence of the growing alkyl chains. The value of optical rotation also depended on the length of the alkyl chain. Optical rotation declined with growing alkyl chain (Table 1). ^1H and ^{13}C NMR spectra were in good agreement with the expected structures and showed essentially no contaminants (Table 2). Extensive NMR spectral data, including 2D COSY experiment, provided strong support for the structure of the product **6R** and analogically for other QUATs. Purity of substances was also confirmed by TLC. IR spectra showed characteristic vibrations of the following groups of atoms: ν(O-H), ν(C-O), ν(C-H, Ph), ν(C=C). Abundance of atoms of C, H, N was specified with elemental analysis.

Consequently, the microbicidal activities of the target molecules **5S-9S** and **5R-8R** and their equimolar mixtures **rac-5–rac-8** were measured with *Escherichia coli* CNCTC 377/79 as a representative of Gram-negative bacteria; *Staphylococcus aureus* ATCC 6538 as a

Table 1. Yields and physicochemical properties of prepared compounds

Compound	Number of atom in the alkyl chain	Formula M_r	w_i(calc.)/% w_i(found)/%			Yield	M.p.[a]	$[\alpha]_D^{25}$	TLC[b]
			C	H	N	%	°C		R_f
5S	10	$C_{21}H_{38}BrNO$ 400.44	62.99 63.13	9.57 9.84	3.50 3.60	56.8	112.5 – 114	-6.19	0.47
6S	12	$C_{23}H_{42}BrNO$ 428.49	64.47 64.21	9.88 10.07	3.27 3.33	85.6	114.5 – 115.5	-5.96	0.49
7S	14	$C_{25}H_{46}BrNO$ 456.54	65.77 64.74	10.15 10.23	3.07 3.09	68.0	116 – 117.5	-5.69	0.47
8S	16	$C_{27}H_{50}BrNO$ 484.60	66.92 66.79	10.40 10.70	2.89 2.94	73.5	117 – 118.5	-5.36	0.49
9S	18	$C_{29}H_{54}BrNO$ 512.65	67.94 67.82	10.62 10.89	2.73 2.78	72.3	119 – 120	-5.02	0.51
5R	10	$C_{21}H_{38}BrNO$ 400.44	62.99 63.18	9.57 9.84	3.50 3.56	63.9	113 – 114	6.08	0.47
6R	12	$C_{23}H_{42}BrNO$ 428.49	64.47 64.76	9.88 10.14	3.27 3.37	76.8	114.5 – 115	6.01	0.48
7R	14	$C_{25}H_{46}BrNO$ 456.54	65.77 66.45	10.15 10.54	3.07 3.17	65.2	116 – 117,5	5.82	0.49
8R	16	$C_{27}H_{50}BrNO$ 484.60	66.92 67.25	10.40 10.78	2.89 2.96	75.5	118 – 119	5.13	0.51

[a] *Solvent for recrystallization CH_3COCH_3-petrolether (5S, 5R), CH_3COCH_3-MeOH (6S-9S, 6R-8R)*
[b] *$CHCl_3$: MeOH – 5 : 1 (V/V)*

representative of Gram-positive bacteria; and *Candida albicans* CCM 8186 as a representative of fungi. The minimal bactericidal or fungicidal concentration (MBC) values were defined as the minimal concentration of the antimicrobial agent that killed the test organism after 24 h of incubation. The MBC values determined for the compounds prepared are listed in Table 3.

Microbicidal activities of the prepared compounds were determined as a function of the length of the alkyl chain, which ranged from 10 – 18 for (*2S*)-*N*-alkyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromides and 10 – 16 for (*2R*)-*N*-alkyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromides, respectively (Table 3). Fig. 2 shows the relative bactericidal and fungicidal activities (log1/MBC) of (*2S*)-*N*-alkyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromides based on the length of the alkyl chain. The highest bactericidal activity against Gram-negative bacteria Escherichia coli and Gram-positive bacteria Staphylococcus aureus were exhibited by compounds **7S**, **7R** and **rac-7** with alkyl chain length n=14. The remaining compounds with either longer or shorter alkyl chains exhibited lower activity compared to compounds **7S**, **7R** and **rac-7**. For the *S. aures*, the cut-off effect was observed at 14.7, *i.e.*,15 carbon atoms in the chain (Table 4). For *E.coli* this parameter is 13.5, *i.e.*,14 carbon atoms in the chain. Similarly for *C. albicans* the $N_{optimal}$ is 14.6, *i.e.*,15 carbon atoms in the chain. The compounds **8S**, **8R** and **rac-8** exhibited the highest activity against *Candida albicans* with their alkyl chain lengths being 16 carbon atoms. Comparable activity was found in compounds **7S**, **7R** and **rac-7** also. The MBC values of compounds **7S** and **7R** and their racemate taken against *Candida albicans* were approx. 0.5 μM lower than the MBC values of compounds **8S**, **8R** and r**ac-8** (Table 3). We also observed that the

Table 2. Spectral data of prepared compounds

Compound	Spectral data
5S	IR: $\tilde{\nu}$/cm^{-1}: 1032 (C-O), 1605 (C=C), 2923 (C-H, alkyl chain), 3025 (C-H, Ph), 3295 (O-H) ^{1}H NMR ($CDCl_3$), δ: 0.88 (t, J = 6.7 Hz, 3H, CH_2CH_3), 1.26 (m, 14H, alkyl chain), 1.72 (2H, m NCH_2CH_2), 3.12 (m, 1H, 3-HH), 3.31 (m, 1H, 3-HH), 3.38 (s, 3H, $N(CH_3)(CH_3)$), 3.45 (s, 3H, $N(CH_3)(CH_3)$), 3.55 (m, 1H, N-CHHCH$_2$), 3.61 (m, 1H, 2-H) 3.71 (m, 1H, CHHOH), 3.82 (m, 1H, N-CHHCH2), 4.21 (m, 1H, CHHOH) 5.09 (t, J = 5.0 Hz, 1H, CHHOH), 7.2 – 7.4 (m, 5H, Ph) ^{13}C NMR ($CDCl_3$), δ: 14.13 (CH3), 22.68, 22.92, 26.31, 29.26, 29.37, 29.42, 31.06, 31.83 (8 × CH_2), 50.43 (2 × N-CH_3), 56.36 (N-CH2), 64.75 (C-2), 73.82 (C-1), 127.52 (C-4'), 129.13 (2 × C), 129.43 (2 × C), 135.48 (C-1')
6S	IR: $\tilde{\nu}$/cm^{-1}: 1032 (C-O), 1605 (C=C), 2921 (C-H, alkyl chain), 3024 (C-H, Ph), 3296 (O-H) ^{1}H NMR ($CDCl_3$), δ: 0.88 (t, 3H, J = 6.7 Hz, CH_2CH_3), 1.25 (m, 18H, alkyl chain), 1.74 (2H, m NCH_2CH_2), 3.12 (m, 1H, 3-HH), 3.33 (m, 1H, 3-HH), 3.38 (s, 3H, N(CH_3)(CH_3)), 3.44 (s, 3H, N(CH_3)(CH_3)), 3.52 (m, 1H, N-CHHCH$_2$), 3.60 (m, 1H, 2-H) 3.71 (m, 1H, CHHOH), 3.82 (m, 1H, N-CHHCH2), 4.21 (m, 1H, CHHOH) 5.08 (t, J = 5.0 Hz, 1H, CHHOH), 7.2 – 7.4 (m, 5H, Ph) ^{13}C NMR ($CDCl_3$), δ: 14.12 (CH3), 22.66, 22.93, 26.31, 29.25, 29.33 29.37, 29.42, 29.59, 31.06, 31.83 (10 × CH_2), 50.43 (2 × N-CH_3), 56.38 (N-CH2), 64.74 (C-2), 73.82 (C-1), 127.51 (C-4'), 129.12 (2 × C), 129.44 (2 × C), 135.52 (C-1')
7S	IR: $\tilde{\nu}$/cm^{-1}: 1032 (C-O), 1604 (C=C), 2923 (C-H, alkyl chain), 3024 (C-H, Ph), 3294 (O-H) ^{1}H NMR ($CDCl_3$), δ: 0.88 (t, J = 6.7 Hz, 3H, CH_2CH_3), 1.26 (m, 22H, alkyl chain), 1.73 (2H, m NCH_2CH_2), 3.12 (m, 1H, 3-HH), 3.29 (m, 1H, 3-HH), 3.38 (s, 3H, N(CH_3)(CH_3)), 3.45 (s, 3H, N(CH_3)(CH_3)), 3.55 (m, 1H, N-CHHCH$_2$), 3.59 (m, 1H, 2-H) 3.71 (m, 1H, CHHOH), 3.82 (m, 1H, N-CHHCH2), 4.21 (m, 1H, CHHOH) 5.09 (t, J = 5.1 Hz, 1H, CHHOH), 7.2 – 7.4 (m, 5H, Ph) ^{13}C NMR ($CDCl_3$), δ: 14.12 (CH3), 22.69, 22.92, 26.31, 29.26, 29.36, 29.48, 29.60, 29.64, 29.68, 31.06, 31.92 (12 × CH_2), 50.43 (2 × N-CH_3), 56.36 (N-CH2), 64.78 (C-2), 73.86 (C-1), 127.50 (C-4'), 129.14 (2 × C), 129.42 (2 × C), 135.49 (C-1')
8S	IR:$\tilde{\nu}$/cm^{-1}: 1032 (C-O), 1605 (C=C), 2920 (C-H, alkyl chain), 3024 (C-H, Ph), 3300 (O-H) ^{1}H NMR ($CDCl_3$), δ: 0.88 (t, J = 6.7 Hz, 3H, CH_2CH_3), 1.26 (m, 26H, alkyl chain), 1.73 (2H, m NCH_2CH_2), 3.13 (m, 1H, 3-HH), 3.32 (m, 1H, 3-HH), 3.39 (s, 3H, N(CH_3)(CH_3)), 3.47 (s, 3H, N(CH_3)(CH_3)), 3.55 (m, 1H, N-CHHCH$_2$), 3.60 (m, 1H, 2-H) 3.71 (m, 1H, CHHOH), 3.81 (m, 1H, N-CHHCH2), 4.20 (m, 1H, CHHOH) 5.08 (t, J = 5.0 Hz, 1H, CHHOH), 7.2 – 7.4 (m, 5H, Ph) ^{13}C NMR ($CDCl_3$), δ: 14.14 (CH3), 22.69, 22.92, 26.32, 29.27, 29.37, 29.49, 29.61, 29.66, 29.70, 31.06, 31.92 (14 × CH_2), 50.42 (2 × N-CH_3), 56.38 (N-CH2), 64.74 (C-2), 73.81 (C-1), 127.50 (C-4'), 129.12 (2 × C), 129.46 (2 × C), 135.52 (C-1')
9S	IR: $\tilde{\nu}$/cm^{-1}: 1032 (C-O), 1605 (C=C), 2923 (C-H, alkyl chain), 3025 (C-H, Ph), 3298 (O-H) ^{1}H NMR ($CDCl_3$), δ: 0.88 (t, J = 6.7 Hz, 3H, CH_2CH_3), 1.26 (m, 30H, alkyl chain), 1.73 (2H, m NCH_2CH_2), 3.12 (m, 1H, 3-HH), 3.30 (m, 1H, 3-HH), 3.38 (s, 3H, N(CH_3)(CH_3)), 3.44 (s, 3H, N(CH_3)(CH_3)), 3.55 (m, 1H, N-CHHCH$_2$), 3.60 (m, 1H, 2-H) 3.71 (m, 1H, CHHOH), 3.82 (m, 1H, N-CHHCH2), 4.20 (m, 1H, CHHOH) 5.09 (t, J = 5.1 Hz, 1H, CHHOH), 7.2 – 7.4 (m, 5H, Ph) ^{13}C NMR ($CDCl_3$), δ: 14.12 (CH3), 22.66, 22.93, 26.31, 29.25, 29.37, 29.42, 31.06, 31.83 (16 × CH_2), 50.43 (2 × N-CH_3), 56.38 (N-CH2), 64.74 (C-2), 73.82 (C-1), 127.50 (C-4'), 129.12 (2 × C), 129.45 (2 × C), 135.52 (C-1')

antimicrobial activities of QUATs depend strongly on the structure of their alkyl chain. If they possessed an unbranched alkyl chain and the head group did not contain a hydrophilic or lipophilic moiety, the optimal alkyl chain length is usually 14-16 carbon atoms [42-48]. *N*-alkyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromides fulfills these conditions. The alkyl chain is straight, unbranched, bearing no aromatic rings or heteroatoms. The head group contains one lipophilic moiety – a benzyl group and one hydrophilic moiety – a hydroxyl group and their hydrophilic-lipophilic balance can be compensated. Compounds **7S**, **7R** and **rac-7** contain a C_{14} alkyl chain and so one can expect the highest microbicidal activity in the series of QUATs investigated with these compounds.

We also studied the quantitative relationship between structure and activity (QSAR) using the equation log (1/MBC) = f(n), where n is the number of carbon atoms in the long alkyl chain. This non-linear course was quantified by the Kubinyi's bilinear model [49,50]

$$\log (1/MBC) = An + B\log (\beta\, 10^{n} + 1) + C$$

This Kubinyi´s bilinear model was also used by evaluation of rich series of compounds described in our earlier papers [42,43,51-55] and its predictive power was proved many times. The values for compounds described in this paper are presented in Table 4. From the computations it follows (Table 4) that the highest activity of this type of compounds would be with the alkyl chain length C_{15} for *S. aureaus* (MBC = 7.6 μM) and *C. albicans* (MBC = 4.6 μM), and C_{14} for *E. coli* (MBC = 8.5 μM), respectively. The values given in parentheses are calculated values from the bilinear equation.

We have also studied the relationship between the optical activity of these compounds and their microbicidal activity. We observed that the optical activity of enantiomers of *N*-alkyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromides had no effect on their bactericidal or fungicidal activity. Differences in microbicidal activity between S and R enantiomers of QUATs with same length of the alkyl chain were observed only in one dilution. The microbicidal activity of racemates (equimolar mixtures of pure enatiomers) was the same as the MBC values of individual enantiomers. Similar results were published by Osanai and Abe [56] with QUATs derived from 1-phenylethanamine.

We compared the microbicidal activity of *N*-alkyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromides with the activity of commercially available benzalkonium bromide (BAB). MBC values of QUATs **7S**, **8S**, **7R**, **8R**, **rac-7**, **rac-8** against Gram-positive bacteria *Staphylococcus aureus* were found to be lower than that of **BAB**. The MBC of compound **9S** was higher against *Escherichia coli* than the MBC of **BAB**. All the other compounds have lower MBCs than **BAB**. The fungicidal activity of QUATs with alkyl chain length n = 12 – 16 against *Candida albicans* were observed to be higher than that of **BAB**. Compounds **7S**, **8S**, **7R**, **8R**,

Table 3. The MBC valuesa of prepared QUATs

Compound	*S. aureus* ATCC 6538	*E. coli* CNCTC 377/79	*C.albicans* CCM 8186
5S	195.1	195.1	390.2
6S	36.4	18.1	18.1
7S	8.5	8.5	8.5
8S	16.1	64.4	8.0
9S	121.9	975.3	61.0
5R	195.1	195.1	390.2
6R	18.2	18.1	18.1
7R	8.5	8,5	8.5
8R	16.1	64.4	8.0
rac-5	195.1	195.1	390.2
rac-6	36.4	18.1	18.1
rac-7	8.5	8.5	8.5
rac-8	16.1	64.4	8.0
BAB	26.0	260.1	26.0

Table 4. Regression coefficients for bilinear relationship between microbicidal activity and structure for (2S)-N-alkyl-N,N-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromides

Relationship	A	B	C	log	r[a]	s_D[b]	F[c]	$N_{optimal}$	$MBC_{optimal}$[d]
S. aureus	0.357±0.011	-0.803±0.025	0.148±0.136	-14.70	1.000	0.014	2158.634	14.6	7.0
E. coli	0.512±0.077	-1.063±0.125	-1.402±0.904	-13.51	0.999	0.074	162.697	13.5	6.4
C. albicans	0.453±0.306	-0.859±0.649	-0.987±3.736	-14.59	0.963	0.383	4.229	14.6	4.3

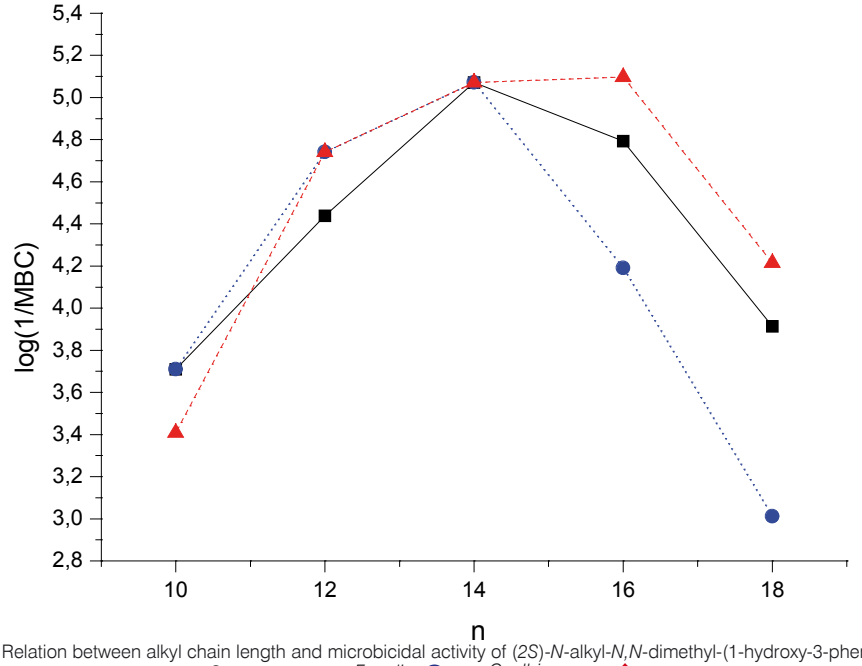

Figure 2. Relation between alkyl chain length and microbicidal activity of (*2S*)-*N*-alkyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammoniumbromides; Symbols: *S. aureus* ■ *E. coli* ● *C. albicans* ▲.

rac-7, **rac-8** exhibit very good microbicidal activity, and in comparison to **BAB**, they are more effective against all the microorganisms studied.

4. Conclusions

The structure−activity relationship of QUATs derived from phenylalanine was investigated to determine the influence of alkyl chain length and stereochemistry of surfactants on their microbicidal activities. The cut-off effect which was observed, wherein the antimicrobial activity depended on the length of alkyl chain, was quantified by a bilinear model. Compounds No. **7S**, **7R** and **rac-7** exhibited the highest microbicidal activity from among the compounds studied. The results suggest that stereochemistry in terms of optical activity of phenylalanine−derived QUATs have apparently no influence on their microbicidal activity. The relationship between MBC and alkyl chain length of (*2S*)-*N*-alkyl-*N*,*N*-dimethyl-(1-hydroxy-3-phenylpropyl)-2-ammonium bromides was quantified by a bilinear model. The results show that *Staphylococcus aureus* would be most sensitive towards the compound with a C_{15} chain, *Escherichia coli* towards C_{14} and *Candida albicans* towards C_{15}, respectively.

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