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Short communication

Synthesis and anti-microbial activities of choline-like quaternary ammonium chlorides

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Abstract

New choline-like quaternary ammonium chlorides were obtained. The work-up procedure of synthesis was quick and efficient. The obtained chlorides showed anti-microbial activities. Quaternary ammonium chlorides derivatives of deanol esters exhibited strong activity and wide anti-bacterial spectra, similar to the activity of benzalkonium chloride. The relationship between chemical structure and anti-microbial activity was analyzed by the QSAR method.

Keywords: Quaternary ammonium chlorides; Deanol; Anti-microbial activities; QSAR analysis

1. Introduction

Quaternary ammonium salts (quats) have been known for over 100 years. They were obtained for the first time by Menschutkin [1] in the reaction of tertiary amine with alkyl halides early in 1890. As compared to later described procedures, this way of the synthesis, termed the Menschutkin reaction, has definitely been more advantageous (irreversible reaction, high efficiency). Practical application of quats has been rapidly developing during the 20th century. In 1915–16 Jacobs and coworkers [2,3] described the bactericidal activity but they were not used for disinfection until 1935, following publication of the study by Domagk [4]. Following the latter publication, the investigators' attention was drawn to the surface activity of quats containing hydrophobic group of more than 8 carbon atoms. In the sixties, the compounds were found to be excellent fabric softeners. In 1965 Mąkosza and Serafinowa [5] described catalytic activity of the group of compounds. The reactions, in which quats are used as catalysers, are termed the Jarousse–Mąkosza reaction [6] or phase transfer catalysis. In the seventies the compounds were used as antielectrostatic agents and wood preservatives. In the preservation of wood, studies of Butcher et al. [7,8] proved to be of key importance. In the eighties, the compounds were applied as asphalt modifiers and in the nineties as clay modifiers. At the end of 20th century, specifically beginning from 1998 the group of discussed compounds containing BF_4^-, PF_6^-, $(CF_3SO_2)_2N^-$, etc. as anions have been named ionic liquids (ILs). The subject of ionic liquids is well summed up in appropriate reviews [9–15]. The historic outline presented above points to the important role played by quats in industry. They consist of a cation of organic type and an inorganic or organic anion. The number of potential cation/anion combinations is very high and is estimated at 10^{18}. Nevertheless, no more than 500 quats are commercially available at present. Mass application of quats has its positive and negative aspects. The positives include their polyfunctionality. In commercial preparations, such compounds may fulfil several functions in parallel, exerting surface activity, anti-microbial and antielectrostatic activity. Compounds of this type will be of particular interest in 21st century. Development of resistance in microbes and the relatively slow decomposition in the natural environment belong to the most important negative aspects of quats application [16,17]. New quats may supplement the presently applied ones, to which microbes developed resistance. The mechanism of quats action remains the same. The

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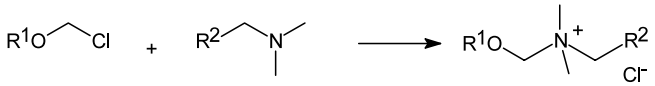
search for new, effective compounds continues [18–27]. We have focused our attention on new derivatives of choline.

2. Chemistry

We prepared five new groups of ammonium chlorides which originated from the following aminoalcohols: 2-dimethylaminethanol (deanol) (**1**), 3-dimethylamino-1-propanol (**2**), 1-dimethylamino-2-propanol (**3**), and aminoesters: 2-(dimethylamino)ethyl decanate (**4**), 2-(dimethylamino)ethyl benzoate (**5**). The quaternary ammonium choline-like derivatives based on deanol structure were obtained by nucleophilic substitution with chloromethyl alkyl ethers, containing 3–18 carbons in alkoxy groups, in hexane.



(**6-10**)

All of the compounds were new and obtained in high yields (Table 1). The structure of new compounds was established by ^{1}H-NMR and ^{13}C-NMR or elemental analysis. The results of elemental analysis were in agreement with the theoretical values. Generally, the most important signals, characteristic for the chlorides came from protons of $N^{+}CH_2O$, which in chlorides **6**–**8** were observed as a singlet at 4.9–5.0 ppm and in **9** and **10** as a singlet at 5.1–5.2 ppm. In the ^{13}C-NMR spectra, the chemical shift of the carbon of $N^{+}CH_2O$ appeared at 90–91 ppm.

All of the obtained compounds proved to be strongly hygroscopic. In the extreme cases they were left in a crystallizer and after a few minutes they formed a water solution. Absorption of water from the surrounding atmosphere was intense and rapid. The synthesized salts could be estimated by a direct two-phase back titration (EN ISO 2871-1). We established that for chlorides **7**, **9** and **10** the alkoxy group had to contain at least seven carbon atoms, for chlorides **8** eight carbon atoms and for chlorides **6** ten carbon atoms. The percentages of chloride in the products are listed in Table 1. The remaining fraction (up to 100%) was formed by water. Despite the restricted contact with water, the product contained significant amounts of water.

3. Pharmacology

3.1. Anti-microbial activity

The quaternary ammonium chlorides with alkoxymethyl substituent in many cases demonstrated anti-microbial activity [18,24,25]. Therefore all synthesized quats were tested in vivo for their reaction against cocci, rods, fungi and bacilli. The minimum inhibitory concentration (MIC) series of compounds were determinated by tube dilution method.

3.2. QSAR analysis

Anti-microbial activity of quats remained, among other factors, lipophilicity-dependent. Good approximation for compound lipophilicity was provided by 1-octanol/water partition coefficient (P). Therefore, the QSAR analysis was constructed mostly using a simplified Hansch equation:

$$\log RBR = a + b \cdot \mathrm{Clog}\ P + \mathrm{c} \cdot \mathrm{Clog}\ P^2$$

The RBR (relative biological response) parameter used to correspond to $1/C$, where C was minimal inhibitory concentration (MIC). The logarithm of 1-octanol/water partition coefficient was calculated using the Meylan–Howard method [28].

4. Results and discussion

New choline-like quaternary ammonium derivatives of **6**–**10,** listed in Table 1, were synthesized by Menschutkin reaction. Quaternisation of **1**–**5** with chloromethyl alkyl ethers was quick and efficient. The mechanism of the reaction was S_N1, in which formation of the cation $R^1OCH_2^+$ by ionization proved to be the slowest step. Disadvantages of chloromethyl alkyl ethers involved its very easy hydrolysis in the presence of traces of moisture. Therefore, quaternisation had to be conducted under strictly anhydrous conditions.

Generally, alcohol derivatives of quaternary ammonium chlorides **6**–**10** were well soluble in water and chloroform and insoluble in hexane for **6**–**8** and **10**, but soluble in hot hexane for **9**. The water solutions of chlorides **6**–**10** were stable at room temperature.

Minimal inhibitory concentration (MIC) values, determined for prepared chlorides **6**–**10,** are given in Tables 2–6. All studied compounds were active against the microorganisms. Activities of investigated compounds were distinctly higher for Gram-positive bacteria than for Gram-negative bacteria. Similarly to previous literature data [24], the highest resistance against the compounds was exhibited by *Pseudomonas aeruginosa.* Surprisingly, the activity against fungi was similar to that against Gram-positive bacteria.

Structure of the molecules permitted us the activity of the examined chlorides. The obtained chlorides could be divided to two groups: alcohol quartenary derivatives (**6a**–**8j**) and ester quartenary derivatives (**9a**–**10j**) with large substituents. In general, MIC values of the ester derivatives were slightly lower than those of alcohols.

Table 1
Quaternary ammonium chloride (**6**–**10**) prepared

Chloride	R^1	R^2	Melting point (°C)	Yield (%)	Purity (%)
6a	C_6H_{13}	$HOCH_2$	oil	90.5	–
6b	C_7H_{15}	$HOCH_2$	hygroscopic	89	–
6c	C_8H_{17}	$HOCH_2$	hygroscopic	94	–
6d	C_9H_{19}	$HOCH_2$	49-51	86	92
6e	$C_{10}H_{21}$	$HOCH_2$	66.5–68.5	88	97
6f	$C_{11}H_{23}$	$HOCH_2$	68.5–70	87.5	98.5
6g	$C_{12}H_{25}$	$HOCH_2$	69.5–71.5	88	99.5
6h	$C_{14}H_{29}$	$HOCH_2$	60–63	90	99
6i	$C_{16}H_{31}$	$HOCH_2$	71–73.5	87	99
6j	$C_{18}H_{35}$	$HOCH_2$	85–87	85	97
7a	C_6H_{13}	$HOCH_2CH_2$	oil	90	–
7b	C_7H_{15}	$HOCH_2CH_2$	hygroscopic	88	88
7c	C_8H_{17}	$HOCH_2CH_2$	hygroscopic	89	92
7d	C_9H_{19}	$HOCH_2CH_2$	hygroscopic	85	94
7e	$C_{10}H_{21}$	$HOCH_2CH_2$	63.5–66	86	96.5
7f	$C_{11}H_{23}$	$HOCH_2CH_2$	66.5–69	87	96
7g	$C_{12}H_{25}$	$HOCH_2CH_2$	69–71	92	98
7h	$C_{14}H_{29}$	$HOCH_2CH_2$	73–76	89	97.5
7i	$C_{16}H_{31}$	$HOCH_2CH_2$	83–85	92	98
7j	$C_{18}H_{35}$	$HOCH_2CH_2$	89–91	91	97
8a	C_6H_{13}	$HO(CH_3)CH$	oil	83.5	–
8b	C_7H_{15}	$HO(CH_3)CH$	oil	81	–
8c	C_8H_{17}	$HO(CH_3)CH$	oil	87	81
8d	C_9H_{19}	$HO(CH_3)CH$	oil	85.5	86
8e	$C_{10}H_{21}$	$HO(CH_3)CH$	hygroscopic	87	90
8f	$C_{11}H_{23}$	$HO(CH_3)CH$	hygroscopic	84	91
8g	$C_{12}H_{25}$	$HO(CH_3)CH$	35–38	82	95
8h	$C_{14}H_{29}$	$HO(CH_3)CH$	41–44	85	96
8i	$C_{16}H_{31}$	$HO(CH_3)CH$	44–47	84	96.5
8j	$C_{18}H_{35}$	$HO(CH_3)CH$	48–51	85	95
8a	C_3H_7	$C_9H_{19}COOCH_2$	oil	85	–
9b	C_4H_9	$C_9H_{19}COOCH_2$	oil	81	–
9c	C_5H_{11}	$C_9H_{19}COOCH_2$	hygroscopic	79	–
9d	C_6H_{13}	$C_9H_{19}COOCH_2$	hygroscopic	85	–
9e	C_7H_{15}	$C_9H_{19}COOCH_2$	50–54	88	95
9f	C_8H_{17}	$C_9H_{19}COOCH_2$	57–60	83	95
9g	C_9H_{19}	$C_9H_{19}COOCH_2$	62.5–64	85.5	99
9h	$C_{10}H_{21}$	$C_9H_{19}COOCH_2$	65–67	87	99
9i	$C_{11}H_{23}$	$C_9H_{19}COOCH_2$	67–69	85	99
9j	$C_{12}H_{25}$	$C_9H_{19}COOCH_2$	75–76	87	99.5
10a	C_6H_{13}	$PhCOOCH_2$	oil	88	–
10b	C_7H_{15}	$PhCOOCH_2$	oil	89	83
10c	C_8H_{17}	$PhCOOCH_2$	hygroscopic	87	86
10d	C_9H_{19}	$PhCOOCH_2$	hygroscopic	86	89
10e	$C_{10}H_{21}$	$PhCOOCH_2$	54–58	87.5	95
10f	$C_{11}H_{23}$	$PhCOOCH_2$	71–74	88	98
10g	$C_{12}H_{25}$	$PhCOOCH_2$	76–79	89	98
10h	$C_{14}H_{29}$	$PhCOOCH_2$	84–86	85	99
10i	$C_{16}H_{31}$	$PhCOOCH_2$	86–88	87	98.5
10j	$C_{18}H_{35}$	$PhCOOCH_2$	87–90	80	97

On the other hand, in the studies, the length of alkoxymethyl group exerted a more pronounced influence on bacteriostatic activity. The most favorable alkoxymethyl group contained 9–14 carbon atoms. The highest activity in the series was revealed: **6g**—26.6–426.8 μM (7.8–125 μg L^{-1}); **8h**—1.7–211.2 μM (0.49–62.5 μg L^{-1}); **9f**—0.9–107.1 μM (0.25–31.25 μg L^{-1}); **10g**—0.3–42.2 μM (0.125–15.63 μg L^{-1}). The MIC values of **9f**, **9g** and **10f**, **10g** was similar to the ones of commercially available benzalkonium chloride (BAC), especially for Gram-positive bacteria. The series of quaternary 3-dimethylamino-1-propanol derivatives (**7a**–**7j**) can be considered as two sets: with an even number of carbons in alkoxy substituents, where **7d** showed maximum activity for the even set (1.1–269.6 μM or 0.25–62.5 μg L^{-1}), and **7h** for the odd set (0.8–

Table 2
MIC values [a] and calculated 1-octanol/water partition coefficients (Clog P) of alkoxymethyl(2-hydroxyethyl)dimethylammonium chlorides (**6a**–**6j**)

Strain	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**	**6g**	**6h**	**6i**	**6j**	BAC [b]
P. aeruginosa	> 2540	> 2350	2190	2040	958.6	451.5	426.8	809.2	1540	> 1470	23.0
E. coli	> 2540	2350	2190	1020	239.6	112.9	106.7	101.2	769.3	> 1470	5.7
K. pneumoniae	> 2540	> 2350	2190	1020	479.3	225.8	213.4	202.3	769.3	> 1470	11.5
P. vulgaris	> 2540	> 2350	2190	1020	479.3	225.8	213.4	404.6	769.3	> 1470	5.7
S. aureus	> 2540	2350	2190	510.7	239.6	112.9	53.4	101.2	384.6	> 1470	0.7
M. luteus	> 2540	2350	2190	510.7	239.6	112.9	26.6	202.3	192.3	733.1	0.7
E. faecalis	> 2540	2350	2190	510.7	239.6	112.9	53.4	202.3	384.6	1470	0.4
C. albicans	> 2540	> 2350	2190	510.7	239.6	225.8	53.4	101.2	384.6	1470	1.4
R. rubrum	> 2540	> 2350	2190	510.7	239.6	112.9	53.4	101.2	192.3	1470	1.4
B. subtilis	> 2540	> 2350	2190	510.7	239.6	112.9	53.4	202.3	384.6	> 1470	0.7
Clog P	− 1.67	− 1.18	− 0.68	− 0.19	0.30	0.79	1.28	2.26	3.24	4.23	3.32

[a] In μM, the inoculum is 0.1 mL of suspension with the number of microorganisms ranged from 10^3 to 10^4.
[b] BAC is benzalkonium chloride.

422.4 μM or 0.25–125 μg L^{-1}). The obtained values for **7** imply the possibility of a different kind of disruption of the cell membrane for these two sets. These results require further research.

Data of Figs. 1 and 2 demonstrate the dependency of the average logarithm of relative biological response values for all strains upon the alkoxymethyl chain length: 6–18 carbon atoms for **6**–**8** and **10** or 3–12 for **9**.

Results of QSAR analysis of the bacteriostatic activity of prepared chlorides **6**–**10** is given in Table 7. The general equation was constructed based on the Hansch equation for parabolic model relating, where RBR was represented by 1/MIC of a tested compound and MIC was the average value for all tested strains. The results were presented in Table 7 as parameters of regression equations with the appropriate statistical data.

A statistically significant correlation was demonstrated between the dependent variable: Clog P and the structural parameter determined in this work. As regards alcohol quaternary derivatives (**6a**–**7a**, **7c**, **7e**, **7g**–**8j**) but without the carbon atoms even number series of quaternary 3-dimethylamino-1-propanol derivatives (**7a**–**7j**), the QSAR equation correlation coefficients were high and fluctuated between 0.9903 to 0.9225. The predicted value of equations ranged between 98 and 85%. The population of carbon atoms odd number series of quaternary 3-dimethylamino-1-propanol derivatives was too small for statistical processing. For ester quaternary derivatives (**9**–**10**), the QSAR equation correlation coefficients were slightly lower than for alcohol quaternary chlorides (**6**–**8**).

The statistically significant correlation of obtained QSAR equations confirm that lipophlicity as Clog P is the main factor of anti-microbial activity. The obtained Clog P_o parameters show that most active alcoholammonium chlorides **6**–**8** are between 1.60 and 2.61 and for esterammonium chlorides: 6.55 for **9** and 5.85 for **10**. The comparison of QSAR parameters for these two quat groups indicate that synthesis of chlorides with two long chain substituents and more lipophilic cause increasing anti-microbial activity.

Our research proves that choline-like quaternary ammonium esters can be successfully used as anti-

Table 3
MIC values [a] and calculated 1-octanol/water partition coefficients (Clog P) of alkoxymethyl(3-hydro-xypropyl)dimethylammonium chlorides (**7a**–**7j**)

Strain	**7a**	**7b**	**7c**	**7d**	**7e**	**7f**	**7g**	**7h**	**7i**	**7j**	BAC [b]
P. aeruginosa	> 2720	> 2500	579.4	269.6	504.4	947.4	223.3	422.4	801.3	1520	23.0
E. coli	2720	1250	144.8	16.8	63.1	118.4	55.8	52.8	400.6	762.1	5.7
K. pneumoniae	2720	1250	289.7	16.8	63.1	118.4	55.8	52.8	400.6	762.1	11.5
P. vulgaris	2720	2500	289.7	33.6	63.1	118.4	55.8	52.8	400.6	762.1	5.7
S. aureus	1361	78.3	36.2	4.2	7.9	14.8	7.0	3.3	12.5	23.8	0.7
M. luteus	680.5	39.0	9.0	1.1	2.0	14.8	0.9	0.8	12.5	23.8	0.7
E. faecalis	680.5	312.9	9.0	2.1	4.0	7.4	1.7	3.3	25.0	23.8	0.4
C. albicans	1361	625.9	36.2	4.2	7.9	14.8	3.5	13.2	12.5	23.8	1.4
R. rubrum	680.5	312.9	18.1	4.2	7.9	14.8	27.9	6.6	12.5	23.8	1.4
B. subtilis	1361	156.5	18.1	4.2	31.5	59.2	14.0	26.4	200.3	190.5	0.7
Clog P	− 1.17	− 0.68	− 0.19	0.30	0.79	1.28	1.77	2.75	3.74	4.72	3.32

[a] In μM, the inoculum is 0.1 mL of suspension with the number of microorganisms ranged from 10^3 to 10^4.
[b] BAC is benzalkonium chloride.

Table 4
MIC values [a] and calculated 1-octanol/water partition coefficient parameters (Clog *P*) of alkoxymethyl(2-hydroxypropyl)dimethyloammonium chlorides (**8a**–**8j**)

Strain	**8a**	**8b**	**8c**	**8d**	**8e**	**8f**	**8g**	**8h**	**8i**	**8j**	BAC [b]
P. aeruginosa	> 2720	2500	2320	1080	1010	473.7	446.6	211.2	400.6	762.1	23.0
Escherichia coli	2720	1250	1160	539.3	252.2	236.8	111.6	105.6	200.3	381.0	5.7
K.pneumoniae	2720	1250	1160	539.3	252.2	236.8	223.3	105.6	200.3	381.0	11.5
P. vulgaris	2720	2500	1160	539.3	252.2	236.8	223.3	211.2	200.3	381.0	5.7
S. aureus	1360	625.9	289.7	67.4	31.5	14.8	7.0	1.7	3.1	190.6	0.7
M. luteus	1360	625.9	289.7	134.8	126.1	59.2	27.9	6.6	25.0	190.6	0.7
E. faecalis	2720	1250	579.4	134.8	63.1	59.2	27.9	6.6	3.1	190.6	0.4
C. albicans	2720	1250	579.4	269.6	126.1	118.4	55.8	13.2	12.5	95.3	1.4
R. rubrum	1360	625.9	289.7	134.8	63.1	29.6	27.9	13.2	6.2	47.6	1.4
B. subtilis	1360	625.9	289.7	134.8	63.1	59.2	27.9	6.6	12.5	190.6	0.7
Clog *P*	−1.25	−0.76	−0.27	0.22	0.72	1.21	1.70	2.68	3.66	4.64	3.32

[a] In μM, the inoculum is 0.1 mL of suspension with the number of microorganisms ranged from 10^3 to 10^4.
[b] BAC is benzalkonium chloride.

microbial agents. Moreover the QSAR equations obtained can be useful in the search for new quaternary ammonium chlorides with stronger anti-microbial activity, which could be applied as new potential disinfectants.

5. Experimental

5.1. Chemistry

Chloromethyl alkyl ether was obtained by passing hydrochloride gas through a mixture of formaldehyde and alcohol. The purity of ethers was determined as a crude product by alkalimetric method: 1 g crude product was added to 10 mL acetone at −20 °C. HCl-substrate was then quickly neutralized with 0.2 M KOH in MeOH. Hot water (50 mL) was added carefully. HCl as a product of hydrolysis of chloromethyl alkyl ether was neutralized with 0.2 M KOH in MeOH. The crude product contained 85–99% chloromethyl alkyl ethers.

NMR spectra were recorded on a Varian model XL 300 spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C at 20 °C with tetramethylsilane as internal reference. Products were confirmed by elemental analyses with error in the range of: C ±0.30%; H ±0.28%; N ± 0.35%. Melting points were measured on Büchi melting point B-540.

Benzalkonium chloride applied in the research represented a mixture of alkyls $C_{12}H_{25}$ (~60%) and $C_{14}H_{29}$ (~40%), available from Aldrich.

The product of quaternization reaction was controlled by TLC on silcagel 60 where the mobile phase was 9:1 chloroform–methanol solution.

5.1.1. General procedure for alkoxymethylation of *1*–*3*

To a stirred solution of dimethylaminoalkanol (0.05 mol) in anhydrous hexane (20 mL) the corresponding chloromethyl alkyl ether (0.055 mol) in anhydrous hexane (10 mL) was added dropwise. After 10 min the precipitate or oil was washed with 3 × 10 mL dry

Table 5
MIC values [a] and calculated 1-octanol/water partition coefficient parameters (Clog *P*) of alkoxymethyl(2-decanoyloxyethyl)dimethylammonium chlorides (**9a**–**9j**)

Strain	**9a**	**9b**	**9c**	**9d**	**9e**	**9f**	**9g**	**9h**	**9i**	**9j**	BAC [b]
P. aeruginosa	> 2360	> 2200	2050	481.1	226.6	107.1	203.0	771.7	735.3	702.2	23.0
E. coli	2360	2200	512.8	120.1	28.3	6.7	12.7	48.2	46.0	175.5	5.7
K. pneumoniae	2360	2200	512.8	120.1	14.1	6.7	6.3	24.1	91.9	87.8	11.5
P. vulgaris	2360	2200	512.8	120.1	14.1	6.7	6.3	12.0	22.9	87.8	5.7
S. aureus	18.4	34.2	8.0	3.8	1.8	1.7	3.2	6.0	5.7	11.0	0.7
M. luteus	295.2	137.2	64.1	15.0	7.1	3.4	6.3	6.0	11.5	21.9	0.7
E. faecalis	9.2	34.2	8.0	3.8	1.8	0.9	1.6	6.0	5.7	11.0	0.4
C. albicans	295.2	274.4	128.2	60.0	7.1	3.4	3.2	6.0	5.7	21.9	1.4
R. rubra	9.2	4.3	2.0	1.9	1.8	1.7	6.3	24.1	46.0	87.8	1.4
B. subtilis	590.5	274.4	64.1	60.0	7.1	1.7	6.3	6.0	5.7	11.0	0.7
Clog *P*	3.79	4.29	4.78	5.27	5.76	6.25	6.74	7.23	7.72	8.21	3.32

[a] In μM, the inoculum is 0.1 mL of suspension with the number of microorganisms ranged from 10^3 to 10^4.
[b] BAC is benzalkonium chloride.

Table 6
MIC values [a] and calculated 1-octanol/water partition coefficient parameters (Clog P) of alkoxymethyl(2-benzoyloxyethyl)dimethylammonium chlorides (**10a**–**10j**)

Strain	**10a**	**10b**	**10c**	**10d**	**10e**	**10f**	**10g**	**10h**	**10i**	**10j**	BAC [b]
P. aeruginosa	1830	862.7	408.7	194.2	92.5	44.2	42.2	323.8	621.8	1200	23.0
E. coli	913.2	862.7	204.4	97.1	46.2	11.0	21.1	81.0	310.9	597.9	5.7
K.pneumoniae	913.2	862.7	204.4	97.1	46.2	11.0	21.1	81.0	310.9	597.9	11.5
P. vulgaris	1830	862.7	408.7	194.2	46.2	22.0	21.1	81.0	310.9	597.9	5.7
S. aureus	228.3	107.8	51.1	12.1	2.9	1.4	0.3	1.3	9.7	18.6	0.7
M. luteus	114.2	26.9	12.8	6.1	2.9	1.4	0.3	1.3	4.8	18.6	0.7
E. faecalis	228.3	107.8	51.1	24.2	11.5	2.8	0.7	2.5	9.7	37.4	0.4
C. albicans	456.6	215.7	102.2	48.6	23.1	5.5	2.6	10.1	19.4	37.4	1.4
R. rubrum	228.3	107.8	51.1	24.2	11.5	2.8	2.6	10.1	19.4	37.4	1.4
B. subtilis	913.2	431.3	25.5	12.1	5.8	2.8	2.6	10.1	77.7	149.5	0.7
Clog P	2.79	3.29	3.78	4.27	4.76	5.25	5.74	6.72	7.71	8.69	3.32

[a] In μM, the inoculum is 0.1 mL of suspension with the number of microorganisms ranged from 10^3 to 10^4.
[b] BAC is benzalkonium chloride.

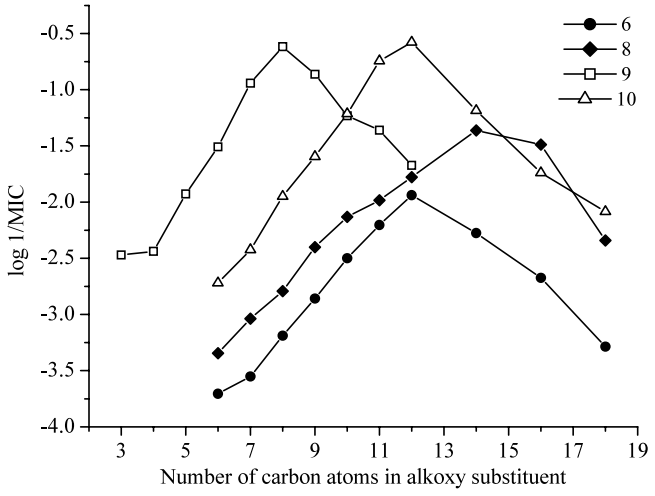


Fig. 1. Relation between alkoxy chain length and average log 1/MIC for **6**, **8**–**10**.

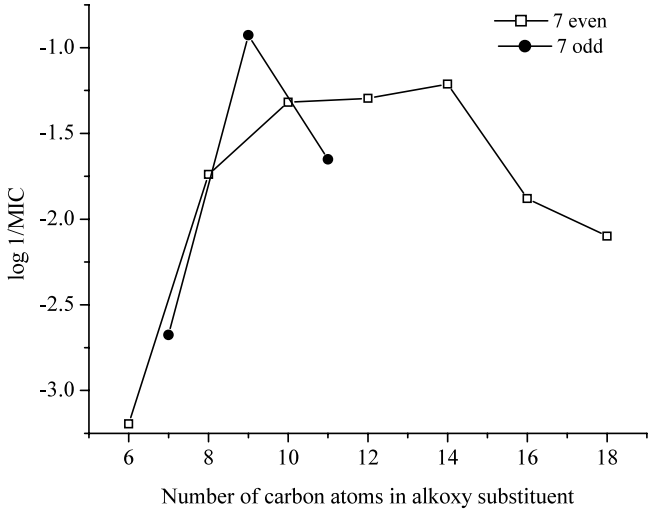


Fig. 2. Relation between alkoxy chain length and average log 1/MIC for **7**.

hexane. The solid products were filtered and then recrystallised from acetone.

*5.1.1.1. Dodecyloxymethyl(2-hydroxyethyl)-dimethylammonium chloride (**6g**).* ^{1}H-NMR ($CDCl_3$) δ ppm = 5.85 (t, J = 5.4 Hz, 1H), 4.99 (s, 2H), 4.08 (s, 2H), 3.85 (t, J = 6.6 Hz, 2H), 3.69 (t, J = 4.8 Hz, 2H), 3.34 (s, 6H), 1.63 (t, J = 6.8 Hz, 2H), 1.26 (m, 18H), 0.88 (t, J = 6.8 Hz, 3H). ^{13}C-NMR ($CDCl_3$) δ ppm = 91.1, 73.4, 62.6, 55.3, 48.1, 31.5, 29.3, 29.2, 29.1, 29.0, 25.4, 22.3, 13.7.

*5.1.1.2. Dodecyloxymethyl(3-hydroxypropyl)-dimethylammonium chloride (**7g**).* ^{1}H-NMR ($CDCl_3$) δ ppm = 4.93 (s, 2H), 3.85 (q, J = 6.6 Hz, 1H), 3.71 (dt, 4H), 3.25 (s, 6H), 2.05 (m, J = 5.5 Hz, 2H), 1,62 (t, J = 6.9 Hz, 2H), 1.26 (m, 18H), 0.88 (t, J = 6,9 Hz, 3H). ^{13}C-NMR ($CDCl_3$) δ ppm = 90.3, 73.5, 59.3, 58.1, 47.3, 31.7, 29.4, 29.4, 29.3, 29.13, 29.09, 25.6, 25.5, 22.4, 13.9.

*5.1.1.3. (2-Hydroxypropyl)-dimethylundecyloxymethylammonium chloride (**8f**).* ^{1}H-NMR ($CDCl_3$) δ ppm = 5.00 (q, J = 7.7 Hz, 2H), 4.49 (m, 1H), 3.84 (t, J = 6.6 Hz, 2H), 3.48 (d, J = 2.7 Hz, 2H), 3.37 (s, 3H), 3.34 (s, 3H), 1.29 (d, J = 6.0 Hz, 3H), 1.62 (m, J = 7.1 Hz, 2H), 1.26 (m, 16H), 0.88 (t, J = 7.1 Hz, 3H). ^{13}C-NMR ($CDCl_3$) δ ppm = 91.2, 73.4, 66.3, 61.3, 48.6, 48.2, 31.6, 29.4, 29.32, 29.26, 29.1, 25.6, 22.4, 22.0, 13.8.

*5.1.2. General procedure for alkoxymethylation of **4** and **5***

To stirred solution of aminoester (0.05 mol) in anhydrous hexane (30 mL), the corresponding chloromethyl alkyl ether (0.06 mol) was added slowly. The mixture was stirred for 10 min at 50 °C. The precipitate or oil was washed with 3 × 10 mL dry hexane.

Table 7
Slopes b, c and intercepts a of linear relationship between logarithm of reciprocal of average MIC against all tested microbial strains and theoretically calculated logarithm of water-1-octanol partition coefficient for quaternary ammonium chlorides (**6**–**10**), Hansch equation form: $\log(1/\mathrm{MIC}) = a + b \cdot \mathrm{Clog}\ P + c \cdot \mathrm{Clog}\ P^2$

Chlorides	a	b	C	r	r^2	s	p	Clog P_o
6a–6j	−2.6690 (±0.0600)	0.4880 (±0.0456)	−0.1528 (±0.0151)	0.9720	0.9447	0.1499	<0.0004	1.60
7odd	−2.2567 (±0.1005)	0.5846 (±0.0869)	−0.1472 (±0.0221)	0.9601	0.9219	0.1950	<0.0061	1.99
8a–8j	−2.6513 (±0.0310)	0.5289 (±0.0299)	−0.1013 (±0.0080)	0.9903	0.9808	0.0793	<0.0000	2.61
9a–9j	−12.2694 (±1.8398)	3.2871 (±0.6364)	−0.2511 (±0.0527)	0.9225	0.8510	0.2921	<0.0013	6.55
10a–10j	−7.4396 (±0.8321)	2.0963 (±0.3140)	−0.1792 (±0.0272)	0.9297	0.8643	0.2694	<0.0009	5.85

r denotes the correlation coefficient; r^2 is the predicted value of equation; s is the standard error of estimation; p is significance level of regression equations; numbers in parenthesis are S.D. of regression coefficients and Clog P_o is the apex of the parabola.

The solid products were filtered and, than, recrystallized from hexane–acetone system for **9a**–**9j** and from ethyl acetate for **10a**–**10j**.

*5.1.2.1. (2-Decanoyloxyethyl)-dodecyloxymethyldimethylammonium chloride (**9g**).* ^{1}H-NMR ($CDCl_3$) δ ppm = 5.15 (s, 2H), 4.55 (t, J = 4.7 Hz, 2H), 4.00 (t, J = 4.7 Hz, 2H), 3.88 (t, J = 6.6 Hz, 2H), 3.44 (s, 6H), 2.34 (t, J = 7.7 Hz, 2H), 1.62 (dt, 4H), 1.265 (m, 24H), 0.88 (t, J = 6.9 Hz, 6H); ^{13}C-NMR ($CDCl_3$) δ ppm = 172.6, 90.9, 73.7, 59.3, 57.3, 47.9, 33.8, 31.6, 29.5, 29.3, 29.21, 29.16, 29.0, 28.9, 25.6, 24.5, 22.5, 13.9.

*5.1.2.2. (2-Benzoyloxyethyl)-dodecyloxymethyldimethylammonium chloride (**10g**).* ^{1}H-NMR ($CDCl_3$) δ ppm = 8.01 (d, J = 7.4 Hz, 2H), 7.60 (t, J = 7.4 Hz, 1H), 7.45 (t, J = 7.4 Hz, 2H), 5.22 (s, 2H), 4.83 (t, 2H), 4.20 (t, 2H), 3.84 (t, J = 6.3 Hz, 2H), 3.52 (s, 6H), 1.55 (m, 2H), 1.25 (m, 18H), 0.88 (t, J = 6.9 Hz, 3H). ^{13}C-NMR ($CDCl_3$) δ ppm = 165.2, 133.4, 129.5, 128.4, 128.4, 90.7, 73.6, 59.3, 58.1, 48.0, 31.7, 29.46, 29.43, 29.2, 25.6.

5.2. Anti-microbial activity

The following microbial strains were used as test organisms: Gram-negative rods; *Pseudomonas aeruginosa* NCTC 6749, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae var. pneumoniae* ATCC 33495, *Proteus vulgaris* NCTC 4635, Gram-positive cocci; *Staphylococcus aureus* NCTC 4163, *Micrococcus luteus* NCTC 7743, *Enterococcus faecalis* ATCC 29212, Gram-positive bacillus; *Bacillus subtilis* ATCC 6633, and yeast-like fungi; *Candida albicans* ATCC 10231, *Rhodotorula rubra* (Demml 1889, Lodder 1934). Standard strains were supplied by National Collection of Type Cultures (NCTC), London and American Type Culture Collection (ATCC). The *R. rubra* was obtained from the Department of Pharmaceutical Bacteriology, Poznań, Poland.

Minimal inhibitory concentration (MIC) was determined by the tube dilution method. Twofold dilutions of quats were prepared in Müller–Hinton broth medium (bacteria) or Sabouraud broth medium (fungi). The samples were incubated at 37 °C (bacteria) and at 26 °C (fungi) and after 48 h the results were recorded. Growth of the microorganisms was determined visually. The lowest concentration at which there was no visible growth (turbidity) was taken as the MIC.

5.3. QSAR analysis

5.3.1. Structural parameters

The theoretically calculated logarithm of 1-octanol/water partition coefficient parameters (Clog P) were chosen as structural parameters for the obtained quaternary ammonium chlorides **6**–**10**. For the quats, the parameters (Clog P) were calculated by KOWIN™ v.1.66 program (Syracuse Research Corporation, New York, NY) from software package EPI Suite™ v.3.10 (U.S. Environmental Protection Agency), which is based on the Meylan–Howard method.

5.3.2. Statistics

The statistical analysis was carried out using Statistica v.6 software (StatSoft, Inc., Tulsa, OK). In the multivariate regression analysis, the tested microorganisms average minimal inhibitory concentration value for chlorides **6**–**10** was related to structural parameters (independent variables).

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References

[1] N. Menschutkin, Z. Phys. Chem. 5 (1890) 589–601.

[2] (a) W.A. Jacobs, M. Heidelberger, J. Biol. Chem. 20 (1915) 659–683;
(b) W.A. Jacobs, M. Heidelberger, J. Biol. Chem. 20 (1915) 685–694;
(c) W.A. Jacobs, M. Heidelberger, J. Biol. Chem. 21 (1915) 103–143.
[3] W.A. Jacobs, J. Exp. Med. 23 (1916) 563–568.
[4] G. Domagk, Dtsch. Med. Wochenschr. 61 (1935) 829–832.
[5] M. Mąkosza, B. Serafinowa, Rocz. Chem. 39 (1965) 1223–1231.
[6] A. Hassner, C. Stumer, Organic Syntheses Based on Name Reactions and Unnamed Reactions, Pergamon, Oxford, 1994.
[7] J.A. Butcher, A.F. Preston, J.A. Drysdale, For. Prod. J. 27 (1977) 19–22.
[8] J.A. Butcher, J.A. Drysdale, Mater. Org. 12 (1977) 271–277.
[9] T. Welton, Chem. Rev. 99 (1999) 2071–2083.
[10] P. Wasserscheid, W. Keim, Angew. Chem. Int. Ed. Engl. 39 (2000) 3772–3789.
[11] R. Sheldon, Chem. Commun. (2001) 2399–407.
[12] H. Olivier-Bourbigou, L. Magna, J. Mol. Catal. A Chem. 182–183 (2002) 419–437.
[13] J. Dupont, R.F. de Souza, P.A.Z. Suarez, Chem. Rev. 102 (2002) 3667–3692.
[14] R.D. Rogers, K.R. Seddon (Eds.), Ionic Liquids, Industrial Applications to Green Chemistry, ACS, Washington, DC, 2002.
[15] P. Wasserscheid, T. Welton (Eds.), Ionic Liquids in Synthesis, Wiley–VCH GmbH & Co. KGaA, Weinheim, 2002.
[16] A.D. Russell, W.B. Hugo, G.A.J. Ayliffe, Principles and Practice of Disinfection, Preservation and Sterilization, Blackwell Science, Oxford, 1999.
[17] T. Nishihara, T. Okamoto, N. Nishiyama, J. Appl. Microbiol. 88 (2000) 641–647.
[18] J. Pernak, I. Mirska, R. Kmiecik, Eur. J. Med. Chem. 34 (1999) 765–771.
[19] K.M. Khan, Z.S. Saify, K.A. Zeeshan, M. Ahmed, M. Saeed, R.J. Abdel-Jalil, G. Grubler, W. Voelter, Z. Naturforsch. B Chem. Sci. 54 (1999) 1210–1218.
[20] T. Maeda, Y. Manabe, M. Yamamoto, M. Yoshida, K. Okazaki, H. Nagamune, H. Kourai, Chem. Pharm. Bull. 47 (1999) 1020–1023.
[21] H. Kohma, T. Yabuhara, JP 2000 159,608; CA133: 27655 (2000).
[22] J. Wang, H. Zhu, CN 1,163,885; CA132: 65770 (2000).
[23] S.R. Subirana, G.N. Bonastre, J. Bigorra, DE 19,829,743; CA132: 32166 (2000).
[24] J. Pernak, J. Rogoża, I. Mirska, Eur. J. Med. Chem. 36 (2001) 313–320.
[25] J. Pernak, J. Kalewska, H. Ksycińska, J. Cybulski, Eur. J. Med. Chem. 36 (2001) 899–907.
[26] V. Klimešová, J. Kocí, M. Pour, J. Stachel, K. Waisser, J. Kaustová, Eur. J. Med. Chem. 37 (2002) 409–18.
[27] V.J. Staats, USP 2002 136,768; CA 137: 253035 (2002).
[28] W.M. Meylan, P.H. Howard, J. Pharm. Sci. 84 (1995) 83–92.