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SYNTHESIS AND ANTIMICROBIAL EFFICIENCY OF SOME NEW TYPES OF THE QUATERNARY AMMONIUM COMPOUNDS

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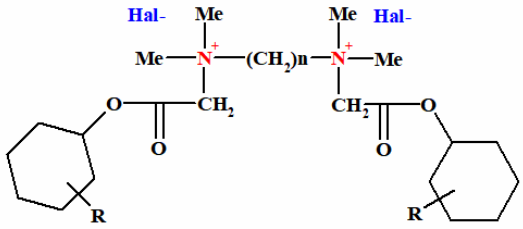
The manuscript deals with newly synthesized bis-quaternary ammonium compounds with non-polar alicyclic chains. The synthesized compounds have been investigated in the context of their antimicrobial activity against some widely distributed bacterial strains, and the most active compounds were identified among the series of synthesized products. An analysis of the influence of the structure of the compounds on their antimicrobial efficiency has been performed. It was shown that the synthesized compounds are highly active against the group of gram-positive bacteria (streptococci and staphylococci), and their minimum inhibition concentrations are ranged from 0.016 to 14.6 μg/mL. The strain *P. aeruginassa ATCC-2523* showed the highest resistance against the synthesized agents.



INTRODUCTION

The quaternary ammonium compounds (QAC) are used regularly as surfactants, laundry conditioners, hair softeners and antistatic agents. Some representatives of this class of compounds, cetyltrimethylammonium bromide (CTAB) or benzalkonium chloride, are used as phase-transfer catalyst to increase the rate of some heterogeneous processes.[1] Besides, they are used in the organic synthesis as reactants in Sommelet–Hauser rearrangement, Stevens rearrangement, and as source compounds in the construction of C-C, C-H and C-Het bonds in Hofmann elimination and Emde degradation.[2]

QAC are of a diphylic nature, and that is why they destroy the cell membranes when interacting with them. This property opens a possibility to use these compounds as disinfectants in the medicine and food processing industry.[3-4] On the other hand, they exhibit high washing activity and can be used for disinfection of the food processing machines and equipment. As a result, an uncontrolled application of QAC for these purposes causes the development of resistance against them in some groups of microbes. This resistance was reported since the early 90th in some strains of *S. aureus* against the common QAC disinfectants of the industrial equipment, benzalkonium chloride and cetylpyridinium chloride.[5-8] Therefore, some actions must be taken to counteract this tendency, which can potentially result in the foods bacterial contamination during the production process.

That is why the synthesis of QAC and investigation of their antimicrobial activity against the resistant strains, provided that they retain their effective washing properties, seems important for the search for new antiseptic agents to be used in food processing technologies and medicine.

Polyhexamethylene biguanides (PHMB) are known among the most effective antiseptics. Such common medicines as Alexidine and Chlorhexidine

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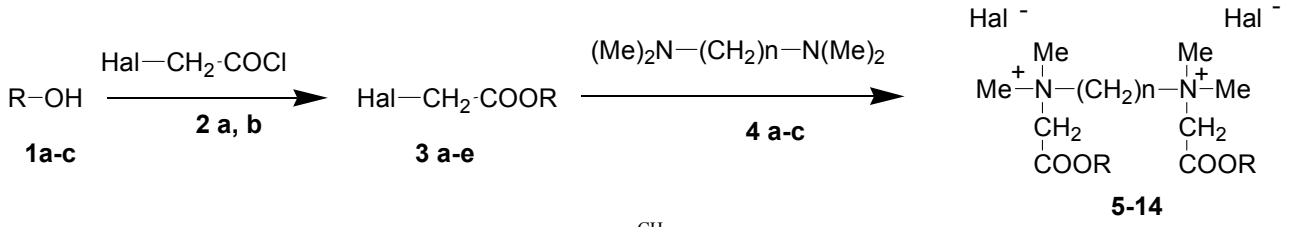
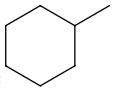
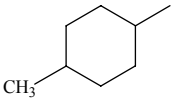
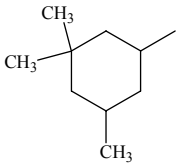
are, in fact, compounds of this category.[9-10] It can be noted that their high antimicrobial activity is based on their structural composition that includes two cationic centers separated by the nonpolar hexamethylene segment.[11] Since QAC and PHMB share some similarity in their structures, it is expected that the former class of compounds would also exhibit high antimicrobial efficiency.

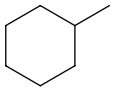
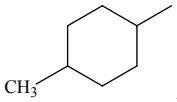
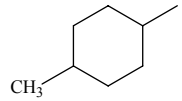
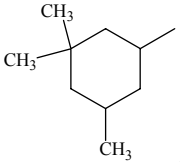
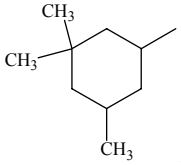
RESULTS AND DISCUSSION

Synthesis of the quaternary ammonium salts. A generalized scheme of synthesis of the quaternary ammonium salts is shown in Figure 1. The synthesized ammonium salts (**5-14**) are white crystalline compounds well soluble in water and poorly soluble in organic solvents. Their structure and composition were determined by qualitative elemental analysis, ^{1}H NMR, ^{13}CNMR and mass spectra.



1a, R =  ; **1b,** R =  ; **1c,** R =  .

2a, Hal = Cl, Br; **2b,** Hal = Br.

3a, R =  , Hal = Cl; **3b,** R =  , Hal = Cl; **3c,** R =  , Hal = Br; **3d,** R =  , Hal = Cl; **3e,** R =  , Hal = Br.

4a, n= 2; **4b,** n= 6; **4c,** n= 10.

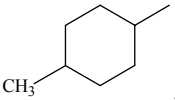
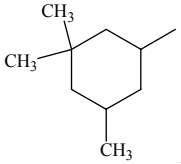
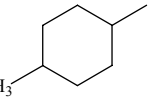
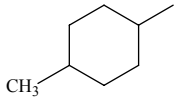
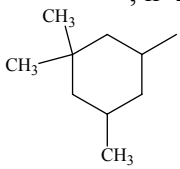
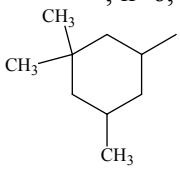
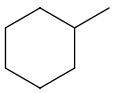
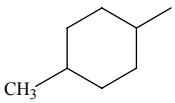
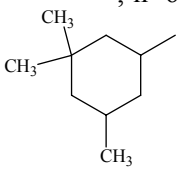
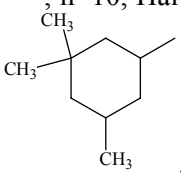
5, R=  , n=2, Hal = Br; **6**, R=  , n=2, Hal = Br; **7**, R=  , n=6, Hal = Br; **8**, R=  , n=6, Hal = Cl; **9**, R=  , n=6, Hal = Br; **10**, R=  , n=6, Hal = Cl; **11**, R=  , n=10, Hal = Cl; **12**, R=  , n=10, Hal = Br; **13**, R=  , n=10, Hal = Br; **14**, R=  , n=10, Hal = Cl.

Fig. 1 – General scheme of synthesis of the quaternary ammonium salts **5-14** (see explanations in the text).

Table 1

Antimicrobial activity of the quaternary salts **5-14** against the archived microbial strains

Compound	*S. aureus* ATCC-25923		*E. coli* ATCC-25922		*P. aeruginasa* ATCC-2523		*B. subtilis* ATCC-6633		*C. albicans* ATCC-885-653	
	MIC	MEC	MIC	MEC	MIC	MEC	MIC	MEC	MIC	MEC
5	500	1000	1000	1000	1000	1000	500	1000	500	1000
6	7,8	15,6	31,2	62,5	500	1000	250	500	250	500
7	250	500	1000	1000	1000	1000	500	1000	500	1000
8	500	1000	500	1000	1000	1000	500	1000	500	1000
9	3,9	7,8	31,2	62,5	500	1000	500	1000	125	250
10	3,9	7,8	15,6	31,2	250	500	500	1000	125	250
11	15,6	31,2	62,5	125	500	1000	500	1000	250	500
12	1,95	3,9	62,5	125	250	500	250	500	62,5	125
13	1,95	3,9	3,9	7,8	250	500	125	250	31,2	62,5
14	0,06	0,49	1,95	3,9	250	500	125	250	31,2	62,5

Table 2

Antimicrobial activity of the quaternary salts **5-14** against some antibiotic-resistant strains

Compound	*S. aureus**		*S. pyogenes***		*C. albicans**	
	MIC	MEC	MIC	MEC	MIC	MEC
5	31,2	62,5	3,9	7,8	125	250
6	3,9	7,8	1,95	3,9	125	250
7	62,5	125	125	500	31,2	62,5
8	125	250	31,2	62,5	62,5	125
9	0,97	1,95	0,97	1,95	31,2	62,4
10	0.97	1,95	0,97	1,95	31,2	62,5
11	7,8	15,6	3,9	7,8	62,5	125
12	0,97	1,95	0,97	1,95	15,6	31,2
13	0,24	0,48	0,48	0,96	7,8	15,6
14	0,06	0,49	0,24	0,48	7,8	15,6

* - 10 freshly isolated starins
** - 5 freshly isolated

As seen from the results of the investigation of the bactericide/antifungal effect, the inhibition of growth of bacteria or fungi strains involved in this investigation required different concentration of the synthesized compounds ranged between 0.06 and 1000 μg/L (Table 1). It was found that the disinfection efficiency of the compounds depends on the nature of the alicyclic substitute in the ester part of the molecule and the distance between quaternary nitrogen atoms in the salt. The bactericide efficiency of compound **14** is the highest. The inter-nitrogen interval in it is 10 carbon units, and there is a fragment of a 3,3,5-thrimethylcyclohexanol in its ester part. MEC of this compound is ranged between 0.06 and 250 μg/L depending on the strain. It can be noted that the replacement of bromide instead of chloride causes a halving of antibacterial activity for almost all compounds. The replacement of the 3,3,5-thrimethylcyclohexanol fragment with 4-methyl-cyclohexanol causes a 5-10 times lower bactericide activity. If the interval between the quaternary ammonium centers decreases, it expectedly results in a decrease in the bactericide activity of the compounds.

As seen from the data shown in Table 1, the highest bacteriostatic activity of the synthesized QAC was registered against gram-positive microbes (staphylococci and streptococci) with MIC ranging from 0.06 to 14.6 μg/L. It should also be emphasized that the QAC also revealed high activity against antibiotic-resistant staphylococci and fungi (Table 2). The strain *P. aeruginasa* ATCC-2523 was found the least sensitive to the synthesized compounds.

EXPERIMENTAL

Chemicals **1a-c, 2a,b** and **4a-c** were purchased from Enamine Ltd company. Melting points were measured on a Kofler melting point-device and are uncorrected. ^{1}H NMR spectra were acquired in pulsed Fourier transform mode on a Varian VXR-400 spectrometer (400 MHz), while ^{13}CNMR spectra were acquired on a Bruker Avance DRX-500 spectrometer (125 MHz), using DMSO-d_6 as solvent. Mass spectra were recorded on an Agilent LC/MSD SL chromatograph equipped with Zorbax SB-C_{18} column

(4.6x15mm), particle size 1.8 μm (PN 82(c)75-932), solvent DMSO, electrospray ionization at atmospheric pressure. Elemental analysis was performed on a PerkinElmer 2400 CHN Analyzer. The individuality of the obtained compounds was monitored by TLC on Silufol UV-254 plates.

1. Synthesis of esters of the halogen ethanoic acids (3 a-e). A solution of 0.011 moles of the aliphatic alcohol (**1a-c)** in 100 mL of benzene were mixed with 0.01 mole of the halogen ethanoic acid chloride (**2a-b**) and boiled for 4 hours. Then the solvent was evaporated at low pressure. According to the chromatographic analysis results, the esters content was approximately 95-98%, and that is why the esters were used at the next stage without additional purification.

2. Synthesis of the quaternary ammonium salts (**5-14**). A solution of 0.005 moles of the diamine (**4**) in 50 ml of the dry benzene was mixed with 0.01 mole of the ester of halogen ethanoic acid (**3**) and kept at the room temperature for 48 hours. Then the sediment, formed after the reaction, was filtered out, washed with the diethyl, and then the solvent was evaporated at low pressure. The product obtained in this reaction should be kept in a desiccator in contact with phosphorus pentoxide.

Compound 3a. Yield 89%, boiling point 90-91°C (10Torr)[12]. ^{1}H NMR: δ = 4.86 (qt, 1H, OC<u>H</u>), 4.09 (s, 2H, O=CC<u>H</u>$_2$Cl), 1.84-1.79 (m, 2H, C<u>H</u>$_2$cycloalk.), 1.75-1.68 (m, 2H, C<u>H</u>$_2$cycloalk.), 1.63-1.21 (m, 6H, C<u>H</u>$_2$cycloalk.). LC-MS: m/z = 177 [M+1] (100%).

Compound 3b. Yield 87%, boiling point 110-112°C (10Torr). ^{1}H NMR: δ = 4.84 (qt, 1H, OC<u>H</u>), 4.08 (s, 2H, O=CC<u>H</u>$_2$Cl), 1.82-1.78 (m, 2H, C<u>H</u>$_2$cycloalk.), 1.73-1.64 (m, 2H, C<u>H</u>$_2$cycloalk.), 1.61-1.18 (m, 6H, C<u>H</u>$_2$cycloalk.), 0.95 (d, 3H, C<u>H</u>$_3$) LC-MS: m/z = 191 [M+1] (100%).

Compound 3c. Yield 84%, boiling point 121-122°C (10Torr)[13]. ^{1}H NMR: δ = 4.81-4.84 (m, 1H, OC<u>H</u>), 3.83 (s, 2H, O=CC<u>H</u>$_2$Br), 1.80-1.77 (m, 2H, C<u>H</u>$_2$cycloalk.), 1.72-1.62 (m, 2H, C<u>H</u>$_2$cycloalk.), 1.60-1.21 (m, 6H, C<u>H</u>$_2$cycloalk.), 1.01 (d, 3H, C<u>H</u>$_3$) LC-MS: m/z = 235 [M+1] (100%).

Compound 3d. Yield 88%, boiling point 130-131°C (10Torr). ^{1}H NMR: δ = 4.75-4.77 (m, 1H, OC<u>H</u>), 4.10 (s, 2H, O=CC<u>H</u>$_2$Cl), 1.80-1.56 (m, 7H, <u>H</u>cycloalk.), 1.13 (s, 3H, C<u>H</u>$_3$), 1.07 (s, 3H, C<u>H</u>$_3$), 0.90 (d, 3H, C<u>H</u>$_3$) LC-MS: m/z = 219 [M+1] (100%).

Compound 3e. Yield 88%, boiling point 152-153°C (10Torr). ^{1}H NMR: δ = 4.77-4.80 (m, 1H, OC<u>H</u>), 4.10 (s, 2H, O=CC<u>H</u>$_2$Br), 1.80-1.54 (m, 7H, Hcycloalk.), 1.12 (s, 3H, C<u>H</u>$_3$), 1.03 (s, 3H, C<u>H</u>$_3$), 0.92 (d, 3H, C<u>H</u>$_3$) LC-MS: m/z = 264 [M+1] (100%).

Compound 5. Yield 87%, melting point 187-188°C. ^{1}H NMR: δ =4.93-4.91 (m, 4H, 2(O=CC<u>H</u>$_2$N$^+$)), 4.80-4.78 (m, 4H, 2(OC<u>H</u>)), 4.21-4.18 (m, 4H, N$^+$C<u>H</u>$_2$C<u>H</u>$_2$N$^+$), 3.72-3.69 (m, 12H, 4(C<u>H</u>$_3$N$^+$)), 1.84-1.32 (m, 20<u>H</u>, Hcycloalk.), 1.04-0.95 (m, 6H, C<u>H</u>$_3$). ^{13}C NMR: δ = 167.5 (<u>C</u>OO), 75.2 (O<u>C</u>H), 61.8 (O=C<u>C</u>H$_2$N$^+$), 58.9 (N$^+$<u>C</u>H$_2$<u>C</u>H$_2$N$^+$), 53.5 (<u>C</u>H$_3$N$^+$), 34.9, 31.6, 31.00, 22.42 (<u>C</u>ycloalk., <u>C</u>H$_3$). LC-MS: m/z = 427 [M^{2+}](100%).[14] Determined %: C 49.25; H 7.87; N 4.71; Br 27.35. $C_{24}H_{46}N_2O_4Br_2$. Calculated %: C 49.15; H 7.91; N 4.78; Br 27.25.

Compound 6. Yield 94%, melting point 192-193°C. ^{1}H NMR: δ =4.90-4.87 (m, 4H, 2(O=CC<u>H</u>$_2$N$^+$)), 4.78-4.76 (m, 4H, 2(OC<u>H</u>)), 4.21-4.16 (m, 4H, N$^+$C<u>H</u>$_2$C<u>H</u>$_2$N$^+$), 3.75-3.68 (m, 12H, 4(C<u>H</u>$_3$N$^+$)), 1.80-1.34 (m, 14<u>H</u>, Hcycloalk.), 1.01-0.93 (m, 18H, C<u>H</u>$_3$). ^{13}C NMR: δ = 167.2 (<u>C</u>OO), 69.5 (O<u>C</u>H), 61.6 (O=C<u>C</u>H$_2$N$^+$), 57.3 (N$^+$<u>C</u>H$_2$<u>C</u>H$_2$N$^+$), 53.1 (<u>C</u>H$_3$N$^+$), 38.0, 31.9, 27.4, 25.5, 22.7 (<u>C</u>ycloalk., <u>C</u>H$_3$). LC-MS: m/z = 483 [M^{2+}] (100%). Determined %: C 52.39; H 8.40; N 4.39; Br 24.81. $C_{28}H_{54}N_2O_4Br_2$. Calculated %: C 52.34; H 8.47; N 4.36; Br 24.87.

Compound 7. Yield 91%, melting point 174-175°C. ^{1}H NMR: δ =4.93-4.90 (m, 4H, 2(O=CC<u>H</u>$_2$N$^+$)), 4.80-4.76 (m, 4H, 2(OC<u>H</u>)), 4.22-4.18 (m, 4H, N$^+$C<u>H</u>$_2$), 3.71-3.65 (m, 12H, 4(C<u>H</u>$_3$N$^+$)), 1.84-1.30 (m, 20<u>H</u>, Hcycloalk. + 4H, N$^+$CH$_2$C<u>H</u>$_2$), 1.00-0.93 (m, 6H, C<u>H</u>$_3$ + 4H, N$^+$CH$_2$CH$_2$C<u>H</u>$_2$). ^{13}C NMR: δ = 169.3 (<u>C</u>OO), 75.1 (O<u>C</u>H), 66.5 (O=C<u>C</u>H$_2$N$^+$), 62.9 (N$^+$<u>C</u>H$_2$), 54.2 (<u>C</u>H$_3$N$^+$), 35.0, 31.6, 31.01, 24.5, 24.3, 22.4 (<u>C</u>ycloalk., + $^+$CH$_2$<u>C</u>H$_2$C<u>H</u>$_2$ + <u>C</u>H$_3$). LC-MS: m/z = 483 [M^{2+}] (100%). Determined %: C 52.32; H 8.44; N 4.43; Br 24.83. $C_{28}H_{54}N_2O_4Br_2$. Calculated %: C 52.34; H 8.47; N 4.36; Br 24.87.

Compound 8. Yield 85%, melting point 168-169°C. ^{1}H NMR: δ =4.93-4.90 (m, 4H, 2(O=CC<u>H</u>$_2$N$^+$)), 4.80-4.78 (m, 4H, 2(OC<u>H</u>)), 4.20-4.14 (m, 4H, N$^+$C<u>H</u>$_2$), 3.71-3.63 (m, 12H, 4(C<u>H</u>$_3$N$^+$)), 1.84-1.30 (m, 20<u>H</u>, Hcycloalk. + 4H, N$^+$CH$_2$C<u>H</u>$_2$), 1.01-0.94 (m, 6H, C<u>H</u>$_3$ + 4H, N$^+$CH$_2$CH$_2$C<u>H</u>$_2$). ^{13}C NMR: δ= 169.8 (<u>C</u>OO), 75.3 (O<u>C</u>H), 66.5 (O=C<u>C</u>H$_2$N$^+$), 62.8 (N$^+$<u>C</u>H$_2$), 54.3 (<u>C</u>H$_3$N$^+$), 35.0, 31.4, 31.03, 24.2, 24.1, 22.4 (<u>C</u>ycloalk., + $^+$CH$_2$<u>C</u>H$_2$C<u>H</u>$_2$ + <u>C</u>H$_3$). LC-MS: m/z = 483 [M^{2+}] (100%). Determined %: C 60.80; H 9.88; N 5.11; Cl 12.80. $C_{28}H_{54}N_2O_4Cl_2$. Calculated %: C 60.74; H 9.83; N 5.06; Cl 12.81.

Compound 9. Yield 89%, melting point 134-135°C. ^{1}H NMR: δ =5.10-5.04 (m, 4H, 2(O=CC<u>H</u>$_2$N$^+$)), 4.38-4.30 (m, 4H, 2(OC<u>H</u>)), 3.48-3.45 (m, 4H, N$^+$C<u>H</u>$_2$), 3.35-3.30 (m, 12H, 4(C<u>H</u>$_3$N$^+$)), 1.84-1.30 (m, 14<u>H</u>, Hcycloalk. + 4H, N$^+$CH$_2$C<u>H</u>$_2$), 1.08-0.92 (m, 18H, C<u>H</u>$_3$ + 4H, N$^+$CH$_2$CH$_2$C<u>H</u>$_2$). ^{13}C NMR: δ= 163.2 (<u>C</u>OO), 69.4 (O<u>C</u>H), 66.5 (O=C<u>C</u>H$_2$N$^+$), 62.7 (N$^+$<u>C</u>H$_2$), 54.2 (<u>C</u>H$_3$N$^+$), 48.1, 38.0, 32.9, 31.9, 27.4, 25.5, 24.5, 24.3 (<u>C</u>ycloalk., + $^+$CH$_2$<u>C</u>H$_2$C<u>H</u>$_2$ + <u>C</u>H$_3$). LC-MS: m/z = 539 [M^{2+}] (100%). Determined %: C 55.09; H 8.90; N 4.05; Br 22.75. $C_{32}H_{62}N_2O_4Br_2$. Calculated %: C 55.01; H 8.94; N 4.01; Br 22.87.

Compound 10. Yield 87%, melting point 122-123°C. ^{1}H NMR: δ =5.10-5.03 (m, 4H, 2(O=CC<u>H</u>$_2$N$^+$)), 4.38-4.32 (m, 4H, 2(OC<u>H</u>)), 3.48-3.43 (m, 4H, N$^+$C<u>H</u>$_2$), 3.36-3.29 (m, 12H, 4(C<u>H</u>$_3$N$^+$)), 1.82-1.30 (m, 14<u>H</u>, Hcycloalk. + 4H, N$^+$CH$_2$C<u>H</u>$_2$), 1.06-0.92 (m, 18H, C<u>H</u>$_3$ + 4H, N$^+$CH$_2$CH$_2$C<u>H</u>$_2$). ^{13}C NMR: δ= 163.1 (<u>C</u>OO), 69.4 (O<u>C</u>H), 66.6 (O=C<u>C</u>H$_2$N$^+$), 62.7 (N$^+$<u>C</u>H$_2$), 54.3 (<u>C</u>H$_3$N$^+$), 48.1, 38.2, 32.9, 31.9, 27.4, 25.5, 24.5, 24.2 (<u>C</u>ycloalk., + $^+$CH$_2$<u>C</u>H$_2$C<u>H</u>$_2$ + <u>C</u>H$_3$). LC-MS: m/z = 539 [M^{2+}] (100%). Determined %: C 63.10; H 10.20; N 4.52; Cl 11.60. $C_{32}H_{62}N_2O_4Cl_2$. Calculated %: C 63.03; H 10.25; N 4.59; Cl 11.63.

Compound 11. Yield 96%, melting point 124-125°C. ^{1}H NMR: δ =5.03-4.96 (m, 4H, 2(O=CC<u>H</u>$_2$N$^+$)), 4.38-4.30 (m, 4H, 2(OC<u>H</u>)), 3.45-3.40 (m, 4H, N$^+$C<u>H</u>$_2$), 3.38-3.27 (m, 12H, 4(C<u>H</u>$_3$N$^+$)), 1.85-0.89 (m, 22<u>H</u>, Hcycloalk. + 16H, N$^+$CH$_2$C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$). ^{13}C NMR: δ = 163.1 (<u>C</u>OO), 69.4 (O<u>C</u>H), 66.6 (O=C<u>C</u>H$_2$N$^+$), 62.7 (N$^+$<u>C</u>H$_2$), 54.3 (<u>C</u>H$_3$N$^+$), 48.1, 38.2, 32.9, 31.9, 27.4, 25.5, 24.5, 24.2 (<u>C</u>ycloalk., + $^+$CH$_2$<u>C</u>H$_2$C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$). LC-MS: m/z = 511 [M^{2+}] (100%). Determined %: C 61.99; H 10.12; N 4.89; Cl 12.09. $C_{30}H_{58}N_2O_4Cl_2$. Calculated %: C 61.94; H 10.05; N 4.82; Cl 12.19.

Compound 12. Yield 90%, melting point 120-121°C. δ =5.04-4.96 (m, 4H, 2(O=CC<u>H</u>$_2$N$^+$)), 4.33-4.29 (m, 4H, 2(OC<u>H</u>)), 3.46-3.42 (m, 4H, N$^+$C<u>H</u>$_2$), 3.34-3.29 (m, 12H, 4(C<u>H</u>$_3$N$^+$)), 1.85-0.86 (m, 20<u>H</u>, Hcycloalk. + 16H,

$N^+CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2$ + 6H, CH_3). ^{13}C NMR: δ = 163.3 (COO), 67.4 (OCH), 66.0 ($O{=}C\underline{C}H_2N^+$), 62.4 ($N^+\underline{C}H_2$), 54.6 ($\underline{C}H_3N^+$), 48.1, 38.2, 32.9, 31.9, 27.4, 25.5, 24.5, 24.2 (Ccycloalk., + $^+CH_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2$ + $\underline{C}H_3$). LC-MS: m/z = 539 [M^{2+}] (100%). Determined %: C 55.05; H 8.94; N 4.05; Br 22.82. $C_{32}H_{62}N_2O_4Br_2$. Calculated %: C 55.01; H 8.94; N 4.01; Br 22.87.

Compound 13. Yield 93%, melting point 118-119°C. δ =5.04-4.94 (m, 4H, 2($O{=}C\underline{C}H_2N^+$)), 4.30-4.26 (m, 4H, 2(OCH)), 3.46-3.41 (m, 4H, $N^+\underline{C}H_2$), 3.32-3.27 (m, 12H, 4($\underline{C}H_3N^+$)), 1.88-0.89 (m, 14H, Hcycloalk. + 16H, $N^+CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2$ + 18H, CH_3). ^{13}C NMR: δ = 169.3 (COO), 69.4 (OCH), 66.5 ($O{=}C\underline{C}H_2N^+$), 62.7 ($N^+\underline{C}H_2$), 54.2 ($\underline{C}H_3N^+$), 48.1, 48.2, 38.2, 32.9, 31.9, 27.9, 27.3, 27.2, 27.1, 26.5, 25.5, 22.7 (Ccycloalk., + $^+CH_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2$ + $\underline{C}H_3$). LC-MS: m/z = 595 [M^{2+}] (100%). Determined %: C 57.32; H 9.39; N 3.70; Br 21.11. $C_{36}H_{70}N_2O_4Br_2$. Calculated %: C 57.29; H 9.35; N 3.71; Br 21.17.

Compound 14. Yield 94%, melting point 105-106°C. δ =5.07-4.96 (m, 4H, 2($O{=}C\underline{C}H_2N^+$)), 4.30-4.24 (m, 4H, 2(OCH)), 3.45-3.41 (m, 4H, $N^+\underline{C}H_2$), 3.30-3.24 (m, 12H, 4($\underline{C}H_3N^+$)), 1.88-0.92 (m, 14H, Hcycloalk. + 16H, $N^+CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2C\underline{H}_2$ + 18H, CH_3). ^{13}C NMR: δ = 169.7 (COO), 69.6 (OCH), 66.5 ($O{=}C\underline{C}H_2N^+$), 62.4 ($N^+\underline{C}H_2$), 54.6 ($\underline{C}H_3N^+$), 48.2, 48.2, 38.4, 32.8, 31.6, 27.9, 27.4, 27.3, 27.1, 26.4, 25.6, 22.6 (Ccycloalk., + $^+CH_2\underline{C}H_2\underline{C}H_2\underline{C}H_2\underline{C}H_2$ + $\underline{C}H_3$). LC-MS: m/z = 595 [M^{2+}]. Determined %: C 64.97; H 10.63; N 4.22; Cl 10.60. $C_{36}H_{70}N_2O_4Cl_2$. Calculated %: C 64.94; H 10.60; N 4.21; Cl 10.65.

A primary microbiological screening and determination of the antimicrobial and antifungal efficiency of the synthesized compounds (**5-14**) were performed by the method of double serial dilutions. A liquid or solid nutrient media were used depending on the nature and type of the test microbes. The 1% meat peptone broth (MPB) with pH=7.2 was used for all bacterial groups, while the 1% glucose MPB was used in the case of β-hemolytic streptococcus.

Liquid Sabouraud agar with pH=6.8 was used to investigate the biological activity of the synthesized compounds against the archival and freshly isolated strains of pathogenic fungi of the genus *Candida*.

The daily bacterial cultures grown on the appropriate substrates at 37°C were used to determine their sensitivity to the synthesized compounds. The yeast-like fungi of the genus *Candida* were used after two days of cultivation.

The concentration of bacterial cultures introduced to the titration row test-tubes was 100 000 cells/mL, and the content of the fungi was 10 000 cells/mL.

All calculations were performed in 20-24 hours after the beginning of incubation, and the last dilution of the antibacterial/antifungal compound that provided a bacteriostatic or fungistatic effect was considered as the corresponding minimum bacteriostatic (fungistatic) concentration (MIC – minimum inhibition concentration). The minimum bactericide (fungicide) concentrations (MEC – minimum extinguishing concentration) were determined similarly, as the minimal concentrations that caused a complete cessation of the growth of the bacterial or fungal culture on the solid substrate after 20-24 hours (48 hours in the case of fungi).

CONCLUSION

The bis-quaternary ammonium salts synthesized from esters of the halogen ethanoic acids and tetramethyldiamines have been found active against a wide range of the archival and freshly isolated bacteria and fungi. Their antimicrobial activity against gram-positive germs is higher than that against gram-negative organisms.

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