Synthesis and Antimicrobial Activity of a Series of *N,N*-Dimethyl-*N*-alkyl-D-glucaminium Bromides

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N,N-Dimethyl-*N*-alkyl-D-glucaminium bromides, with the number of carbon atoms in the alkyl chain varying from 8 to 22, have been synthesized by an S_N reaction of *N,N*-dimethyl-D-glucamine with 1-bromoalkanes. The target compounds were characterized and their structure proved by NMR and IR spectroscopy. The effect on *S. aureus*, *E. coli*, and *C. albicans* was also examined and the antimicrobial activity expressed by the minimum inhibitory concentration (MIC). The structure—activity relationship was evaluated by the QSAR method using the *Kubínyi*'s bilinear model.

Quaternary ammonium salts (QAS), especially those possessing amphiphilic properties, are well known and widely used in multifunctional formulations with cleansing, disinfecting, antimicrobial, detergent, and similar effects [1, 2]. One of the main factors which affects the properties of QAS is lipophilicity. Many authors studied dependence of antimicrobial activity on the lipophilicity by varying the number of carbon atoms in one of the alkyl chains bonded to the nitrogen atom of QAS [2—5]. It was found that the function $\log(1/\mathrm{MIC}) = f(x)$, where x is m (number of carbons), $\log P$, R_M or c_k (critical micelle concentration), is nonlinear and can be expressed by parabolic *Hansch*'s [6] or the *Kubínyi*'s bilinear model [7].

One of the most favourable properties of QAS is their low toxicity and decomposition to simpler molecules by hydrolytic oxidation or biodegradation. It is therefore advantageous when the long alkyl chains of QAS contain groups which are susceptible to such reactions (*e.g.* esters, amides, alcohols) [2—5]. QAS derived from *N*-methyl-D-glucamine belong to such a group of cation-active compounds bearing hydroxyl groups on the alkyl chain. These types of QAS display surface-active behaviour as well as antimicrobial and antifungicidal activities [8, 9]. They are routinely prepared by starting from *N*-methyl-D-glucamine [10] or *N,N*-dimethyl-D-glucamine [11] which in turn can be obtained by condensation of methylamine (or dimethylamine) with D-glucose. Some surface-active and solubilization properties have also been observed for *N*-methyl-D-glucamine as well as *N,N*-dialkyl-D-glucamines [12].

EXPERIMENTAL

I (99 %), m.p. = 129—131.5 °C, was commercially available product from Aldrich. Melting points were measured on a Kofler hot stage. Samples for elemental analysis were dried over phosphorus pentoxide (30—80 °C; 2 kPa) for 8—10 h. Elemental analyses (with the following deviations from calculated values: ± 0.30 % C, 0.20 % H, and 0.15 % N) were performed on C. Erba analyzer, model 1102. IR spectra were obtained on a M-80 (Zeiss, Jena) spectrophotometer, compounds *II* and *III* were measured in KBr pellets and compounds *IV—XIII* in a chloroform solution. NMR spectra (^{1}H at 299.93 MHz and ^{13}C at 75.43 MHz) were measured in CD_3OD (99.6 %) at room temperature on a Varian VXR-300 spectrometer. The protonated part of the solvent was used as the reference (δ = 3.34) for ^{1}H shifts and the deuterated part (δ = 49.0) for ^{13}C shifts. To assign the signals unambiguously, the two-dimensional ^{1}H-^{1}H correlation (DQCOSY) and ^{1}H-^{13}C heteronuclear correlation (HETCOR) experiments were carried out for the samples.

All the compounds were checked for purity by TLC on silica gel Silufol UV-254 (Kavalier, Czechoslovakia). Mobile phase for compounds *II* and *III* – acetic acid : water (φ_r = 1 : 5). For compounds *IV—XIII* (Table 1) plates impregnated by 5 % solution of silicon oil in heptane were used, mobile phase – 1 M-HCl—acetone (φ_r = 3 : 2) (partition TLC), detected by the Dragendorff's spray reagent.

The antimicrobial activity was examined on *Staphylococcus aureus* 45/54 *Oxford Man* 29/58, *Escherichia coli* 377/79, and *Candida albicans* 45/44

Table 1. Characterization of the Prepared Compounds

Compound	R	m	Formula	M_r	Yield/%	M.p./°C	R_f^a	Time/h[b]
III		0	$C_8H_{19}NO_5$	209.24	90	93—94	0.71[c]	
IV	Octyl	8	$C_{16}H_{36}NO_5Br$	402.37	82	65—66	0.77	24
V	Nonyl	9	$C_{17}H_{38}NO_5Br$	416.40	85	109—110	0.69	26
VI	Decyl	10	$C_{18}H_{40}NO_5Br$	430.43	80	119—122	0.58	28
VII	Undecyl	11	$C_{19}H_{42}NO_5Br$	444.45	72	160—162	0.47	30
VIII	Dodecyl	12	$C_{20}H_{44}NO_5Br$	458.48	60	165—167	0.40	32
IX	Tetradecyl	14	$C_{22}H_{48}NO_5Br$	486.54	69	192—193	0.21	34
X	Hexadecyl	16	$C_{24}H_{52}NO_5Br$	514.57	85	223—225	0.14	38
XI	Octadecyl	18	$C_{26}H_{56}NO_5Br$	542.54	86	252—254	0.08	42
XII	Eicosyl	20	$C_{28}H_{60}NO_5Br$	570.70	72	256—258	0.06	50
XIII	Docosyl	22	$C_{30}H_{64}NO_5Br$	598.75	75	266—268	0.04	60

a) Mobile phase: 1 M-HCl—acetone ($\varphi_r = 3 : 2$); *b*) reaction time; *c*) mobile phase: acetic acid—water ($\varphi_r = 1 : 5$).

from the Czecho-Slovak collection of type cultures. A method of dilution test [13] was used and the antimicrobial activity is expressed by the minimum inhibitory concentration (MIC), standard: *N,N*-dimethylbenzyldodecylammonium bromide. The toxicity of some selected compounds was evaluated after the subcutaneous application of 1 % solution of the compound to white mice and expressed as an estimation of LD_{50} values (determined 24 h after application of the compound).

N,N-Dimethyl-D-glucaminium Chloride (*II*)

99 % formic acid (113.7 g; 2.5 mol) was placed into a 500 cm^3 flask and under stirring and cooling a 35 % aqueous formaldehyde (94.3 g; 1.1 mol) was added so as the temperature of the mixture was 20—30 °C. Then *I* (195.2 g; 1 mol) was added portionwise under stirring and the mixture was heated under reflux for 16 h. After cooling to room temperature, 37 % hydrochloric acid (103 g; 1.05 mol) was added under stirring and the mixture was stirred for additional 20 min at room temperature. Then the liquid components were removed by distillation under reduced pressure and the rest of moisture was removed by azeotropic distillation with toluene. The residue – sirup oil – was extracted with hot propanol (2 × 200 cm^3). After 10—15 min a solid colourless product appeared, which was filtered, washed with ethanol and dried. Crystallization from anhydrous ethanol afforded 206 g (84 %) of *II*, m.p. = 133—135 °C. IR spectrum (KBr pellets), $\tilde{\nu}/cm^{-1}$: 3360 (O—H), 2996 (H—CH_2N^+), 2716 (N^+—H), 1456 $\delta_s(H_3C—N^+)$.

N,N-Dimethyl-D-glucamine (*III*)

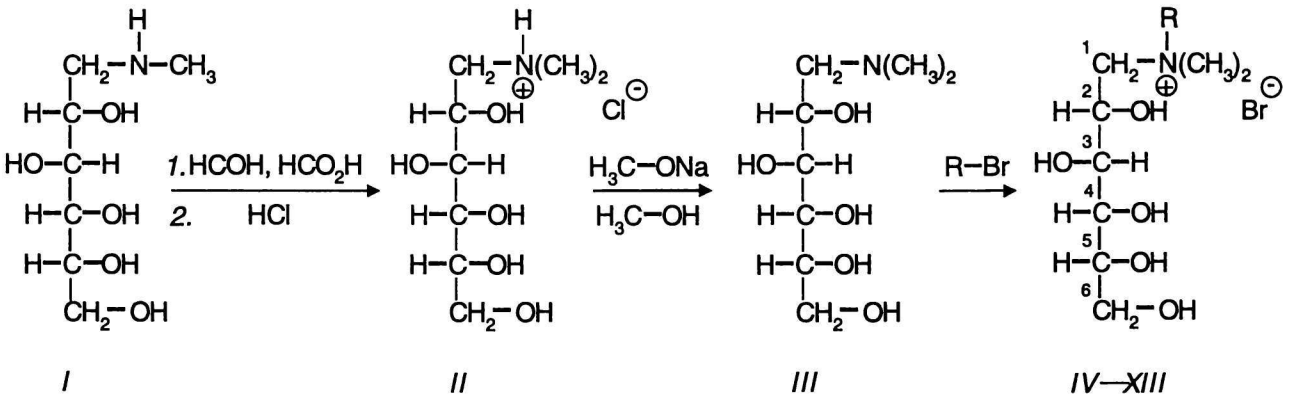
To a solution of sodium (11.5 g; 0.5 mol) in anhydrous methanol (300 cm^3) a hot solution of *II* (122.8 g; 0.5 mol) in anhydrous methanol (250—300 cm^3) was added and the reaction mixture was refluxed for 10 min. After cooling to 20 °C, the solid sodium chloride was filtered off and methanol was removed by distillation under reduced pressure. The residue (viscous oil) was crystallized from anhydrous ethyl acetate to yield 94 g (90 %) of *III* as a colourless solid, m.p. = 92—94 °C. Characterization of the compound is given in Table 1, spectral data in Tables 2—4.

N,N-Dimethyl-*N*-alkyl-D-glucaminium Bromides (*IV*—*XIII*)

A mixture of *III* (3.6 g; 30 mmol), 1-bromoalkane (35 mmol), and solvent (20—40 cm^3) was refluxed for 24—60 h so as to prevent it from atmospheric moisture. Then the mixture was cooled and allowed to stand for a day in a refrigerator. The product which appeared (as a solid or an oil) was separated and impurities were removed by repeated extraction with hot acetonitrile. Crystallization was performed from anhydrous acetone to obtain *IV*—*XIII* as colourless solids, the characteristics of which are given in Table 1. A mixture of 2-propanol—acetonitrile was used, the ratio of the solvents and the reaction time being varied depending on the number of carbon atoms of the corresponding 1-bromoalkane. For 1-bromoacetate the volume ratio of the solvents was $\varphi_r = 3 : 1$ and the reaction time 24 h, whereas for 1-bromodocosane $\varphi_r = 1 : 3$ and the reaction time 60 h. For other 1-bromoalkanes these parameters are given in Table 1.

RESULTS AND DISCUSSION

A homological series of *N,N*-dimethyl-*N*-alkyl-D-glucaminium bromides *IV*—*XIII* was prepared by reaction of *N,N*-dimethyl-D-glucamine (*III*) with 1-bromoalkanes with the number of carbon atoms m = 8—12, 14, 16, 18, 20, 22. A mixture of 2-propanol and methylcyanide was used as solvent in this reaction. The volume ratio of these solvents as well as the reaction time varied depending on the length of the 1-bromoalkane. They were $\varphi_r = 3 : 1$ and 24 h for 1-bromooctane, whereas for 1-bromodocosane $\varphi_r = 1 : 3$ and the reaction time 60 h. For the other 1-bromoalkanes the two parameters were intermediate



$R = C_mH_{2m+1}$; m = 8—12, 14, 16, 18, 20, 22

Scheme 1

Table 2. IR Spectra of the Prepared Compounds

Compound	$\tilde{\nu}$/cm^{-1}				
	ν(OH)	ν(C—H)	ν(CH$_3$—N)	ν(C—O)	δ(CH$_2$)
III	3356		1464	1090	720
IV	3340	2928	1468	1082	724
V	3348	2928	1468	1080	724
VI	3336	2928	1468	1080	724
VII	3340	2928	1466	1082	724
VIII	3348	2928	1466	1080	722
IX	3340	2928	1466	1080	724
X	3348	2928	1466	1080	724
XI	3358	2928	1467	1080	724
XII	3348	2929	1468	1080	724
XIII	3354	2929	1466	1076	722

between these limits (Table 1). Yields of the products *IV*—*XIII* ranged from 60 to 82 % while the yields were substantially lower when either 2-propanol or methylcyanide alone was used as solvent (10—20 %). The mixture of the above solvents was chosen since both *III* and higher 1-bromoalkanes are insoluble in methylcyanide, so the ratio of the solvents was adjusted on the following grounds: for each 1-bromoalkane a constant volume of methylcyanide (20 cm^3) was added to the reaction mixture and under boiling 2-propanol was added portionwise unless the mixture appeared in solution. The starting *III* which is described in the literature as an oil obtained by reaction of D-glucose with dimethylamine [11], was prepared in this work by reductive methylation (Wallach—Leuckart reaction) [14] of *N*-methylglucamine (*I*) by formaldehyde and formic acid (Scheme 1). The first intermediate in this reaction is *N,N*-dimethyl-D-glucaminium chloride (*II*). It was found that the commonly used method based on liberation of the amine from an aqueous solution of its salt by treating with sodium or potassium hydroxide followed by isolation of the amine by ex-

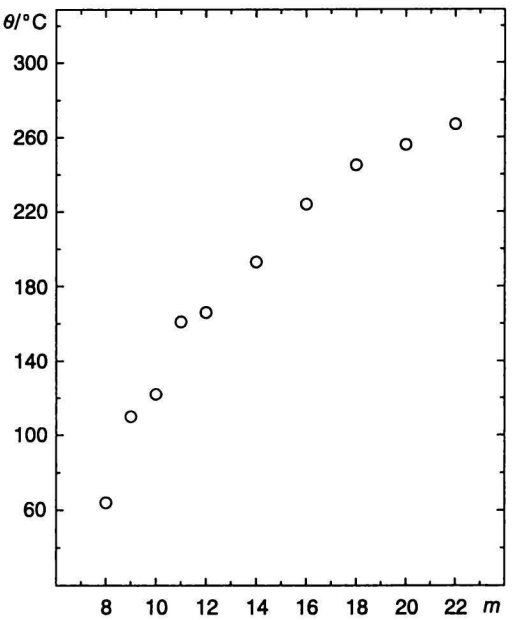


Fig. 1. Dependence m.p. = $f(m)$, m – number of carbon atoms in alkoxy group of the prepared compounds.

Table 3. ^{1}H NMR Chemical Shifts of the Prepared Compounds

Compound	δ					
	H-1	H-2	H-3	H-4, H-5, H-6	$N(CH_3)_2$	Alkyl
III	2.52 2.54	3.92	3.76	3.62—3.88	2.35	
IV	3.57 3.59	4.39	3.90	3.63—3.84	3.25	3.48; 1.85; 1.41; 1.33 (8H); 0.91
V	3.56 3.58	4.40	3.89	3.62—3.84	3.21	3.46; 1.83; 1.40; 1.31 (10H); 0.90
VI	3.59	4.41	3.89	3.62—3.87	3.22	3.47; 1.83; 1.40; 1.30 (12H); 0.90
VII	3.59	4.41	3.89	3.62—3.87	3.22	3.47; 1.83; 1.40; 1.30 (14H); 0.90
VIII	3.59	4.42	3.90	3.63—3.88	3.23	3.49; 1.83; 1.40; 1.30 (16H); 0.90
IX	3.59	4.41	3.89	3.62—3.87	3.22	3.48; 1.82; 1.39; 1.28 (20H); 0.88
X	3.59	4.42	3.90	3.63—3.88	3.23	3.50; 1.84; 1.40; 1.29 (24H); 0.90
XI	3.60	4.42	3.90	3.63—3.88	3.23	3.50; 1.84; 1.41; 1.30 (28H); 0.90
XII	3.60	4.42	3.91	3.63—3.87	3.23	3.50; 1.84; 1.41; 1.30 (32H); 0.90
XIII	3.60	4.42	3.91	3.62—3.88	3.22	3.51; 1.84; 1.41; 1.30 (36H); 0.90

Table 4. ^{13}C NMR Chemical Shifts of the Prepared Compounds

Compound	δ							
	C-1	C-2	C-3	C-4	C-5	C-6	$(CH_3)_2$	Alkyl
III	63.1	73.2	72.6	71.7	73.0	64.6	46.1	
IV	67.3	68.7	71.9	71.6	72.9	64.5	52.5 52.7	66.7; 32.7; 30.1; 30.1; 27.3; 23.6; 23.5; 14.4
V	67.0	68.2	71.5	71.2	72.2	63.8	52.4 52.5	66.6; 32.5; 30.0; 29.8; 29.7; 26.9; 23.3; 23.2; 14.5
VI	67.2	68.3	71.5	71.2	72.2	63.8	52.5	66.6; 32.7; 30.3 (2C); 30.1; 22.9; 27.0; 23.4; 14.6
VII	67.2	68.3	71.5	71.2	72.2	63.9	52.5	66.7; 32.7; 30.4; 30.4; 30.3; 30.2; 29.9; 27.0; 23.4; 23.3
VIII	67.3	68.3	71.5	71.3	72.2	63.9	52.5	66.7; 32.8; 30.6; 30.6; 30.5; 30.5; 30.3; 30.1; 27.2; 23.5; 23.5; 14.7
IX	67.4	68.3	71.5	71.3	72.2	63.9	52.5 52.5	66.7; 32.9; 30.8 (3C); 30.7 (2C); 30.7; 30.4; 30.2; 27.3; 23.6; 23.5; 14.8
X	67.4	68.3	71.5	71.3	72.2	63.9	52.5 52.5	66.7; 32.9; 30.9 (4C); 31.0; 30.9; 30.8; 30.7; 30.5; 30.2; 27.3; 23.6; 14.9
XI	67.5	68.3	71.5	71.3	72.2	63.9	52.5 52.5	66.7; 33.0; 31.0 (5C); 31.0; 30.9; 30.9; 30.8 (2C); 30.5; 30.3; 27.3; 23.7; 23.6; 14.9
XII	67.6	68.3	71.5	71.3	72.2	63.9	52.5 52.5	66.7; 33.0; 31.0 (6C); 30.9 (3C); 30.8 (3C); 30.5; 30.4; 27.4; 23.8; 23.6; 14.9
XIII	67.6	68.4	71.5	71.4	72.2	64.0	52.5 52.5	66.7; 33.1; 31.1 (7C); 31.0 (4C); 30.9 (3C); 30.6; 30.4; 27.4; 23.8; 23.7; 15.0

traction with benzene or ethyl acetate could not be applied in this case since the liberated *III* did not accumulate in the organic phase during the extraction. For these reasons the intermediate *II* was first isolated from the reaction mixture, subsequently purified by crystallization and then the liberation of compound *III* was achieved by treating with a methanolic solution of sodium methoxide. This newly proposed method was shown to be very simple and highly effective.

The final compounds *IV*—*XIII* are colourless solids and their melting points increase nonlinearly with the number of carbon atoms. As shown in Fig. 1, the dependence m.p. = $f(m)$ is different for m even and odd. QAS *III*—*XIII* are soluble in water, lower alcohols, and chloroform and insoluble in methylcyanide, hexane, and benzene. As expected, the aqueous solubility decreases with increasing m. Preliminary tests have shown that the prepared QAS display solubilization properties (induce *e.g.* solubilization of I_2 in water). When a concentrated aqueous solution of QAS is heated with benzene, a homogenization of the mix-

Table 5. Antimicrobial Activity (MIC) and log (1/MIC) Values of the Prepared Compounds and their Lipophilicity (R_M Values)

Compound	MIC/(mol dm^{-3}) log ((MIC)$^{-1}$/(mol dm^{-3})$^{-1}$)			R_M^a
	S. aureus	*E. coli*	*C. albicans*	
IV	6.213×10^{-3} 2.206	1.243×10^{-2} 1.905	1.243×10^{-2} 1.905	−0.524
VI	2.904×10^{-3} 2.537	5.800×10^{-3} 2.236	2.904×10^{-3} 2.537	−0.140
VIII	8.506×10^{-5} 4.070	2.720×10^{-3} 2.565	1.567×10^{-4} 3.781	0.176
IX	2.006×10^{-5} 4.698	6.414×10^{-4} 3.193	9.930×10^{-6} 5.003	0.575
X	9.483×10^{-6} 5.023	1.250×10^{-3} 2.903	1.896×10^{-5} 4.722	0.788
XI	8.993×10^{-6} 5.046	4.607×10^{-3} 2.336	3.593×10^{-5} 4.444	1.060
XII	3.916×10^{-5} 4.466	4.380×10^{-3} 2.358	6.842×10^{-5} 4.164	1.195
XIII	5.210×10^{-4} 3.283	8.350×10^{-3} 2.078	1.304×10^{-4} 3.885	1.380
Standard[b]	2.509×10^{-5}	2.509×10^{-4}	2.509×10^{-5}	

a) $R_M = \log(1/R_f - 1)$; *b*) *N,N*-dimethylbenzyldodecylammonium bromide.

ture, *i.e.* formation of microemulsion takes place which is stable even after cooling. The QAS display strong amphiphilic properties, *i.e.* they are capable to associate with water as well as with nonpolar solvents.

Purity of the target compounds *IV*—*XIII* was checked by partition TLC by using silica gel impregnated by 5 % solution of silicone oil in heptane. Identification of the compounds was based on elemental analysis, IR (Table 2), and ^{1}H and ^{13}C NMR spectra. ^{1}H NMR spectrum in the region $\delta = 3.6$—3.9 contains an unresolvable multiplet which was not further analyzed (Table 3). For higher homologues the signals of the carbon atoms from the middle parts of the alkyl chains are also poorly resolvable (Table 4).

The prepared QAS were found to display antimicrobial activity expressed as minimum inhibitory concentration (MIC) (Table 5). The antimicrobial activity was analyzed in relation to the structural parameter m (number of carbons in the alkyl chain) as well as the lipophilicity parameter R_M (correlation between R_M and m was verified to be linear: $R_M = 0.138\,m - 1.509$, $r = 0.989$). The relationship between log (1/MIC) and m or R_M was quantified by using the *Kubínyi*'s bilinear model [7] and the resultant coefficients and statistical parameters are given in Table 6 (see also Fig. 2).

As shown in Fig. 2, the maximum of the antimicrobial activity for the three bacterial species is reached for compounds with $m = 14$—16, *i.e.* at substantially higher number of carbons compared to other homological series of QAS where the maximum activity is usually observed at $m = 8$—12 [2—4]. This is most likely caused by a high hydrophilicity of our compounds *IV*—*XIII*. These compounds display the highest activity against *S. aureus*, whereas the activity

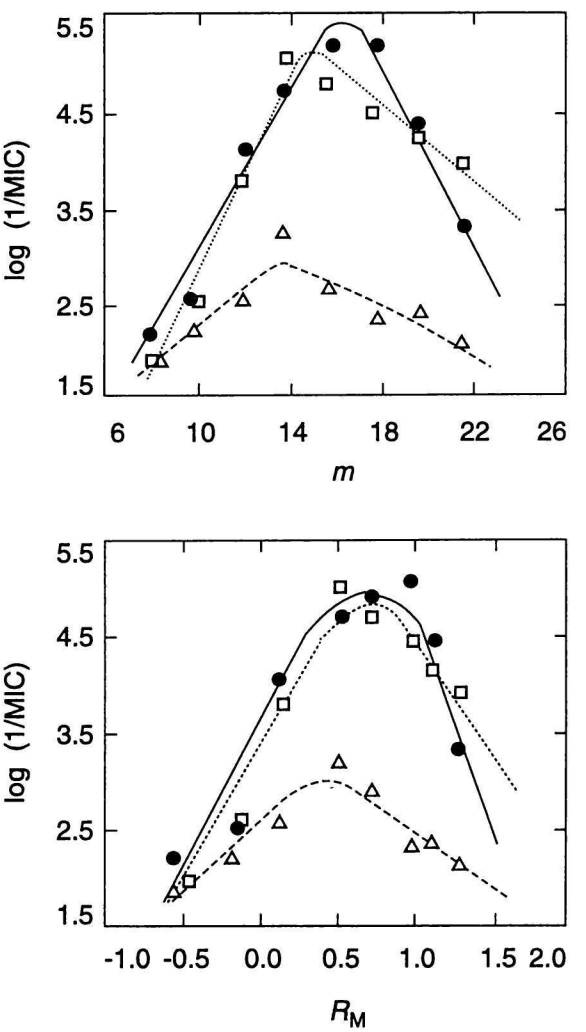


Fig. 2. *Kubínyi*'s bilinear relationship log (1/MIC) = $f(x)$, x = m; R_M. □ *C. albicans*; ● *S. aureus*; △ *E. coli*.

Table 6. Regression Coefficients for Bilinear Relationships between Antimicrobial Activity and Structure (m) and Lipophilicity for the Eight Prepared Compounds (*IV, VI, VIII—XIII*)

$$\log(1/\mathrm{MIC}) = A \cdot x + B \cdot \log(\beta \cdot 10^{x} + 1) + C;\ x = m \text{ or } R_{\mathrm{M}}$$

Bacteria	x	A	B	C	$\log\beta^{a}$	r^{b}	$s_{\mathrm{d}}{}^{c}$	F^{d}	Optimum m or R_{M}	Eqn
S. aureus	m	0.404	−0.829	−1.120	−16.59	0.978	0.301	44.05	17	(*1*)
E. coli	m	0.204	−0.331	0.222	−13.72	0.934	0.188	53.71	14	(*2*)
C. albicans	m	0.478	−0.673	−2.014	−15.00	0.983	0.254	59.10	15	(*3*)
S. aureus	R_{M}	3.598	−17.40	3.814	−1.380	0.958	0.371	37.83	0.80	(*4*)
E. coli	R_{M}	1.717	−5.279	2.798	−0.904	0.919	0.198	33.71	0.59	(*5*)
C. albicans	R_{M}	3.660	−11.03	3.705	−1.117	0.973	0.292	44.72	0.81	(*6*)

a) The nonlinear parameter in the bilinear equation; *b*) correlation coefficient; *c*) standard deviation from regression; *d*) values of the Fisher—Snedecar test.

against *E. coli* is the lowest. The difference in activity against *S. aureus* and *C. albicans* is small, which is a further distinction compared to other types of QAS [1—5]. The most active compounds of our series are comparable to the standard *N,N*-dimethyl-*N*-benzyldodecylammonium bromide (Table 5). Compounds *VIII* and *X* were also evaluated for acute toxicity and the LD_{50} values indicate that the toxicity of the compounds is reasonably low.

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