

Synthesis, Characterization and Antibacterial Activity of Quaternary Ammonium Compounds Bearing Mixed *N*-Substituted Groups

SOFIANE DAOUDI¹, ADIL A. OTHMAN^{1*}, TAHAR BENAÏSSA²
and ZAHRA OULD KADA¹

¹Laboratory of Bioactive Organic Chemistry, Department of Chemistry, Faculty of Sciences, University of Sciences and Technology of Oran -Mohamed Boudiaf, P.O.Box 1505 El-M'naouer, Oran 31003, Algeria

²Chemical Physics Laboratory, University of Moulay Tahar, Saida, 20000 Algeria
adelaliothman@gmail.com

Received 10 September 2012 / Accepted 23 April 2013

Abstract: Sixteen quaternary ammonium bromides [QAB]4(a-d)-7(a-d) bearing mixed *N*-branches were synthesized by treating *n*-butyl **2a**, cyclohexyl **2b**, 2,3,4,6-tetra-*O*-acetyl - *D*- glucosyl **2c** and cholesteryl **2d** bromides, separately with *n*-butyl amine **3a**, benzyl amine **3b**, *N,N*-dimethyl amino ethyl-2- methyl acrylate **3c** and *N,N*-diethyl amino ethyl-2- methyl acrylate **3d**. *N,N*-Diphenyl amine **3e** failed to react with any bromides within the scope of this work. The synthesized bromides and QAB were characterized by FT-IR, ¹H-NMR and ¹³C-NMR. All compounds were tested for their activity against some gram-positive and gram-negative bacteria and some of them exhibited significant antibacterial activities.

Keywords: Alkyl and cycloalkyl bromides, Benzyl bromides, Dialkylaminomethyl acrylate bromides, Quaternary ammonium compounds, Antibacterial activity

Introduction

Quaternary ammonium salts [QAS] found in very important life compounds such as amino acids, central nervous system of animals and cationic bile salts¹. Some synthesized bile salts were used as good cholesterol-lowering agents^{2,3} and have also been used as antimicrobials⁴, gall stone dissolution enhancers⁵ and DNA transfection agents^{6,7}. Several generations of synthetic quaternary ammonium compounds [QAC] which have been used as antimicrobial agents being recognized in the past seven decades. Variations in the four branches attached to quaternary ammonium nitrogen and multiplicity of N⁺ centers were the major features of the classes of QACs⁸. The *N*-alkyl-*N,N*-dimethyl ammonium methyl ethyl acrylates⁹⁻¹³ and *N*-alkyl-*N,N*-diethyl ammonium methyl ethyl acrylates¹⁰ are the raw material for dental antibacterial agents. Two of the new groups with good antimicrobial activity are the heterocyclic piperidinium¹⁴ and glucopyranose QAS¹⁵. Other increasing applications of QAC were reported in the literature, such as food, beverages preserving agents¹⁶, fabric¹⁷, cosmetic¹⁸, medical¹⁹, environmental²⁰ and electrolytes for liquids batteries²¹ uses. Because of the increasing resistance of microorganisms to

commonly used disinfectants make the synthesis of new types of microbiocides is very important topic. Very few examples of carbo- and heterocyclic branches such as cyclohexane, glycosyl, cholesterol moieties attached directly to the quaternary nitrogen were mentioned in the literature. In this work we report the synthesis of sixteen QAB belong to four classes of amines (*n*-butyl, benzyl, *N,N*-dimethyl ethyl-2- methyl acrylate amine, *N,N*-diethyl ethyl-2- methyl acrylate amine) and four classes of alkyl bromides bearing cyclohexyl, glycosyl and cholesteryl groups.

Experimental

All reactions were monitored by TLC, silica gel F₂₅₄, made by Merck, Germany. The melting points were measured with a BÜCHI 540 melting point apparatus and are uncorrected. The IR spectra were recorded using KBr discs in a GENESIS II FTIR spectrophotometer, in units of cm⁻¹. The ¹H and ¹³C NMR spectra were recorded on a Bruker AM 300 MHz spectrometer (University of Oran, Essenia) in CDCl₃ and referenced to TMS, chemical shifts in ppm. Microorganisms in this study were supplied by the university hospital of Oran and identified in our laboratory. The Mueller Hinton medium was supplied by (Difco).

General procedure for the synthesis of alkyl bromides **2a**, **2b** and **2d**

The alcohols **1a**, **1b** and **1d** (0.067 mol), KBr (0.33 mol), H₂O (20 mL), H₂SO₄ (10 mL) were added carefully and the mixture was refluxed at 80 °C for appropriate time until bromide spots on TLC exhibited no change in intensity [average time 7 h]. After cooling to room temperature, water (25 mL) was added and neutralized with Na₂CO₃. Extracted with chloroform for three times and dried over anhydrous MgSO₄, filtered and evaporated down to dryness to give the bromides **2a**, **2b** and **2d** in yields: 73%, 57% and 87% respectively.

Preparation of bromo-2, 3, 4, 6-*O*-acetyl-*D*-glucopyranoside **2c**²²

D-Glucose (**1c**) 4.0 g, 0.022 mol was dissolved in acetic anhydride (20 mL), acetyl bromide (5 mL) and HClO₄ (0.3 mL) added to it and the mixture was left aside for 48 h at room temperature. The reaction mixture was neutralized with aqueous Na₂CO₃ (25 mL) and extracted with chloroform, dried over anhydrous MgSO₄, filtered and evaporated to dryness to give a solid crystalline, recrystallized from diethyl ether to a colorless crystalline **2c** (7.5 g), 85%, m.p. 82 °C.

General procedure for the synthesis of quaternary ammonium bromides **4(a-d)** - **7(a-d)**

Alkyl bromides **2(a-d)** (0.0036 mol), alkyl amines **3(a-e)** in dichloromethane (20 mL) were refluxed for appropriate times and temperatures until TLC showed complete conversions to products. The volatiles were removed under vacuum to give solid products which recrystallized from diethyl ether to give colorless crystalline or as syrup **4(a-d)**-**7(a-d)**. Results are summarized in Table 1.

Table 1. Reactants, reflux time and melting points for synthesis of QAB **4(a-d)**-**7(a-d)**

QAS	Bromide	Amine	Reflux h	m.p. °C	QAS %	QAS	Bromide	Amine	Reflux h	m.p. °C	QAS %
4a	2a	3a	6	240	95	6a	2a	3c	36	118	62
4b	2b	3a	8		91	6b	2b	3c	56		46
4c	2c	3a	6		92	6c	2c	3c	8		57
4d	2d	3a	8	127	94	6d	2d	3c	96	137	56
5a	2a	3b	8	96	90	7a	2a	3d	8	117	94
5b	2b	3b	8	92	78	7b	2b	3d	8	108	94
5c	2c	3b	6		93	7c	2c	3d	8		89
5d	2d	3b	8	122	92	7d	2d	3d	8	148	89

Spectral data of QAB**Group-A***n*-Butyl aminobutyl bromide, [*n*-butyl-ABB] **4a**

ν (cm⁻¹): 3444.24 (N⁺-H)²⁴, 2955.96(C-H), 1041.37(C-N). δ (ppm):5.60(H-N⁺), 3.32(H₂C-N⁺), 1.98(-CH₂-),46.94(H₂C-N⁺)²⁵, 26.34(-CH₂-).

Cyclohexyl aminobutyl bromide, [cyclohexyl -ABB] **4b**

ν (cm⁻¹): 3445.21 (N⁺-H), 2963.09 (C-H), 1040.41 (C-N), δ (ppm): 5.53 (H-N⁺), 3.30(H₂C-N⁺), 1.94(-CH₂-), 1.92(-CH-cycl), 47.26(H₂C-N⁺), 37.92(-CH₂-), 36.21 (-CH-cycl).

2,3,4,6-Tetra-O-acetylglucosyl aminobutyl bromide, [glucosyl-ABB] **4c**

ν (cm⁻¹): : 3284.18 (N⁺-H), 2931.27 (C-H), 1736.58 (C=O), 1244.83 (C-N) 1031.73(C-O), δ (ppm): 5.37 (H-N⁺), 3.40(H₂C-N⁺), 2.08(-CH₂-), 6.40(-CH₂CO), 6.41(CHO-glu), 46.95(H₂C-N⁺), 29.54(-CH₂-), 93.21(CHO-glu).

Cholesteryl aminobutyl bromide, [cholesteryl-ABB] **4d**

ν (cm⁻¹): 3419.17 (N⁺-H), 2935.13 (C-H), 1633.41 (C=C), 1373.93 (C-N), δ (ppm):5.76(H-N⁺), 3.30 (H₂C-N⁺), 2.54(-CH₂-), 1.48 (-CH-cycl), 50.17(H₂C-N⁺), 29.50 (-CH₂-), 36.21 (-CH-cycl).

Group-B*n*-Butyl aminobenzyl bromides [*n*-butyl-ABzB] **5a**

ν (cm⁻¹): 3444.17 (N⁺-H), 3020.73 (=C-H), 2957.30(C-H), 1459.85 (C=C), 1054.87 (C-N), δ (ppm):5.77 (H-N⁺), 3.33 (-CH₂-CH₂-N⁺), 1.94(-CH₂-CH₂-N⁺), 1.70 (-CH₂-(CH₂)₂-N⁺), 2.57 (-N⁺-CH₂-Ph), 7.57 (H-Ar), 131.95, 128.67, 128.02, 127.19 (C-Ar), 55.74 (-N⁺-CH₂-Ph), 46.92 (N⁺-CH₂-), 6.35(-(CH₂)₂-CH₂-N⁺).

Cyclohexyl aminobenzyl bromides [Cyclohexyl-ABzB] **5b**

ν (cm⁻¹): 3361.32 (N⁺-H), 2930.31 (C-H), 1643.05 (C=C), 1070.30 (C-N).

2,3,4,6-Tetra-O-acetylglucosyl aminobenzyl bromides [glucosyl-ABzB] **5c**

ν (cm⁻¹): 3423.04 (N⁺-H), 2961.16 (C-H), 1649.80 (C=O), 1449.24(C=C), 1293.04 (C-N), 1031.73 (C-O), δ (ppm):6.46 (H-N⁺), 5.78 (N⁺-CH-O-), 6.81 (CH₂CO), 1.68 (-N⁺-CH₂-Ph), 8.26 (H-Ar), 125.56; 125.83; 126.88; 127.56 (C-Ar), 49.65 (-N⁺-CH₂-Ph), 88.01 (N⁺-CH-O-), 170.40 (-CH₂CO).

Cholesteryl aminobenzyl bromides [glucosyl-ABzB] **5d**

ν (cm⁻¹):3400.55 (N⁺-H), 2935.13 (C-H), 1644.02 (C=C), 1050. 80 (C-N), δ (ppm):5.37 (H-N⁺), 3.55 (N⁺-CH-cycl), 2.00 (-N⁺-CH₂-Ph), 8.41, 7.82 (H-Ar), 50.17 (-N⁺-CH₂-Ph), 140.79 (C=C aliphatic), 130.78, 128.30, 128.62, 128.50 (C-Ar).

Group-C*n*-Butyl amino dimethylaminoethyl-2-methyl acrylate bromides [*n*-butyl-ABzB] **6a**

ν (cm⁻¹): 2945.10 (C-H), 1718.50 (C=O), 1633.60 (C=C), 1045.30 (C-N), 1164.90(C-O), δ (ppm): 3.67 (H₃C-N⁺), 4.07 (-H₂C-N⁺), 4.07 (-CH₂CO), 6.06 (CH₂CO), 4.59 (H₂C-N⁺), 62.21 (H₂C-N⁺), 51.79 (-H₂C-N⁺), 62.23(N⁺-CH₂CH₂-O), 24.69 (-CH₂CO), 166.30 (-CH₂CO).

Cyclohexyldimethylaminoethyl-2-methyl acrylate bromides [cyclohexyl-DMAEMAB] 6b

ν (cm⁻¹): 2954.05 (C-H), 1719.23 (C=O), 1633.48 (C=C), 1154.39 (C-O), 1045.23 (C-N), δ (ppm): 3.33 (-N⁺-CH₃); 4.18 (N⁺-H₂C-), 4.61 (-CH₂-O-CO), 3.08 (H-C-cyclohexyl), 4.62 (H-C=C), 1.58 (CH₃-C=C), 61.26 (-N⁺-CH₃); 53.96 (-N⁺-CH₂); 63.00 (-CH₂-O-CO); 56.56 (H-C-cyclohexyl); 134.51 (CH₂-C=C).

2,3,4,6-Tetra-O-acetylglucosyl dimethylaminoethyl-2-methyl acrylate bromides [glucosyl-DMAEMAB] 6c

ν (cm⁻¹): 2964.05 (C-H), 1742.37 (C=O), 1645.05 (C=C), 1141.65 (C-N), 1035.55 (C-O), δ (ppm): 4.10 (-N⁺-CH₃); 4.30 (N⁺-H₂C-), 4.58 (-CH₂-O-CO), 4.31 (N⁺-CH-O-glucosyl), 4.72 (CH₃-CO).

Cholesteryl dimethylaminoethyl-2-methyl acrylate bromides [cholesteryl -DMAEMAB] 6d

ν (cm⁻¹): 2929.7 (CH), 1728.10 (C=O), 1602.70 (C=C), 1126.40 (C-N), 1066.90 (C-O), δ (ppm): 3.33 (-N⁺-CH₃); 5.34 (N⁺-H₂C-), 4.64 (-CH₂-O-CO), 3.41 (H-C-cyclohexyl), 5.67 (H-C=C), 76.95 (-N⁺-CH₃); 59.35 (N⁺-H₂C-), 50.48 (-CH₂-C=O), 135.58 (CH₃-C=C), 141.12 (-CH=C), 166.88 (CO-CH₂).

Group D*n-Butyl amino diethylaminoethyl-2-methyl acrylate bromides [n-butyl-DEAEMAB] 7a:*

ν (cm⁻¹): 2945.10 (C-H), 1718.50 (C=O), 1633.60 (C=C), 1164.90 (C-O), δ (ppm): 3.57 (H₂C-N⁺), 3.87 (-OCH₂-CH₂-N⁺), 4.57 (-OCH₂-CH₂-N⁺), 6.04 (H-C=C), 57.86 (H₂C-N⁺), 58.74 (N⁺-CH₂CH₂-O), 24.02 (N⁺-CH₂CH₂-O), 166.35 (-CH₂-CO), 135.1 (C=C-CH₂).

Cyclohexyldiethylaminoethyl-2-methyl acrylate bromides [cyclohexyl-DEAEMAB] 7b

ν (cm⁻¹): 2931.27 (C-H), 1720.19 (C=O), 1635.3 (C=C), 1165.72 (C-O), 1295 (C-N).

2,3,4,6-Tetra-O-acetylglucosyldiethylaminoethyl-2-methyl acrylate bromides [glucosyl-DEAEMAB] 7c

ν (cm⁻¹): 2971.77 (C-H), 1728.22 (C=O), 1636.30 (C=C), 1150.94 (C-N), 1037.62 (C-O), δ (ppm): 3.33 (CH₃-CH₂-N⁺), 3.94 (H₂C-N⁺), 4.53 (-OCH₂-CH₂-N⁺), 4.74 (N⁺-CH₂CH₂-O), 4.20, 4.06 (H-C-O-Ac).

Cholesteryl diethylaminoethyl-2-methyl acrylate bromides [cholesteryl -DEAEMAB] 7d

ν (cm⁻¹): 2936.13 (C-H), 1727.91 (C=O), 1647.33 (C=C), 1135.07 (C-N), 1053.91 (C-O).

Antibacterial susceptibility testing

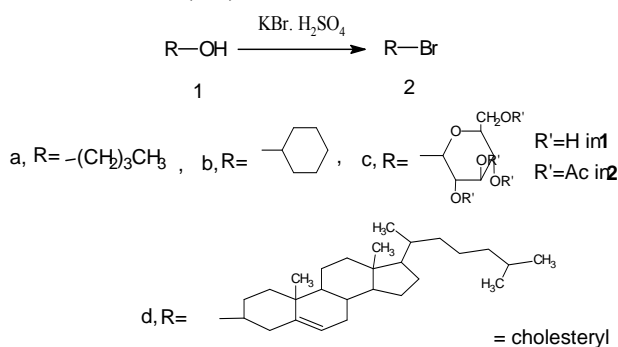
A disk diffusion assay according to the standard protocols (NCCLS, 2003, 2005; CLSI, 2006) was used²⁶⁻²⁸ in duplicates to determine the susceptibility of three gram-negative bacteria *Shigella*, *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* ATCC 10145 and one gram-positive bacteria *Staphylococcus aureus* ATCC 25923, using gentamycin and amoxicillin as references. The bacterial suspension (in 0.9% NaCl) turbidity were adjusted to 0.5 Mc Farland, then the suspensions were spread with a sterile cotton swab confluent over the entire surface of Mueller Hinton agar (Merck, Germany). This agar medium was inoculated with 0.5 mL of cultures containing about 10⁶ CFU/mL. Filter paper disks (5 mm diameter) saturated with dimethylsulphoxide (10% DMSO v:v) solutions of each compound was placed on the indicated agar medium. The incubation time was 24 h at 37 °C. The blank test disk with DMSO was used. Inhibitory activity was evaluated by measuring the diameter of clear zone observed around the disk in mm (c.f. Table 5).

The minimum inhibition concentration (MIC) Tests Microorganisms not affected by compounds tested, no further dilution tests were conducted. Each 1 mL of the original concentration ($10 \mu\text{g mL}^{-1}$) in DMSO of the compounds **4(a-d)**-**7(a-d)** were diluted with DMSO in test tubes to $5.0 \mu\text{g mL}^{-1}$ and optical density at 600 nm was measured at 24 h.

Results and Discussion

Synthesis of bromides

n-Butyl bromide **2a**, cyclohexyl bromide **2b**, glycosyl bromide **2c** and cholesteryl bromide **2d**²³ were prepared by refluxing the corresponding alcohols **1a**, **1b** and **1d** with KBr in H_2SO_4 , while 2,3,4,6-tetra-*O*-acetyl bromide **2c** was obtained according to literature method²² by treating *D*-glucose with acetyl bromide in acetic anhydride and catalytic amount of HClO_4 . The resulted bromides **2(a-d)** are exhibited in Scheme 1.



Scheme 1, Synthesis of bromides **2(a-d)**

All bromides **2(a-d)** were isolated and characterized by measuring melting points of solid substances and by spectral analysis of IR as shown in Table 2.

Table 2, Melting points and IR of bromides **2(a-d)**

Comp	Mp °C	IR ν_{max} , cm^{-1}	
		OAC	C-Br
2a			611
2b			557
2c	82	1737.7	605
2d	151		621

Synthesis of quaternary ammonium bromides [QAB] **4(a-d)**-**7(a-d)**

The four bromides **2(a-d)** were treated with five readily available amines **3(a-e)** namely: *n*-butyl amine (**3a**), benzyl amine (**3b**), *N,N*-dimethyl ethyl-2-methyl acrylate amine (**3c**), *N,N*-diethyl ethyl-2-methyl acrylate amine (**3d**) and *N,N*-diphenyl amine (**3e**) revealed only sixteen QAB **4(a-d)**-**7(a-d)**.

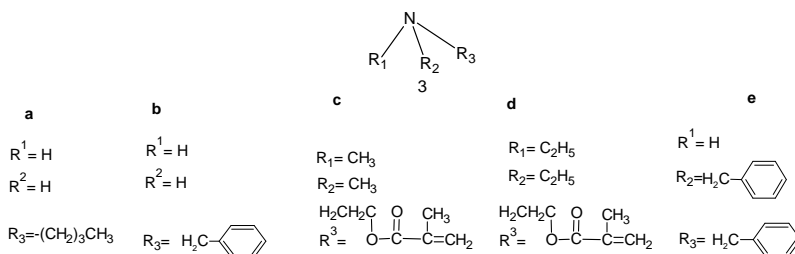
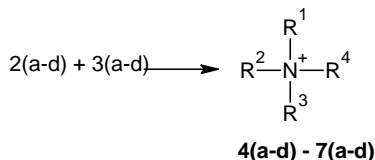


Figure 1. The amines used for the synthesis of QAB

N,N-Diphenyl amine **3e** was failed to react with any of the already synthesized bromides **2(a-d)** due to its low nucleophilicity caused by resonance phenomenon between the lone pair of electrons in nitrogen-amine and the two adjacent phenyl groups.



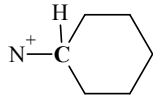
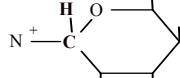
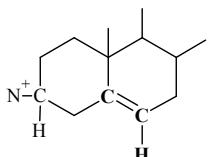
Scheme 2. Synthesis of QAB from bromides **2(a-d)** and amines **3(a-d)**

The sixteen QAB will be categorized into Four Groups named after their amines. **Group-A** are made of four alkyl aminobutyl bromides [alkyl-ABB]**4(a-d)**, they are: [*n*-butyl-ABB]**4a**, [cyclohexyl-ABB]**4b**, [glucosidyl-ABB]**4c** and [cholesteryl-ABB]**4d**. **Group-B** are comprise of four alkyl aminobenzyl bromides [alkyl-ABzB]**5(a-d)**, they are: [*n*-butyl-ABzB]**(5a)**, [cyclohexyl-ABzB]**(5b)**, [glucosidyl-ABzB]**(5c)** and [cholesteryl-ABzB]**5d**. **Group-C** are made of four alkyl dimethylaminoethyl-2-methyl acrylate bromides [alkyl-DMAEMAB]**6(a-d)**, they are: [*n*-butyl-DMAEMAB]**6a**, [cyclohexyl-DMAEMAB]**6b**, [glucosidyl-DMAEMAB]**6c** and [cholesteryl-DMAEMAB]**(6d)**. **Group-D** are composed of four alkyl diethyl-aminoethyl-2-methyl acrylate bromides [alkyl-DEAEMAB]**7(a-d)**, they are: [*n*-butyl-DEAEMAB]**7a**, [cyclohexyl-DEAEMAB]**7b**, [glucosidyl-DEAEMAB]**7c** and [cholesteryl-DEAEMAB]**7d**. Table 3 shows the compositions, melting points of solid products, IR, ¹H- and ¹³C-NMR spectroscopic data for some common characteristic groups.

Characterization of QAB by ¹H- and ¹³C-NMR

There are some selected structural fragments which are common in all QAB groups can be used to detect the formation of the particular QAB such as of **Group A** as shown in Table 3.

Table 3. Literature data ¹H-, ¹³C-NMR²⁴ in ppm of selected fragments which are common in QAB **4(a-d)**-**7(a-d)**.

Structural fragment	¹ H-NMR ¹³ C-NMR
⁺ N-H	6-9
⁺ N-CH ₂ CH ₂ -	3.27, 1.27 60.40, 16.0
	3.3 54.4
	5.9 95
	3.3 54.4

Note: Data are for the bold letters

Characterization of Group-A=[alkyl-ABB] 4(a-d)

The formations of *n*-butyl ABB were detected by thin layer chromatography (TLC) via their R_f which were different from starting materials. The IR spectra of 4(a-d) showed bands between 3445-3284 cm^{-1} for H-N⁺ bonds, while 4c²⁴ exhibited an additional band at 1736 cm^{-1} for acetate groups and 4d showed an absorption at 1633 cm^{-1} indicating the presence of C=C for cholesterol. ¹H-NMR spectra showed the following signals between 5.76 to 5.37 ppm for N⁺-H, while N⁺-CH₂- signals exhibited between 3.30- 3.40 ppm. ¹³C-NMR spectra for compounds 4(a-d), the groups N⁺-CH₂- exhibited signals 46.94, 47.26, 46.95 and 50.17 ppm while the another -CH₂-pieces in N⁺-CH₂CH₂- fragments were shown in 26.34, 37.92, 29.54 and 29.50 ppm in 4(a-d) respectively and they are very close to literature values (Tables 3 and 4)²⁵.

Characterization of Group-B = [alkyl-ABzB] 5(a-d)

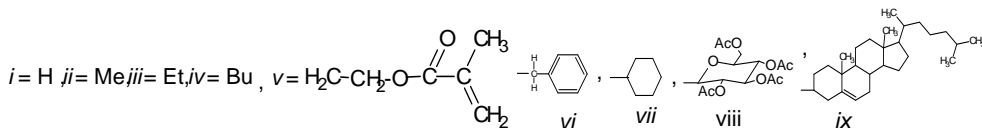
The [alkyl-ABzB] 5(a-d) have almost similar groups N⁺-H, N⁺-CH₂CH₂- as in Group A, in addition they have the benzyl group -CH₂Ph. IR spectra of the members of this group showed similar N⁺-H stretching between 3444-3400 cm^{-1} and additional aromatic C=C stretching at the region 1640 cm^{-1} . ¹H-NMR spectra for N⁺-H showed at 6.46-5.77 ppm while N⁺-CH₂-Ph signals exhibited between 2.57-1.86 ppm and phenyl protons entered at 8.26, 8.16 and 7.57 ppm. ¹³C-NMR spectra for the same fragments are shown in 55.74 (N⁺-CH₂-Ph) and 131.95-127.19 ppm for aromatic ring carbon. Rest spectral data of IR, ¹H- and ¹³C-NMR are summarized in Table 4.

Table 4, IR, ¹H and ¹³C-NMR Data of some common groups of QAS 4(a-d)-7(a-d)

Compositions and m.ps of synthesized QAS 4(a-d)-7(a-d)						IR ν_{max} , cm^{-1}	¹ H-NMR δ , (ppm) ¹³ C-NMR δ , (ppm)
R ¹	R ²	R ₃	R ⁴	m.p. ^o C			
4a	i	i	iv	iv	240	3444 (H-N ⁺)	5.60(H-N ⁺), 3.32(H ₂ C-N ⁺), 1.98(-CH ₂ -) 46.94(H ₂ C-N ⁺), 26.34(-CH ₂ -).
4b	i	i	iv	vii		3445 (H-N ⁺)	5.53(H-N ⁺), 3.30(H ₂ C-N ⁺), 1.94(-CH ₂ -), 1.92(-CH-cycl) 47.26(H ₂ C-N ⁺), 37.92(-CH ₂ -), 36.21 (- CH-cycl).
4c	i	i	iv	viii		3284 (H-N ⁺) 1736(C=O)	5.37 (H-N ⁺), 3.40(H ₂ C-N ⁺), 2.08(-CH ₂ -), 6.40(-CH ₂ CO), 6.41(CHO-glu) 46.95(H ₂ C-N ⁺), 29.54(-CH ₂ -), 93.21(CHO-glu)
4d	i	i	iv	ix	127	3419 (H-N ⁺) 1633(C=C)	5.76(H-N ⁺), 3.30 (H ₂ C-N ⁺), 2.54(-CH ₂ -), 1.48 (-CH-cycl). 50.17(H ₂ C-N ⁺), 29.50 (-CH ₂ -), 36.21 (- CH-cycl).
5a	i	i	vi	iv	96	3444 (H-N ⁺) 1640 (C=C Ar.)	5.77 (H-N ⁺), 3.33 (-CH ₂ -CH ₂ -N ⁺), 1.94(- CH ₂ -CH ₂ -N ⁺), 1.70 (-CH ₂ -(CH ₂) ₂ -N ⁺), 2.57 (-N ⁺ -CH ₂ -Ph), 7.57 (CH-Ar) 131.95, 128.67, 128.02, 127.19 (CH-Ar), 55.74 (-N ⁺ -CH ₂ -Ph), 46.92 (N ⁺ -CH ₂ -), 6.35(-CH ₂) ₂ -CH ₂ -N ⁺)

Contd...

5b	i	i	vi	vii	92	3361 (H-N⁺), 1643 (C=C Ar.)	
5c	i	i	vi	vii i		3423 (H-N⁺), 1725(C=O),	6.46 (H-N⁺), 5.78 (N⁺-CH-O-), 6.81 (CH₂CO), 1.68 (-N⁺-CH₂-Ph), 8.26 (CH- Ar). 125.56; 125.83; 126.88; 127.56 (CH-Ar), 49.65 68 (-N⁺-CH₂-Ph), 88.01 (N⁺-CH-O-), 170.40 (-CH₂CO).
5d	i	i	vi	ix	122	3400 (H-N⁺), 1644(C=C Ar.)	5.37 (H-N⁺), 3.55 (N⁺-CH-cycl), 2.00 (- N⁺-CH₂-Ph), 8.41, 7.82 (CH-Ar). 50.17 (-N⁺-CH₂-Ph), 140.79 (C=C aliphatic), 130.78, 128.30, 128.62, 128.50 (CH-Ar).
6a	ii	ii	v	iv	118	1718 (C=O), 1633(C=C)	4.07 (H₂C-N⁺), 3.67 (-H₂C-N⁺), 4.07 (- CH₂CO), 6.06 (CH₂CO), 4.59 (H₂C-N⁺). 62.21 (H₂C-N⁺), 51.79 (-H₂C-N⁺), 62.23(N⁺-CH₂CH₂-O), 24.69 (-CH₂CO), 166.30 (-CH₂CO).
6b	ii	ii	v	vii		1729(C=O), 1633 (C=C)	3.33 (-N⁺-CH₃); 4.18 (N⁺-H₂C-), 4.61 (- CH₂-O-CO), 3.08 (H-C-cyclohexyl), 4.62 (H-C=C), 1.58 (CH₃-C=C). 61.26 (-N⁺-CH₃); 53.96 (-N⁺-CH₂); 63.00 (-CH₂-O-CO); 56.56 (H-C-cyclohexyl); 134.51(CH₂-C=CH₂).
6c	ii	ii	v	viii		1724 (C=O), 1645(C=C)	4.10 (-N⁺-CH₃); 4.30 (N⁺-H₂C-), 4.58 (- CH₂-O-CO), 4.31(N⁺-CH-O-gluco), 4.72 (CH₃-CO).
6d	ii	ii	v	ix	137	1728(C=O), 1633 (C=CH₂)	3.33 (-N⁺-CH₃); 5.34 (N⁺-H₂C-), 4.64 (- CH₂-O-CO), 3.41(H-C-cyclohexyl), 5.67 (H-C=C), 76.95 (-N⁺-CH₃); 59.35 (N⁺- H₂C-), 50.48 (-CH₂C=O), 135.58 (CH₃- C=C), 141.12 (-CH=C), 166.88 (CO- CH₂).
7a	iii	iii	v	iv	117	1728 (C=O), 1636 (C=CH₂)	3.57 (H₂C-N⁺), 3.87 (-CH₂-CH₂-N⁺), 4.57 (-CH₂-CH₂-N⁺), 6.04 (H-C=C). 57.86 (H₂C-N⁺), 58.74 (N⁺-CH₂CH₂-O), 24.02 (N⁺-CH₂CH₂-O), 166.35 (-CH₂CO), 135.1 (C=C-CH₂).
7b	iii	iii	v	vii	108	1720(C=O), 1635 (C=CH₂)	
7c	iii	iii	v	viii		1728(C=O), 1636 C=CH₂)	3.33 (H₂C-N⁺), 3.94 (-CH₂-CH₂-N⁺), 4.53 (-CH₂-CH₂-N⁺), 4.74 (N⁺-CH₂CH₂-O), 4.20, 4.06 (H-C-O-Ac).
7d	iii	iii	v	ix	148	1727(C=O), 1647(C=CH₂)	



Characterization of Groups-C & D = [alkyl-DMAEMAB] 6(a-d) & [alkyl-DEAEMAB] 7(a-d)

Groups C and D have almost similar structural formulas represented by *N,N*-dialkyl amino ethyl-2- methyl acrylate fragments, for Group-C and for R=H, Group-D, R= -CH₃:

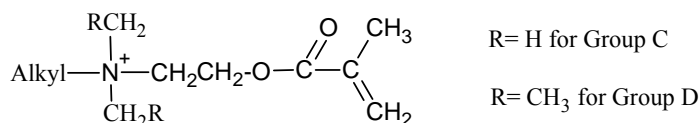


Figure 2. The basic structural formulas for **Groups C and D**

Thus all compounds **6(a-d)**-**7(a-d)** showed IR absorption between 1728-1718 cm⁻¹(CO), 1647-1633 cm⁻¹ (C=C). Also compounds exhibited signals between 4.10-3.33 ppm corresponding to N⁺-CH₂-R, 4.74- 4.07 due to -N⁺-CH₂-CH₂-O-, 124-127 ppm and 166-167 ppm related to C=CH₂ and CO respectively²⁵.

Antibacterial activity

The sixteenth QAB **4(a-d)**-**7(a-d)** were screened for their activity against Gram-positive *Staphylococcus aureus* and Gram-negative bacteria, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Shigella*. The antibacterial activities of the tested compounds were evaluated using the paper disk diffusion method. DMSO which is known as bacterial static in the above mentioned concentration was used as negative control and standard disks (Mast Diagnostics, UK), saturated with known antibiotic Gentamycin and Amoxicillin as positive control were applied. After incubation at 37 °C for 24 h, the zone of inhibition of growth around each disk was measured in millimeters and zone diameters were interpreted in accordance with CLSI and NCCLS (for *Campylobacter* spp.) guidelines (CLSI, 2006; NCCLS, 2003, 2005)²⁶⁻²⁸. The experiments were performed in duplicates and the average results are summarized in Table 5.

Table 5. Antibacterial activity of the synthesized compounds

Compounds	Inhibition Zone, mm			
	<i>Shigella</i>	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
4a	10.00	18.00	00.00	25.00
4b	08.00	18.00	00.00	25.00
4c	15.00	00.00	00.00	36.00
4d	00.00	00.00	00.00	10.00
5a	15.00	15.00	20.00	18.00
5b	00.00	15.00	00.00	30.00
5c	00.00	10.00	00.00	20.00
5d	00.00	00.00	00.00	20.00
6a	10.00	10.00	00.00	25.00
6b	15.00	17.00	30.00	15.00
6c	13.00	13.00	20.00	15.00

Contd...

6d	00.00	00.00	00.00	16.00
7a	15.00	13.00	00.00	20.00
7b	00.00	12.00	30.00	08.00
7c	20.00	10.00	00.00	15.00
7d	00.00	00.00	00.00	10.00
DMSO	00.00	00.00	00.00	00.00
Gentamicine	30.00	30.00	45.00	25.00
Amoxicilline	25.00	25.00	40.00	45.00

All QAB **4(a-d)**-**7(a-d)** have affected gram-positive *Staphylococcus aureus* in active range compatible to known antibiotic Gentamicin and moderately active comparable to Amoxicillin. Compound glucosidyl-ABB (**4c**) showed the highest activity against *S. aureus* followed by compound cyclohexyl -ABzB (**5b**).

All QAB **4(a-d)**-**7(a-d)** showed less activity against gram-negative bacteria under study. *Shigella* exhibited about 44% resistance to all synthesized QAB while *Pseudomonas fluorescens* showed about 31% resistance. *Pseudomonas aeruginosa* was resistant to about 76% of the synthesized QAB except four compounds **5a**, **6b**, **6c** and **7b** which showed moderate to weak activity.

Microorganisms not affected by compounds tested, no minimum inhibition concentration (MIC) test were conducted. Those which showed activity, MIC was done by dilution with DMSO to 5.0 $\mu\text{g mL}^{-1}$ but they show almost no significant activity.

Conclusion

The work in this study is beneficial and valuable to those who are interested in studying antibacterial activity for new QAC and particularly with resin composites such as the acrylate QABs.

Acknowledgment

Thanks are due to Mrs.F.Z. Benmiloudi, M.Sc, Miss. F. Taieb Brahimi, M.Sc. and Miss. I. Taghaneth, M.Sc., of the Laboratory of Bioactive Organic Chemistry, Department of Chemistry, University of Sciences and Technology of Oran for performing the biological test.

References

1. Bhat S, Leikin-Gobbi D, Konikoff F M and Maitra U, *Biochimica et Biophysica Acta (BBA) - General Subjects*, 2006, **1760(10)**, 1489-1496.
2. Zhang Z H, Janout V, Renner J L, Urgami M and Regan S L, *Bioconjugate Chem.*, 2000, **11(3)**, 397-400; DOI:10.1021/bc990151v.
3. Fears R, Brown R, Ferres H, Grenier F and Tyrrell A W R, *Biochem Pharmacol.*, 1990, **40(9)**, 2029-2037; DOI:10.1016/0006-2952(90)90233-B.
4. Fini A, Roda A, Billini A M, Mencini E and Quarneri M, *J Pharm Sci.*, 1990, **79(7)**, 603-605; DOI:10.1002/jps.2600790712.
5. Kwan K H, Higuchi W I, Molokhia A M and Hofmann A F, *J Pharm Sci.*, 1977, **66(8)**, 1105-1108; DOI:10.1002/jps.2600660813.
6. Walker S, Sofia M J and Axelrod H R, *Adv Drug Delivery Rev.*, 1988, **30(1-3)**, 61-71; DOI:10.1016/S0169-409X(97)00107-5.
7. Bandyopadhyaya P, Lanout V, Zhang L H, Sawko J A and Regan S L, *J Am Chem Soc.*, 2000, **122(51)**, 12888-12889; DOI:10.1021/ja0056466.
8. Brycki B, Kowalczyk I and Kozirog A, *Molecules*, 2011, **16(1)**, 319-335; DOI:10.3390/molecules16010319.

9. Ohta Y, Kondo Y, Kawada K, Ternaka T and Yoshino N, *J Oleo Sci.*, 2008, **8**, 445-452.
10. Xiao Y H, Chen J H, Fang M, Xing X D, Wang H, Wang Y J and Li F, *J Oral Sci.*, 2008, **50**(3), 323-327.
11. Makhlofua M, Benaissa T, Derdour A and Henni D E, *J Chem.*, 2010, **7**(S1), 561-566.
12. He J, Soderling E, Osterblad M, Vallittu P K and Lassila L V J, *Molecules*, 2011, **16**(11), 9755-9763; DOI:10.3390/molecules16119755.
13. Weng Y, Guo X, Chong V J, Howard L, Gregory R L and Xie D, *J Biomed Sci Eng.*, 2011, **4**, 147-157.
14. Dega-Szafron Z, Dulewicz E and Brycki B, *Arkivoc*, 2007, (vi), 90-102.
15. de Britto D, Goy R C, Filho S P C and Assis O B G, *Int J Carbohy Chem.*, 2011, Article ID 312539, 12 pages; DOI:10.1155/2011/312539
16. Richter F L and Cords B R, Disinfection, Sterilization and Preservation, Block S S, Editor, 5th Ed., Lippincott Williams & Wilkins, Philadelphia, 9106, USA. 2001.
17. Zhu P and Sun G, *J Appl Polym Sci.*, 2004, **93**(3), 1037-1041; DOI:10.1002/app.20563.
18. Lang G, Wendel H and Birkel S, in “*Chitin Handbook*”, Muzzarelli R A A and Peter M G, Eds., European Society Press, Grottanmmare, Italy, 1997, 384-389.
19. Ringdahl B, Roch M and Jenden D J, *J Med Chem.*, 1988, **31**(1), 160-164; DOI:10.1021/jm00396a024.
20. Mac Farlane D R, Forsyth S A, Golding J and Deacon G B, *Green Chem.*, 2002, **4**, 444-448; DOI:10.1039/B205641K.
21. Tigelaar D M, Meador M A B and Bennett W R, *Macromolecules*, 2007, **40**(12), 4159-4164; DOI:10.1021/ma062804q.
22. Furniss B S, Hannford A J, Smith P W G and Tatchell A R, Vogel’s Text Book of Practical Organic Chemistry, 5/e, Longman Scientific & Technical, New York, 1989, 647.
23. Gao M, Wang M, Miller K D, Sledge G W, Hutchins G D and Zheng Q H, *Steroids*, 2010, **75**, 715-720.
24. Pretsch E, Buhlmann P and Affolter C, Structure Determination of Organic Compounds – tables of spectral data, Springer-Verlage Berlin Heidelberg, 2000, 254.
25. Pretsch E, Buhlmann P and Affolter C, Structure Determination of Organic Compounds–tables of spectral data, Springer-Verlage Berlin Heidelberg, 2009, p 161, 71.
26. Dallal M M S, Doyle M P, Rezadehbashi M, Dabiri H, Sanaei M, Modarresi S, Bakhtiari R, Sharify K, Taremi M, Zali M R and Sharifi-Yazdi M K, *Food Control*, 2010, **21**(4), 388-392; DOI:10.1016/j.foodcont.2009.06.001.
27. Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth International Supplement. 2006, **26**, 11-35.
28. National Committee for Clinical and Laboratory Standards Villanova, PA, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 4/e, 1997.