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Research Article

Synthesis and Antimicrobial Activity of Carboxylate Phosphabetaines Derivatives with Alkyl Chains of Various Lengths

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The purpose of the present study was to investigate the antibacterial activity of fifteen novel nanosized alkyl esters of carboxylate phosphabetaine: β-(carboxyalkyl)ethyltriphenylphosphonium bromides **4–8**, β-(carboxyalkyl)-β-methylethyltriphenylphosphonium bromides **9–13**, and β-(carboxyalkyl)-α-methylethyltriphenylphosphonium bromides **14–18**. The in vitro microbiological activity of the synthesized phosphonium bromides against gram-positive and gram-negative bacteria and the yeast *Candida albicans* was determined in comparison to standard agents. Microbiological results indicate that the synthesized phosphonium salts **4–18** possess a broad spectrum of activity against the tested microorganisms. Every newly synthesized compound was characterized by elemental analyses, IR, ^{1}H NMR, and ^{31}P NMR spectral studies.

1. Introduction

Demand for new antimicrobial agents is high because more microorganisms develop resistance against drugs currently available on the market. Resistance of pathogenic bacteria to antibiotics is rapidly becoming a major problem in the medical community and hospital-based healthcare settings. The search for novel agents to combat resistant bacteria has become one of the most important areas of antibacterial research today [1, 2]. Pharmaceutical and organic chemists are trying to synthesize new drugs with better pharmacokinetic and dynamic properties.

In this study, we prepared triphenyl-substituted phosphonium salts **4–18** on the base of phosphabetaines (**1–3**) containing alkyl chains of various lengths. The synthesis of such phosphonium salts is very difficult in comparison with ammonium analogs [3, 4]. In the past years, our group carried out regular research on the synthesis, structure, and reactivity of phosphabetaines of type **1–3**, obtained on the basis of tertiary phosphines and unsaturated carboxylic acids [5–8].

The surging interest in this class of compounds becomes quite understandable if we take into account the fact that phosphabetaines are also the original analogs of organic amino acids, with a wide spectrum of potential chemical and biological properties. In these internal phosphonium salts, cationic phosphonium and anionic centers are interconnected not only by ionic but also by covalent bonds. The structure of all products **1–3** has been confirmed by the direct X-ray method [6–8].

Betaines **1–3** easily react with alkyl halogenides with short alkyl chains to form the corresponding phosphonium salts without biological activity [5–8].

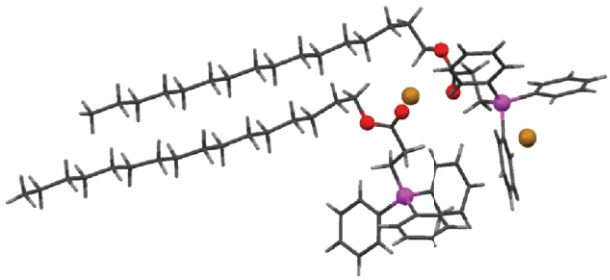


Figure 1: Molecular structure of the asymmetric unit of the $Ph_3PCH_2CH_2C(O)OC_{16}H_{33}$ (**7**) in crystal.

2. Experimental

2.1. Chemistry. All materials were obtained from commercial suppliers and used without purification. Analytical data were obtained from Perkin Elmer 2400 LS and were found within ±0.4% of the theoretical values. Infrared (IR) spectra were recorded on using KBr disk on Specord M-80. ^{1}H NMR (D_2O) and ^{31}P NMR (DMSO-d_6) spectra were determined on a Bruker Avance digital spectrometer 400 MHz.

2.2. Synthesis of β-(Carboxyalkyl)ethyltriphenylphosphonium Bromides (**4–8**)

2.2.1. General Procedure. A mixture of equimolar quantities of *β*-triphenylphosphonium ethylcarboxylate **1** (0.01 mol) and appropriate alkyl halogenides (0.01 mol) was refluxed in dry chloroform (100 mL) for 2 h. Excess of solvent was removed under reduced pressure. The resulting salts **4–8** were obtained as yellow oils and purified by diethyl ether from starting reagents.

4. Yield (64%), oil. IR (KBr): 1718 (C=O), 1135 (P–C) cm^{-1}; ^{1}H NMR (D_2O) δ: 0.75 (t, 3H, CH_3, J = 7.1 Hz), 1.21–1.37 (m, 14H, $7CH_2$), 2.41–2.56 (m, 2H, $OCCH_2$), 3.10–3.25 (m, 2H, $PCCH_2$), 4.11–4.21 (m, 2H, PCH_2), 4.23–4.31 (m, 2H, OCH_2), 7.31–7.54 (m, 15H, phenyl H); ^{31}P NMR (DMSO-d_6) δ: 25.58. $C_{31}H_{40}BrO_2P$ (554.90): calcd. C 67.02%, H 7.20%; found C 66.93%, H 7.51%.

5. Yield (71%), oil. IR (KBr): 1721 (C=O), 1129 (P–C) cm^{-1}; ^{1}H NMR (D_2O) δ: 0.80 (t, 3H, CH_3, J = 7.0 Hz), 1.25–1.39 (m, 18H, $9CH_2$), 2.47–2.63 (m, 2H, $OCCH_2$), 3.11–3.27 (m, 2H, $PCCH_2$), 4.07–4.19 (m, 2H, PCH_2), 4.28–4.39 (m, 2H, OCH_2), 7.33–7.59 (m, 15H, phenyl H); ^{31}P NMR (DMSO-d_6) δ: 25.62. $C_{33}H_{44}BrO_2P$ (582.90): calcd. C 67.92%, H 7.54%; found C 68.03%, H 7.87%.

6. Yield (59%), oil. IR (KBr): 1720 (C=O), 1130 (P–C) cm^{-1}; ^{1}H NMR (D_2O) δ: 0.81 (t, 3H, CH_3, J = 6.8 Hz), 1.20–1.35 (m, 22H, $11CH_2$), 2.43–2.58 (m, 2H, $OCCH_2$), 3.13–3.29 (m, 2H, $PCCH_2$), 4.17–4.27 (m, 2H, PCH_2), 4.30–4.37 (m, 2H, OCH_2), 7.30–7.58 (m, 15H, phenyl H); ^{31}P NMR (DMSO-d_6) δ: 25.55. $C_{35}H_{48}BrO_2P$ (610.90): calcd. C 68.74%, H 7.85%; found C 69.01%, H, 8.13%.

7. Yield (65%), m.p; 208°C. IR (KBr): 1719 (C=O), 1140 (P–C) cm^{-1}; ^{1}H NMR (D_2O) δ: 0.70 (t, 3H, CH_3, J = 6.9 Hz), 1.19–1.40 (m, 26H, $13CH_2$), 2.47–2.63 (m, 2H, $OCCH_2$), 3.17–3.27 (m, 2H, $PCCH_2$), 4.13–4.29 (m, 2H, PCH_2), 4.22–4.39 (m, 2H, OCH_2), 7.43–7.67 (m, 15H, phenyl H); ^{31}P NMR (DMSO-d_6) δ: 25.70. $C_{37}H_{52}BrO_2P$ (638.90): calcd. C 69.48%, H 8.13%; found C 69.47%, H 8.47%.

8. Yield (60%), oil. IR (KBr): 1720 (C=O), 1138 (P–C) cm^{-1}; ^{1}H NMR (D_2O) δ: 0.72 (t, 3H, CH_3, J = 6.9 Hz), 1.17–1.27 (m, 30H, $15CH_2$), 2.47–2.59 (m, 2H, $OCCH_2$), 3.17–3.36 (m, 2H, $PCCH_2$), 4.09–4.19 (m, 2H, PCH_2), 4.21–4.37 (m, 2H, OCH_2), 7.38–7.66 (m, 15H, phenyl H); ^{31}P NMR (DMSO-d_6) δ: 25.87. $C_{39}H_{56}BrO_2P$ (666.90): calcd. C 70.16%, H 8.39%; found C 69.89%, H 8.02%.

2.3. Synthesis of β-(Carboxyalkyl)-β-methylethyltriphenylphosphonium Bromides (**9–13**)

2.3.1. General Procedure. A mixture of equimolar quantities of *β*-triphenylphosphonium *β*-methylethylcarboxylate **2** (0.01 mol) and appropriate alkyl halogenides (0.01 mol) was refluxed in dry chloroform (100 mL) for 10 h. Excess of solvent was removed under reduced pressure. The resulting salts **9–13** were obtained as yellow oils and purified by diethyl ether from starting reagents.

9. Yield (65%), oil. IR (KBr): 1710 (C=O), 1133 (P–C) cm^{-1}; ^{1}H NMR (D_2O) δ: 0.77 (t, 3H, CH_3, J = 7.0 Hz), 0.80–0.95 (m. 3H, $PCCH_3$), 1.23–1.47 (m, 14H, $7CH_2$), 2.48–2.51 (m, 2H, $OCCH_2$), 3.10–3.35 (m, 2H, $PCCH_2$), 4.28–4.39 (m, 2H, OCH_2), 4.69–4.77 (m. 1H, PCH), 7.27–7.84 (m, 15H, phenyl H); ^{31}P NMR (DMSO-d_6) δ: 24.21. $C_{31}H_{42}BrO_2P$ (556.90): calcd. C 66.79%, H 7.58%; found C 66.25%, H 7.40%.

10. Yield (70%), oil. IR (KBr): 1715 (C=O), 1137 (P–C) cm^{-1}; ^{1}H NMR (D_2O) δ: 0.80 (t, 3H, CH_3, J = 6.9 Hz), 0.79–0.93 (m. 3H, $PCCH_3$), 1.27–1.53 (m, 18H, $9CH_2$), 2.37–2.49 (m, 2H, $OCCH_2$), 3.07–3.37 (m, 2H, $PCCH_2$), 4.25–4.40 (m, 2H, OCH_2), 4.73–4.87 (m. 1H, PCH), 7.25–7.81 (m, 15H, phenyl H); ^{31}P NMR (DMSO-d_6) δ: 24.17. $C_{33}H_{46}BrO_2P$ (584.90): calcd. C 67.69%, H 7.86%; found C 67.91%, H 7.79%.

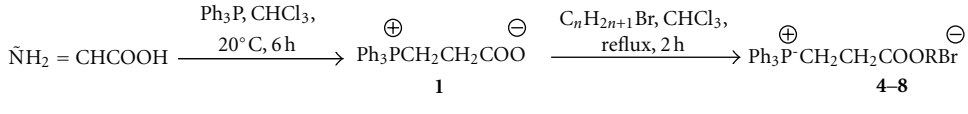
11. Yield (63%), oil. IR (KBr): 1712 (C=O), 1135 (P–C) cm^{-1}; ^{1}H NMR (D_2O) δ: 0.81 (t, 3H, CH_3, J = 7.3 Hz), 0.83–0.97 (m. 3H, $PCCH_3$), 1.25–1.43 (m, 22H, $11CH_2$), 2.43–2.59 (m, 2H, $OCCH_2$), 3.11–3.31 (m, 2H, $PCCH_2$), 4.18–4.29 (m, 2H, OCH_2), 4.81–4.97 (m. 1H, PCH), 7.27–7.87 (m, 15H, phenyl H); ^{31}P NMR (DMSO-d_6) δ: 24.07. $C_{35}H_{50}BrO_2P$ (612.90): calcd. C 68.52%, H 8.16%; found C 69.16%, H, 8.27%.

12. Yield (71%), m.p; 208°C. IR (KBr): 1715 (C=O), 1137 (P–C) cm^{-1}; ^{1}H NMR (D_2O) δ: 0.73 (t, 3H, CH_3, J = 6.9 Hz), 0.81–0.93 (m. 3H, $PCCH_3$), 1.27–1.47 (m, 26H, $13CH_2$), 2.49–2.58 (m, 2H, $OCCH_2$), 3.15–3.40 (m, 2H, $PCCH_2$), 4.18–4.29 (m, 2H, OCH_2), 4.73–4.86 (m. 1H, PCH), 7.31–7.93 (m, 15H, phenyl H); ^{31}P NMR (DMSO-d_6) δ: 23.77. $C_{37}H_{54}BrO_2P$ (640.90): calcd. C 69.27%, H 8.42%; found C 69.58%, H 8.30%.

13. Yield (67%), oil. IR (KBr): 1720 (C=O), 1135 (P–C) cm^{-1}; ^{1}H NMR (D_2O) δ: 0.72 (t, 3H, CH_3, J = 6.9 Hz), 0.76–0.83 (m. 3H, $PCCH_3$), 1.13–1.59 (m, 30H, $15CH_2$), 2.47–2.57 (m, 2H, $OCCH_2$), 3.07–3.25 (m, 2H, $PCCH_2$), 4.08–4.19 (m, 2H, OCH_2), 4.73–4.87 (m. 1H, PCH),

R = Ph; R′ = H; R″ = H **(1)**
R = Ph; R′ = CH_3; R″ = H **(2)**
R = Ph; R′ = H; R″ = CH_3 **(3)**

Scheme 1



R = $C_{10}H_{21}$ (**4**)
R = $C_{12}H_{25}$ (**5**)
R = $C_{14}H_{29}$ (**6**)
R = $C_{16}H_{33}$ (**7**)
R = $C_{18}H_{37}$ (**8**)

Scheme 2: Synthetic routes of β-(carboxyalkyl)ethyltriphenylphosphonium bromides **4–8**; reagents and conditions.

7.33–7.89 (m, 15H, phenyl H); ^{31}P NMR (DMSO-d_6) δ: 23.83. $C_{39}H_{58}BrO_2P$ (668.90): calcd. C 69.96%, H 8.67%; found C 69.86%, H 8.15%.

2.4. Synthesis of β-(Carboxyalkyl)-α-methylethyltriphenylphosphonium Bromides (**14–18**)

2.4.1. General Procedure. A mixture of equimolar quantities of β-triphenylphosphonium-α-methylethylcarboxylate **3** (0.01 mol) and appropriate alkyl halogenides (0.01 mol) was refluxed in dry chloroform (100 mL) for 14 h. Excess of solvent was removed under reduced pressure. The resulting salts **14–18** were obtained as yellow oils and purified by diethyl ether from starting reagents.

14. Yield (73%), oil. IR (KBr): 1720 (C=O), 1133 (P–C) cm^{-1}; ^{1}H NMR (D_2O) δ: 0.73 (t, 3H, CH_3, J = 7.1 Hz), 1.27–1.43 (m, 14H, $7CH_2$), 1.57–1.62 (m. 3H, $PCCCH_3$), 2.47–2.53 (m, 2H, $OCCH_2$), 3.52–3.59 (m. 1H, PCCH), 4.15–4.31 (m, 2H, PCH_2), 4.33–4.49 (m, 2H, OCH_2), 7.29–7.52 (m, 15H, phenyl H); ^{31}P NMR (DMSO-d_6) δ: 23.26. $C_{31}H_{42}BrO_2P$ (556.90): calcd. C 66.79%, H 7.54%; found C 66.93%, H 7.02%.

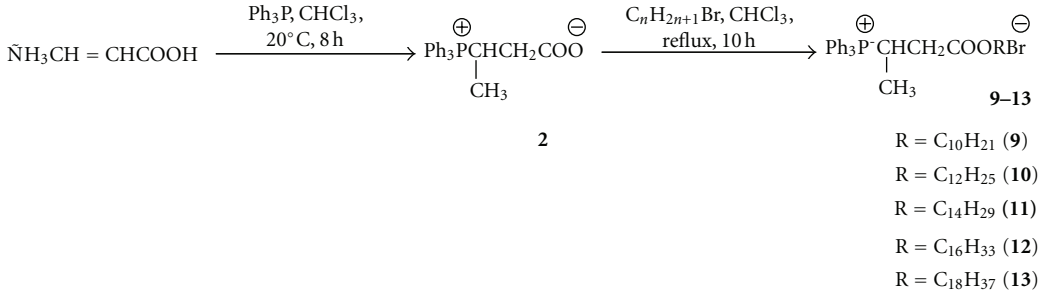
15. Yield (80%), oil. IR (KBr): 1720 (C=O), 1130 (P–C) cm^{-1}; ^{1}H NMR (D_2O) δ: 0.71 (t, 3H, CH_3, J = 7.3 Hz), 1.16–1.49 (m, 18H, $9CH_2$), 1.45–1.67 (m. 3H, $PCCCH_3$), 2.40–2.59 (m, 2H, $OCCH_2$), 3.45–3.55 (m. 1H, PCCH), 4.10–4.21 (m, 2H, PCH_2), 4.23–4.51 (m, 2H, OCH_2), 7.16–7.61 (m, 15H, phenyl H); ^{31}P NMR (DMSO-d_6) δ: 23.48. $C_{33}H_{46}BrO_2P$ (584.90): calcd. C 67.69%, H 7.86%; found C 67.84%, H 7.63%.

16. Yield (77%), oil. IR (KBr): 1715 (C=O), 1132 (P–C) cm^{-1}; ^{1}H NMR (D_2O) δ: 0.75 (t, 3H, CH_3, J = 7.1 Hz), 1.31–1.41 (m, 22H, $11CH_2$), 1.47–1.66 (m. 3H, $PCCCH_3$), 2.43–2.51 (m, 2H, $OCCH_2$), 3.57–3.66 (m. 1H, PCCH), 4.27–4.39 (m, 2H, OCH_2), 4.41–4.53 (m, 2H, PCH_2), 7.30–7.63 (m, 15H, phenyl H); ^{31}P NMR (DMSO-d_6) δ: 23.54. $C_{35}H_{50}BrO_2P$ (612.90): calcd. C 68.52%, H 8.16%; found C 68.77%, H, 7.05%.

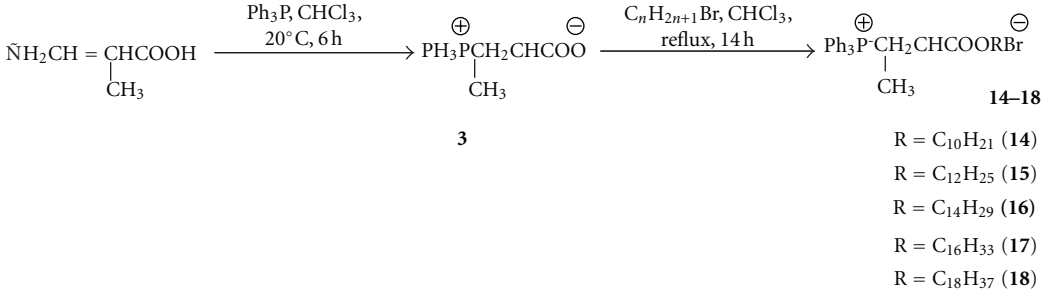
17. Yield (90%), m.p; 208°C. IR (KBr): 1720 (C=O), 1137 (P–C) cm^{-1}; ^{1}H NMR (D_2O) δ: 0.70 (t, 3H, CH_3, J = 7.2 Hz), 1.31–1.49 (m, 26H, $13CH_2$), 1.57–1.72 (m. 3H, $PCCCH_3$), 2.45–2.56 (m, 2H, $OCCH_2$), 3.52–3.59 (m. 1H, PCCH), 4.16–4.30 (m, 2H, PCH_2), 4.33–4.49 (m, 2H, OCH_2), 7.29–7.52 (m, 15H, phenyl H); ^{31}P NMR (DMSO-d_6) δ: 23.58. $C_{37}H_{54}BrO_2P$ (640.90): calcd. C 69.27%, H 8.42%; found C 68.95%, H 8.11%.

18. Yield (81%), oil. IR (KBr): 1720 (C=O), 1133 (P–C) cm^{-1}; ^{1}H NMR (D_2O) δ: 0.73 (t, 3H, CH_3, J = 7.0 Hz), 1.31–1.47 (m, 30H, $15CH_2$), 1.62–1.73 (m. 3H, $PCCCH_3$), 2.51–2.63 (m, 2H, $OCCH_2$), 3.49–3.57 (m. 1H, PCCH), 4.14–4.29 (m, 2H, PCH_2), 4.31–4.41 (m, 2H, OCH_2), 7.37–7.59 (m, 15H, phenyl H); ^{31}P NMR (DMSO-d_6) δ: 23.60. $C_{39}H_{58}BrO_2P$ (668.90): calcd. C 69.96%, H 8.67%; found C 70.24%, H 8.32%.

2.5. Antimicrobial Screening. The antimicrobial activity of the newly synthesized compounds was determined in vitro using the agar disk-diffusion method using Mueller-Hilton agar medium [9, 10] against a variety of pathogenic microorganisms: *Staphylococcus aureus* (ATCC 29213) (Gram-positive bacteria), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Proteus mirabilis* (ATCC 12453) (Gram-negative bacteria) and fungus *Candida albicans* (ATCC 885-653). The inhibition zones of the tested compounds were measured after 24–48 h incubation at 37°C for bacteria and after 5 days of incubation at 28°C for fungi. Penicillin (Sigma-Aldrich) and Chlorhexidine (Sigma-Aldrich) were used as reference drug for bacteria, whereas



Scheme 3: Synthetic routes of β-(carboxyalkyl)-β-methylethyltriphenylphosphonium bromides **9–13**; reagents and conditions.



Scheme 4: Synthetic routes of β-(carboxyalkyl)-α-methylethyltriphenylphosphonium bromides **14–18**; reagents and conditions.

Griseofulvin (Sigma-Aldrich) was used as reference drug for fungi. Every experiment in the antibacterial and antifungal assay was replicated twice. For the antibacterial and antifungal activity, the compounds were dissolved in dimethylsulfoxide (DMSO).

Many years we thought that it is impossible to grow single crystals of oil products **4–18** with long alkyl chains suitable for X-ray diffraction, but after five years we have a real chance to obtain the crystalline structure of the $Ph_3PCH_2CH_2C(O)OC_{16}$ H_{33} (**7**), which gave good quality crystals (Figure 1) [11].

2.5.1. Antibacterial Activity. Different strains of bacteria were used as *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Proteus mirabilis* (ATCC 12453). Cup-plate Agar method was used for evaluation of antibacterial activity. The nutrient agar medium is used. The medium with bacteria was poured into sterilized Petri dishes under aseptic conditions. Standard drugs used were Penicillin (50 μg/0.1 mL) and Chlorhexidine (50 μg/0.1 mL) and test compounds at concentration of 50 μg/0.1 mL. Solvent used was dimethyl sulfoxide (DMSO). Plates were incubated at 37°C for 24 hours. After incubation the average zone of inhibition was recorded in mm [12].

2.5.2. Antifungal Activity. The antifungal activity was carried out by using cup-plate method using Sabouraud's agar medium. Fungal strains used were *Candida albicans* (ATCC 885-653) with incubation period of 48 hours at temperature 28°C. The standard drug used was Griseofulvin (50 μg/0.1 mL) and the test compounds at concentration of 50 μg/0.1 mL by using dimethyl sulfoxide (DMSO) [13].

3. Results and Discussions

3.1. Chemistry. The synthetic routes are given in Schemes 2–4. In this paper we present the synthesis and biological activity of a series of nanosized (30 nm) quaternary phosphonium salts **4–18** with long alkyl chains (R=C_nH_{2n+1}; n = 10, 12, 14, 16, 18; here n is the number of carbon atoms in alkyl groups) on the basis of phosphabetaines **1–3** and higher alkyl halogenides. All the synthesized compounds were characterized by elemental analysis, IR, ^{1}H NMR, and ^{31}P NMR spectroscopy. A crystalline product of β-(carboxyhexadecyl)ethyltriphenylphosphonium bromide **7** was prepared and characterized by single-crystal X-ray analysis [11] (Figure 1).

3.1.1. Scheme 2 Depicts the Synthesis of β-(Carboxyalkyl) ethyltriphenylphosphonium Bromides (**4–8**). Treatment of acrylic acid with triphenylphosphine at room temperature in chloroform during 6 hours yielded (78%) phosphabetaine **1**. Alkylation of the starting phosphabetaine **1**-β-triphenylphosphonium ethylcarboxylate with alkyl halogenides (reflux for two hours in CH_3Cl) gave the corresponding phosphonium bromides **4–8** with long alkyl chains. Molecular structure of product **7** is given in Figure 1.

3.1.2. Scheme 3 Depicts the Synthesis of β-(Carboxyalkyl)-β-methylethyltriphenylphosphonium Bromides **9–13**. Treatment of crotonic acid with triphenylphosphine at room

Table 1: Antimicrobial activity of the newly synthesized compounds and the control drugs (50 μg/0.1 mL).

		Zone of inhibition (mm)				
n	Compound	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Proteus mirabilis*	*Candida albicans*
4	$Ph_3\overset{\oplus}{P}–CH_2CH_2C(O)O\tilde{N}_{10}H_{21}\ \overset{\ominus}{Br}$	15	20	21	18	24
5	$Ph_3\overset{\oplus}{P}—CH_2CH_2C(O)O\tilde{N}_{12}H_{25}\ \overset{\ominus}{Br}$	20	22	13.5	23	28
6	$Ph_3\overset{\oplus}{P}—CH_2CH_2C(O)O\tilde{N}_{14}H_{29}\ \overset{\ominus}{Br}$	25	17	14	10	24
7	$Ph_3\overset{\oplus}{P}—CH_2CH_2C(O)O\tilde{N}_{16}H_{33}\ \overset{\ominus}{Br}$	25	18	11	17	22
8	$Ph_3\overset{\oplus}{P}—CH_2CH_2C(O)O\tilde{N}_{18}H_{37}\ \overset{\ominus}{Br}$	23	14	13	8	24
9	$Ph_3\overset{\oplus}{P}—\underset{\vert}{\underset{CH_3}{CH}}CH_2C(O)OC_{10}H_{21}\ \overset{\ominus}{Br}$	17	10	17	16	23
10	$Ph_3\overset{\oplus}{P}—\underset{\vert}{\underset{CH_3}{CH}}CH_2C(O)OC_{12}H_{25}\ \overset{\ominus}{Br}$	21	17	15	11	20.5
11	$Ph_3\overset{\oplus}{P}—\underset{\vert}{\underset{CH_3}{CH}}CH_2C(O)OC_{14}H_{29}\ \overset{\ominus}{Br}$	26	17	14	13	25
12	$Ph_3\overset{\oplus}{P}—\underset{\vert}{\underset{CH_3}{CH}}CH_2C(O)OC_{16}H_{33}\ \overset{\ominus}{Br}$	20	14	15	11	21
13	$Ph_3\overset{\oplus}{P}—\underset{\vert}{\underset{CH_3}{CH}}CH_2C(O)OC_{18}H_{37}\ \overset{\ominus}{Br}$	19	13	10.5	9	20
14	$Ph_3\overset{\oplus}{P}—CH_2\underset{\vert}{\underset{CH_3}{CH}}C(O)OC_{10}H_{21}\ \overset{\ominus}{Br}$	17	14	15	11	21.5
15	$Ph_3\overset{\oplus}{P}—CH_2\underset{\vert}{\underset{CH_3}{CH}}C(O)OC_{12}H_{25}\ \overset{\ominus}{Br}$	25.5	17	18	15	25
16	$Ph_3\overset{\oplus}{P}—CH_2\underset{\vert}{\underset{CH_3}{CH}}C(O)OC_{14}H_{29}\ \overset{\ominus}{Br}$	25	19	17.5	17	28
17	$Ph_3\overset{\oplus}{P}—CH_2\underset{\vert}{\underset{CH_3}{CH}}C(O)OC_{16}H_{33}\ \overset{\ominus}{Br}$	17	13.5	14	11.5	20
18	$Ph_3\overset{\oplus}{P}—CH_2\underset{\vert}{\underset{CH}{CH}}C(O)OC_{18}H_{37}\ \overset{\ominus}{Br}$	19	13	11	17	19
19	Chlorhexidine	16	15	13	14	16.5
20	Penicillin	23	16	8	10	—
21	Griseofulvin	—	—	—	—	19

n: Compound number.
All tests were performed in triplicate. Zone of inhibition 20 to 28: highly significant, between 11 and 19 mm: less significant, below 10 mm: poor active.

temperature in chloroform during 8 hours yielded (75%) phosphabetaine **2**. Alkylation of the starting phosphabetaine **2**-β-triphenylphosphonium β-methylethylcarboxylate with alkyl halogenides (reflux for 10 hours in CH_3Cl) gave the corresponding phosphonium bromides **9–13** with long alkyl chains. The yield was 60%–70%.

3.1.3. Scheme 4 Depicts the Synthesis of β-(Carboxyalkyl)-α-methylethyltriphenylphosphonium Bromides (**14–18**). Treatment of methacrylic acid with triphenylphosphine at room temperature in chloroform during 6 hours yielded (77%) phosphabetaine **3**. Alkylation of the starting phosphabetaine **3**-β-triphenylphosphonium-α-methylethylcarboxylate with

alkyl halogenides (reflux for 14 hours in CH_3Cl) gave the corresponding phosphonium bromides **14–18** with long alkyl chains. The yield was 70%–90%.

3.2. Antimicrobial Activity. The synthesized compounds—a new class of bioactive nanomolecules (30 nm)—were screened for antibacterial and antifungal activity at 50 μg/0.1 mL concentration by using the cup-plate agar diffusion method, and standard drugs used were Chlorhexidine **19**, Penicillin **20**, and Griseofulvin **21**. The novel synthesized compounds **4–18** with long alkyl chains (n = 10, 12, 14, 16, and 18) show maximal activity against pathogenic microorganisms. Starting phosphabetaines **1–3** and all phosphonium salts with short alkyl chains, synthesized earlier [6], were not active at all. Compounds **4**, **5**, **6**, **7**, **11**, **15**, and **16** were highly significant against tested bacteria as well as fungi. Our results are reported in Table 1.

Such a high biological activity of cationic biocides **4–18** we explain by their ability to be integrated into the lipid layers of biomembranes of pathogenic microflora eventually leading to the destruction of this last [14]. To confirm this idea we studied the interaction mechanism of compounds **4–8**—synthetic phosphorus analogs of biomembranes—with natural biological membranes (lecithin) using the model of Langmuir monolayers [15]. It was discovered that alkylated phosphabetaines **4–8** interact with lecithin, by forming a pores, and thus deteriorating the membrane functions.

4. Conclusion

In conclusion, carboxylate phosphabetaines derivatives with alkyl chains of various lengths were synthesized in good yield, characterized by different spectral studies, and their antimicrobial activity has been evaluated. Compounds **5–8**, **11**, **15**, and **16** demonstrated good inhibitions against all the strains tested comparable to Chlorhexidine, Penicillin, and Griseofulvin as positive standard. So, it may be concluded from our results that the synthesized compounds are potent nanoantimicrobial agents against pathogenic bacteria and fungi.

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