Synthesis and Antibacterial Activity of Quaternary Ammonium Salt-Type Antibacterial Agents with a Phosphate Group

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Abstract: Quaternary ammonium salts are frequently used as antibacterial agent that disrupts cell membrane through the binding of their ammonium cations to anionic sites in the outer layer tissue of bacteria. This article describes the synthesis of quaternary ammonium salt-type antibacterial agents with a phosphate group that strongly binds to hydroxyapatite and bromide ion as counterion. Evaluation of the antibacterial activity of the synthesized compounds in terms of minimum inhibitory concentration (MIC test) showed that the compounds exhibit an excellent antibacterial activity on a variety of bacteria including Gram-positive and Gram-negative bacteria, yeast, and fungi.

Key words: antibacterial agent, synthesis, quaternary ammonium salt, phosphate group, minimum inhibitory concentration

1 INTRODUCTION

Recent increase in people's inclination toward cleanliness in their daily life has led to the antibacterial treatment of many materials. In the field of dentistry, the control of oral sanitary has become increasingly important because the suppression of oral bacteria is expected to be effective in protecting elderly people confined to bed and physically handicapped persons from respiratory tract infection and pneumonia.

About 8% of the causes of Japanese people's death is due to pneumonia and 92% of the people died of the disease are at ages over 65. Meanwhile, according to a report from the Tokyo Metropolitan Geriatric Hospital, pneumonia infection was found in 44.6% autopsy cases for elderly people of ages over 85 among 4591 autopsy cases. Hence, pneumonia is cited as one of the important causes of death for aged people. The infection way of pneumonia for aged people is via aspiration in many cases and the number of pneumonia cases triggered by aspiration is larger for dentulous jaw

patients than for edentulous jaw ones. Oral care is then important for the prevention of aspiratory pneumonia.

Pneumonia is caused generally by many kinds of bacteria, fungi, protozoa, and viruses. There are certain cases where even healthy people are infected with fungi and protozoa and the disease often attacks hosts when their immune strength is weakened. Geriatric pneumonia, namely, aspiratory pneumonia is caused by oral bacteria and hence antibacterial agents are very important that can achieve oral care by controlling oral bacteria.

Quaternary ammonium salt-type compounds are highly sterilizable and disinfective while they are non-irritant and scarcely toxic. Quaternary ammonium salts fixed on the surface of material exhibit their antibacterial activity by inhibiting the functions of cell wall and cytoplasm membrane or by physically disrupting cell membrane¹⁻⁶⁾. Hence, quaternary ammonium salt-type compounds are often used to disinfect and sterilize medical tools and fingers. Imazato *et al.* developed a new antibacterial

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PPh-*n*-QAB (*n* = 8, 10, 12, 14, 16, 18)

Scheme 1

monomer with a pyridinium bromide group and reported that the dental composite resin containing the monomer shows an antibacterial activity on *Streptococcus mutans*, the bacterium that causes carious tooth⁷). Phosphate group is known to have a self-etching primer effect, be a constituent of dentin adhesive suited for human tooth, and strongly adsorb to hydroxyapatite. The group is also known to constitute the hydrophilic part of anionic surfactant, and surfactants with a phosphate group are frequently used in cosmetics and body washing agent because they hardly harm human skin.

The present article deals with the synthesis of quaternary ammonium salt-type antibacterial agents having a phosphate group in their molecule with the aim to develop new oral antibacterial agents that can be used in aqueous solution instead of non-aqueous environment for silane coupling agent-type antibacterial agents and that can keep their properties for long period of time in severe oral circumstances (high humidity, changing temperature, occlusal pressure, etc). Evaluations of the antibacterial activity of the synthesized agents were conducted using the minimum inhibitory concentration test (MIC test). Scheme 1 shows the structure of the compounds synthesized in this article.

2 EXPERIMENTAL

2.1 Materials

THF solution of 0.5 M 9-borabicyclo[3.3.1]nonane ($C_8H_{14}BH$, 9-BBN), 35% hydrogen peroxide (H_2O_2), diphenyl phosphorochloridate ($(C_6H_5O)_2POCl$, Kanto Kagaku), 4-dimethylaminopyridine ($(CH_3)_2NC_5H_4N$, Tokyo Kasei), sodium hydroxide (NaOH, Kanto Kagaku), hydrochloric acid (HCl, Kanto Kagaku), magnesium sulfate ($MgSO_4$, Nakaraitesk), sea sand B (Nakaraitesk), and silica gel (Wako-gel C-300, Wako Pure Chemicals) were used as supplied. 1-Bromooctane ($CH_3(CH_2)_7Br$), 1-bromododecane ($CH_3(CH_2)_{11}Br$), 1-bromooctadecane ($CH_3(CH_2)_{17}Br$) (all from Tokyo Kasei), 1-bromodecane ($CH_3(CH_2)_9Br$), 1-bromotetradecane ($CH_3(CH_2)_{13}Br$), 1-bromohexadecane ($CH_3(CH_2)_{15}Br$) (all from Aldrich) were used after being dehydrated with molecular sieves. *N,N*-diallyl-*N*-methylaniline ($(CH_2=CHCH_2)_2NCH_3$, Wako Pure Chemicals) was

used after being distilled (bp 111°C). Tetrahydrofuran (THF), acetonitrile, hexane, and dichloromethane as solvent were used after being dehydrated over calcium hydride and distilled. The other solvents (chloroform, methanol, pyridine, acetone, 1,4-dioxane, and ethanol) were used as purchased.

2.2 Measurements

A Nicolet Avatar 360-FT-IR spectrometer was used to measure FT-IR spectrum by the attenuated total reflection method. 400 MHz 1H -NMR and 100 MHz ^{13}C -NMR spectra were measured at room temperature in $CDCl_3$ or CD_3OD with TMS as internal standard using a Bruker DPX-400 spectrometer. A JEOL JMS SX102A was used for MS measurement by the electron impact (EI) or the fast atom bombardment (FAB) method.

2.3 Synthesis of quaternary ammonium salts of alkylphosphates

Scheme 2 shows the synthesis route of the compounds.

2.3.1 Synthesis of 3-(*N*-allyl-*N*-methylamino)propan-1-ol (MDAAOH)

N,N-diallyl-*N*-methylaniline 5.56 g (50.0 mmol) was dissolved in anhydrous THF (10 mL) in a nitrogen atmosphere and the solution was cooled to 0°C. To this solution was added dropwise 0.5 M 9-BBN solution 84.9 g (50.0 mmol) in THF and the resultant solution was stirred for 15 h at room temperature. In order to obtain MDAAOH from the borane derivative formed, the reaction mixture was cooled to 0°C and stirred for 3 h at 50°C and then overnight at room temperature after dropwise additions of H_2O (2.0 mL), 3 M NaOH solution (15 mL), and 34.8% H_2O_2 (18 mL). This solution was distilled (bp 105°C /2000 Pa) to give MDAAOH 5.03 g (39.0 mmol) as a viscous colorless liquid in a yield of 78.0%. The results are given in 3.1.1.

2.3.2 Synthesis of 3-(*N*-allyl-*N*-methylamino)propyl diphenylphosphate (MDAAPP2)

MDAAOH 5.03 g (39.0 mmol) and 4-dimethylaminopyridine 7.15 g (58.5 mmol) were dissolved in dichloromethane (40 mL), and pyridine 5.78 g (58.5 mmol) and diphenyl phosphorochloridate 15.7 g (58.5 mmol) were added in this order to the above solution and the resultant solution was stirred overnight at 35°C. After dichloromethane and pyridine were separated by vacuum distillation from the solution, the product was extracted with chloroform and dissolved in a mixed solvent of chloroform and acetone at 90 : 1 (volume ratio). This solution was passed through a silica gel column filled with Wako-gel C-300 (column diameter 6.0 cm, column length 46.0 cm) to adsorb the product, which was then purified by elution with methanol after the impurities were removed. Thus, MDAAPP2 was obtained as a viscous yellow liquid 7.65 g (21.2 mmol) in a yield of 54.3%. The results are given in 3.1.2.

Scheme 2

2.3.3 Synthesis of 3-(*N*-allyl-*N*-methylamino)propyl phenylhydrophosphate (MDAAPP)

MDAAPP2 7.65 g (21.2 mmol) was dissolved in 1,4-dioxane (40 mL) and 4 M NaOH solution (4.5 mL) was added to this solution. The solution was stirred at 50°C for 5 h while heating and the solvents were removed by vacuum distillation after the reaction was completed. The residue was dissolved in water and the chloroform-soluble part was extracted after the aqueous solution was neutralized with 1 M hydrochloric acid. The product was purified by silica gel chromatography using methanol as developer to give MDAAPP 2.11 g (7.40 mmol) as a viscous yellow liquid in a yield of 34.9%.

2.3.4 Synthesis of quaternary ammonium bromide salt of 3-(*N*-allyl-*N*-methyl-*N*-octyl)propyl phenylhydrophosphate (PPh-8-QAB)

1-Bromooctane 0.71 g (3.70 mmol) and acetonitrile (5 mL) were taken into a flask in a nitrogen flow, to which was slowly added MDAAPP 1.05 g (3.7 mmol) solution in acetonitrile (5 mL) with a dropping funnel while refluxing and heating and the reaction mixture was refluxed for 48 h while heating. The unreacted 1-bromooctane was removed by extraction with hexane after the solvent was removed by vacuum distillation. The residue was dissolved in chloroform and the unreacted MDAAPP was removed by washing with water. The product was dried at a reduced pressure to give PPh-8-QAB 0.90 g (1.89 mmol) as a viscous

orange liquid in a yield of 51.1%.

2.3.5 Synthesis of quaternary ammonium bromide salt of 3-(*N*-allyl-*N*-methyl-*N*-decyl)propyl phenylhydrophosphate (PPh-10-QAB), quaternary ammonium bromide salt of 3-(*N*-allyl-*N*-methyl-*N*-dodecyl)propyl phenylhydrophosphate (PPh-12-QAB), quaternary ammonium bromide salt of 3-(*N*-allyl-*N*-methyl-*N*-tetradecyl)propyl phenylhydrophosphate (PPh-14-QAB), quaternary ammonium bromide salt of 3-(*N*-allyl-*N*-methyl-*N*-hexadecyl)propyl phenylhydrophosphate (PPh-16-QAB), and quaternary ammonium bromide salt of 3-(*N*-allyl-*N*-methyl-*N*-octadecyl)propyl phenylhydrophosphate (PPh-18-QAB)

The methods of synthesis and purification were the same as described above (Table 1). The results are given in 3.2.2-3.2.6.

2.4 Minimum inhibitory concentration (MIC) test on quaternary ammonium salts of alkyl phosphates

2.4.1 Preparation of culture media

First, 0.4% PPh-*n*-QAB solution in ethanol (stock solution) was prepared and then ordinary agar medium (for bacteria) or potato dextrose agar medium (for yeast and mold) were prepared using solutions of the quaternary ammonium salts made by diluting successively the stock solution with water to give concentrations of 400, 200, 100, 50, 25, 10, 5, 2.5 and 1.0 ppm. These medium solutions thus

Table 1 Quantity of Materials, Reaction Time and Yield of Products.

product	MDAAPP [g(mmol)]	H(CH ₂) _n Br [g(mmol)]	reaction time [h]	yield [%]
PPh-10-QAB	1.06(3.70)	0.82(3.70)	48.0	48.1
PPh-12-QAB	1.79(6.36)	2.36(9.45)	72.0	50.3
PPh-14-QAB	2.00(7.00)	2.91(10.5)	72.0	50.0
PPh-16-QAB	1.94(6.84)	3.15(10.3)	72.0	48.0
PPh-18-QAB	1.85(6.48)	3.24(9.72)	96.0	48.0

Table 2 Microorganism Strains Used for the MIC Test.

Gram-positive bacterium	
<i>S.aureus</i>	<i>Staphylococcus aureus</i> KB210
<i>B.subtilis</i>	<i>Bacillus subtilis</i> KB211
<i>M.luteus</i>	<i>Micrococcus luteus</i> KB212
Gram-negative bacterium	
<i>E.coli</i>	<i>Escherichia coli</i> KB213
<i>S.typhimurium</i>	<i>Salmonella typhimurium</i> KB20
<i>P.aeruginosa</i>	<i>Pseudomonas aeruginosa</i> KB115
Yeast	
<i>C.albicans</i>	<i>Candida albicans</i> KF1
<i>S.cerevisiae</i>	<i>Saccharomyces cerevisiae</i> KF25
Mold	
<i>T.mentagrophytes</i>	<i>Trichophyton mentagrophytes</i> KF213
<i>M.gypseum</i>	<i>Microsporium gypseum</i> KF64
<i>P.chrysogenum</i>	<i>Penicillium chrysogenum</i> KF270
<i>A.niger</i>	<i>Aspergillus niger</i> KF103

prepared were sterilized in an autoclave for 30 min at 126°C and then transferred to petri dishes to be solidified.

2.4.2 Microbial inoculation and observations

A drop of each of spore suspensions containing each of microorganisms suspended in 2 mL of sterilized physiological saline solution was inoculated in the medium prepared. **Table 2** shows the microorganisms used. The bacterium-inoculated and yeast- or mold-inoculated media were cultured for 2 days at 37°C and for 7 days at 25°C, respectively, and they were observed to check if the microorganism grew or not. The minimum concentration for each salt at which no microorganism growth was found was defined as its MIC. The results obtained for the six synthesized compounds are given in **3.3**.

3 RESULTS AND DISCUSSION

3.1 Synthesis of quaternary ammonium salts of alkylphosphates

3.1.1 Synthesis of MDAAOH

IR (cm⁻¹): 3354($\nu_{\text{O-H}}$), 2920-2845($\nu_{\text{C-H}}$), 1644($\nu_{\text{C=C}}$), 1448(δ_{CH_2}), 1065($\nu_{\text{C-N}}$), 1065 ($\nu_{\text{C-O}}$); ¹H-NMR (CDCl₃): δ 1.70(f, m(multiplet), 2H), 2.25(d, s(singlet), 3H), 2.61(e, t(triplet), 2H; J = 11.48 Hz), 3.02(c, d(doublet), 2H; J = 6.52 Hz), 3.79(g, t, 2H; J = 10.44 Hz), 5.15-5.19 (a, m, 2H), 5.78-5.89 (b, m, 1H) for CH₂=CH^aCH₂^bN(CH₃^d)CH₂^cCH₂^fCH₂^gOH; ¹³C-NMR (CDCl₃): δ 27.8 (f), 42.0 (d), 57.6(e), 61.1 (c), 64.7 (g), 118.0 (a), 135.0 (b) for C^aH₂=C^bHC^cH₂N(C^dH₃)C^eH₂C^fH₂C^gH₂OH; MS (m/z) (rel int): 129 (5.0) [M]⁺, 84 (100) [CH₂=CHCH₂N(CH₃)CH₂]⁺, 41 (30) [CH₂=CHCH₂]⁺; HRMS (EI⁺): obsd. 129.1156 [68.5, (calcd. 129.1153 for C₇H₁₅O₁N₁)].

3.1.2 Synthesis of MDAAPP2

IR (cm⁻¹): 3060-2959($\nu_{\text{C-H}}$), 1589-1483($\nu_{\text{C=C}}$), 1288($\nu_{\text{P=O}}$), 1210($\nu_{\text{C-O}}$), 1092($\nu_{\text{P-O}}$); ¹H-NMR (CD₃OD): δ 1.93 (f, m, 2H), 2.28(d, s, 3H), 2.56(e, t, 2H; J = 15.16 Hz), 3.11(c, d, 2H; J = 6.66 Hz), 4.38(g, m, 2H), 5.51-5.56(a, m, 2H), 5.85-5.98 (b, m,

1H), 7.19-7.28(h, m, 6H), 7.39-7.43(i, m, 4H) for $\text{CH}_2^a=\text{CH}^b\text{CH}_2^c\text{N}(\text{CH}_3^d)\text{CH}_2^e\text{CH}_2^f\text{CH}_2^g\text{OP}(=\text{O})(\text{OC}_6\text{H}_5^{h,i})_2$; ^{13}C -NMR (CD_3OD): δ 27.8(f), 41.4(d), 53.7(e), 60.9(c), 68.7(g), 121.2(i), 124.4(a), 126.8(k), 130.2(b), 131.2(j), 152.3(h) for

MS (m/z) (rel int): 361(40) $[\text{M}]^+$, 251(5) $[\text{OP}(\text{O})(\text{OC}_6\text{H}_5)_2]^+$, 84 (100) $[\text{CH}_2=\text{CHCH}_2\text{N}(\text{CH}_3)\text{CH}_2]^+$, 41 (30) $[\text{CH}_2=\text{CHCH}_2]^+$; HRMS (EI+): obsd. 361.1443 [100.0, (calcd. 361.1442 for $\text{C}_{19}\text{H}_{24}\text{O}_4\text{N}_1\text{P}_1$)].

3.1.3 Synthesis of MDAAPP

IR (cm^{-1}): 3323($\nu_{\text{O-H}}$), 2939-2822($\nu_{\text{C-H}}$), 1593-1495($\nu_{\text{C=O}}$), 1288($\nu_{\text{P=O}}$), 1237($\nu_{\text{C-O}}$), 1026($\nu_{\text{P-O}}$); ^1H -NMR (CD_3OD): δ 1.95(f, m, 2H), 2.75(d, s, 3H), 2.61(e, t, 2H; $J = 11.4$ Hz), 3.10(c, d, 2H; $J = 7.08$ Hz), 4.00(g, m, 2H), 5.17-5.26(a, m, 2H), 5.80-5.94(b, m, 1H), 6.85-6.93(h, m, 3H), 7.18-7.29(i, m, 2H) for $\text{CH}_2^a=\text{CH}^b\text{CH}_2^c\text{N}(\text{CH}_3^d)\text{CH}_2^e\text{CH}_2^f\text{CH}_2^g\text{OP}(=\text{O})(\text{OH})(\text{OC}_6\text{H}_5^{h,i})$; ^{13}C -NMR (CD_3OD): δ 26.5(f), 39.9(d), 54.4(e), 59.5(c), 64.1(g), 121.4(i), 124.4(a), 126.8(k), 127.7(b), 130.4(j), 154.2(h) for

MS (m/z) (rel int): 285 (12) $[\text{M}]^+$, 215(3) $[\text{CH}_2\text{CH}_2\text{CH}_2\text{OP}(=\text{O})(\text{OH})(\text{OC}_6\text{H}_5)]^+$, 192(10) $[\text{CH}_2=\text{CHCH}_2\text{N}(\text{CH}_3)\text{CH}_2\text{CH}_2\text{CH}_2\text{OP}(=\text{O})(\text{OH})]^+$, 112(25) $[\text{CH}_2=\text{CHCH}_2\text{N}(\text{CH}_3)\text{CH}_2]^+$, 84 (100) $[\text{CH}_2=\text{CHCH}_2\text{N}(\text{CH}_3)\text{CH}_2]^+$, 41 (23) $[\text{CH}_2=\text{CHCH}_2]^+$; HRMS (EI+): obsd. 285.1130 [22.8, (calcd. 285.1129 for $\text{C}_{13}\text{H}_{20}\text{O}_4\text{N}_1\text{P}_1$)].

3.2 Synthesis of quaternary ammonium salts of alkylphosphates

3.2.1 Synthesis of quaternary ammonium bromide salt of PPh-8-QAB

IR (cm^{-1}): 3413($\nu_{\text{O-H}}$), 2947 and 2927($\nu_{\text{C-H}}$), 1593 and 1483($\nu_{\text{C=O}}$), 1229($\nu_{\text{P=O}}$), 1229($\nu_{\text{C-O}}$), 1088($\nu_{\text{P-O}}$); ^1H -NMR (CD_3OD): δ 0.89(m, t, 3H; $J = 13.48$ Hz), 1.27-1.28(l, m, 10H), 1.70(k, m, 2H), 2.05(f, m, 2H), 2.65(d, s, 3H), 3.13(j, m, 2H), 3.77(e, m, 2H), 4.08(g, m, 2H), 4.15-4.35(c, m, 2H), 5.56-5.61(a, m, 2H), 5.82-6.00(b, m, 1H), 7.02-7.1, 7.19-7.21, 7.28-7.30(h and i, m, 5H) for $\text{CH}_2^a=\text{CH}^b\text{CH}_2^c\text{N}(\text{CH}_3^d)(\text{CH}_2^j\text{CH}_2^k(\text{CH}_2^l)_5\text{CH}_3^m)\text{CH}_2^e\text{CH}_2^f\text{CH}_2^g\text{OP}(=\text{O})(\text{OH})(\text{OC}_6\text{H}_5^{h,i})$; ^{13}C -NMR (CDCl_3): δ 13.0(q), 21.3(p), 24.2(f and m), 27.8(n), 30.5(o), 37.9(d), 51.5(l), 57.1(e and g), 61.8(c), 119.0(i), 122.3(a), 124.5(k), 125.1(b), 128.2(j), 151.2(h) for

MS (m/z) (rel int): 398(100) $[\text{M-Br}]^+$, 304(4) $[\text{M-Br-C}_6\text{H}_5\text{OH}]^+$,

215(7) $[\text{CH}_2\text{CH}_2\text{CH}_2\text{OP}(=\text{O})(\text{OH})(\text{OC}_6\text{H}_5)]^+$, 112(34) $[\text{CH}_2=\text{CHCH}_2\text{N}(\text{CH}_3)\text{CH}_2]^+$, 84 (19) $[\text{CH}_2=\text{CHCH}_2\text{N}(\text{CH}_3)\text{CH}_2]^+$, 41 (3) $[\text{CH}_2=\text{CHCH}_2]^+$; HRMS (FAB+) $[\text{M-Br}]^+$: obsd. 398.2460 [100.0, (calcd. 398.2459 for $\text{C}_{21}\text{H}_{37}\text{O}_4\text{N}_1\text{P}_1$)].

3.2.2 Synthesis of quaternary ammonium bromide salt of PPh-10-QAB

IR (cm^{-1}): 3393($\nu_{\text{O-H}}$), 2930-2927($\nu_{\text{C-H}}$), 1589-1487($\nu_{\text{C=O}}$), 1237($\nu_{\text{P=O}}$), 1237($\nu_{\text{C-O}}$), 1084($\nu_{\text{P-O}}$); ^1H -NMR (CD_3OD): δ 0.90(m, t, 3H; $J = 12.60$ Hz), 1.20-1.49(l, m, 14H), 1.69(k, m, 2H), 2.06(f, m, 2H), 2.77(d, s, 3H), 3.15(j, m, 2H), 3.77(e, m, 2H), 4.08(g, m, 2H), 4.33(c, m, 2H), 5.57-5.63(a, m, 2H), 5.87-6.00(b, m, 1H), 7.04-7.12, 7.17-7.36, 7.38-7.48(h and i, m, 5H) for $\text{CH}_2^a=\text{CH}^b\text{CH}_2^c\text{N}(\text{CH}_3^d)(\text{CH}_2^j\text{CH}_2^k(\text{CH}_2^l)_7\text{CH}_3^m)\text{CH}_2^e\text{CH}_2^f\text{CH}_2^g\text{OP}(=\text{O})(\text{OH})(\text{OC}_6\text{H}_5^{h,i})$; ^{13}C -NMR (CDCl_3): δ 13.0(q), 21.6(p), 24.4(f and m), 28.2(n), 30.8(o), 38.1(d), 51.7(l), 57.3(e and g), 62.0(c), 119.2(i), 122.5(a), 124.8(k), 125.2(b), 128.3(j), 151.4(h) for

MS (m/z) (rel int): 426(100) $[\text{M-Br}]^+$, 332(4) $[\text{M-Br-C}_6\text{H}_5\text{OH}]^+$, 215(10) $[\text{CH}_2\text{CH}_2\text{CH}_2\text{OP}(=\text{O})(\text{OH})(\text{OC}_6\text{H}_5)]^+$, 112(33) $[\text{CH}_2=\text{CHCH}_2\text{N}(\text{CH}_3)\text{CH}_2]^+$, 84 (22) $[\text{CH}_2=\text{CHCH}_2\text{N}(\text{CH}_3)\text{CH}_2]^+$, 41 (3) $[\text{CH}_2=\text{CHCH}_2]^+$; HRMS (FAB+) $[\text{M-Br}]^+$: obsd. 426.2773 [100.0, (calcd. 426.2771 for $\text{C}_{23}\text{H}_{41}\text{O}_4\text{N}_1\text{P}_1$)].

3.2.3 Synthesis of quaternary ammonium bromide salt of PPh-12-QAB

IR (cm^{-1}): 3409($\nu_{\text{O-H}}$), 2923-2853($\nu_{\text{C-H}}$), 1593-1484($\nu_{\text{C=O}}$), 1221($\nu_{\text{P=O}}$), 1221($\nu_{\text{C-O}}$), 1076($\nu_{\text{P-O}}$); ^1H -NMR (CD_3OD): δ 0.90(m, t, 3H; $J = 12.22$ Hz), 1.20-1.49(l, m, 18H), 1.83(k, m, 2H), 2.06(f, m, 2H), 2.78(d, s, 3H), 3.13(j, m, 2H), 3.77(e, m, 2H), 4.09(g, m, 2H), 4.35(c, m, 2H), 5.55-5.68(a, m, 2H), 5.87-6.03(b, m, 1H), 7.05-7.13, 7.17-7.36, 7.38-7.48(h and i, m, 5H) for $\text{CH}_2^a=\text{CH}^b\text{CH}_2^c\text{N}(\text{CH}_3^d)(\text{CH}_2^j\text{CH}_2^k(\text{CH}_2^l)_9\text{CH}_3^m)\text{CH}_2^e\text{CH}_2^f\text{CH}_2^g\text{OP}(=\text{O})(\text{OH})(\text{OC}_6\text{H}_5^{h,i})$; ^{13}C -NMR (CDCl_3): δ 13.0(q), 21.6(p), 24.5(f and m), 28.4(n), 30.8(o), 38.1(d), 51.7(l), 57.4(e and g), 62.2(c), 119.2(i), 122.8(a), 124.5(k), 125.0(b), 128.3(j), 151.0(h) for

MS (m/z) (rel int): 454(100) $[\text{M-Br}]^+$, 360(4) $[\text{M-Br-C}_6\text{H}_5\text{OH}]^+$, 215(10) $[\text{CH}_2\text{CH}_2\text{CH}_2\text{OP}(=\text{O})(\text{OH})(\text{OC}_6\text{H}_5)]^+$, 112(35) $[\text{CH}_2=\text{CHCH}_2\text{N}(\text{CH}_3)\text{CH}_2]^+$, 84 (32) $[\text{CH}_2=\text{CHCH}_2\text{N}(\text{CH}_3)\text{CH}_2]^+$, 41 (5) $[\text{CH}_2=\text{CHCH}_2]^+$; HRMS (FAB+) $[\text{M-Br}]^+$: obsd. 454.3086 [100.0, (calcd. 454.3084 for $\text{C}_{25}\text{H}_{45}\text{O}_4\text{N}_1\text{P}_1$)].

3.2.4 Synthesis of quaternary ammonium bromide salt of PPh-14-QAB

IR (cm⁻¹): 3397($\nu_{\text{O-H}}$), 2923 and 2850($\nu_{\text{C-H}}$), 1589 and 1487($\nu_{\text{C=C}}$), 1221($\nu_{\text{P=O}}$), 1221($\nu_{\text{C-O}}$), 1084($\nu_{\text{P-O}}$); ¹H-NMR (CD₃OD): δ 0.90(m, t, 3H; J = 12.07 Hz), 1.21-1.45(l, m, 22H), 1.72(k, m, 2H), 2.06(f, m, 2H), 2.77(d, s, 3H), 3.13(j, m, 2H), 3.77(e, m, 2H), 4.09(g, m, 2H), 4.25(c, m, 2H), 5.55-5.68(a, m, 2H), 5.88-6.00(b, m, 1H), 7.05-7.12, 7.17-7.36, 7.38-7.46(h and i, m, 5H) for CH₂^a=CH^bCH₂^cN(CH₃^d)(CH₂^jCH₂^k(CH₂^l)₁₁CH₃^m)CH₂^eCH₂^fCH₂^gOP(=O)(OH)(OC₆H₅^{h,i}); ¹³C-NMR (CDCl₃): δ 12.8(q), 21.4(p), 24.3(f and m), 28.3(n), 30.6(o), 37.9(d), 51.6(l), 57.2(e and g), 61.8(c), 119.0(i), 122.3(a), 124.3(k), 125.3(b), 128.2(j), 151.1(h) for

MS (m/z) (rel int): 482(100)[M-Br]⁺, 388(25)[M-Br-C₆H₅OH]⁺, 215(15)[CH₂CH₂CH₂OP(=O)(OH)(OC₆H₅)]⁺, 112(52) [CH₂=CHCH₂N(CH₃)CH₂]⁺, 84 (35) [CH₂=CHCH₂N(CH₃)CH₂]⁺, 41 (7) [CH₂=CHCH₂]⁺; HRMS (FAB+) [M-Br]⁺: obsd. 482.3398 [100.0, (calcd. 482.3397 for C₂₇H₄₉O₄N₁P₁)].

3.2.5 Synthesis of quaternary ammonium bromide salt of PPh-16-QAB

IR (cm⁻¹): 3413($\nu_{\text{O-H}}$), 2920 and 2850($\nu_{\text{C-H}}$), 1593 and 1491($\nu_{\text{C=C}}$), 1229($\nu_{\text{P=O}}$), 1229($\nu_{\text{C-O}}$), 1084($\nu_{\text{P-O}}$); ¹H-NMR (CD₃OD): δ 0.90(m, t, 3H; J = 12.53 Hz), 1.25-1.49(l, m, 26H), 1.72(k, m, 2H), 2.09(f, m, 2H), 2.78(d, s, 3H), 3.15(j, m, 2H), 3.76(e, m, 2H), 4.09(g, m, 2H), 4.25(c, m, 2H), 5.57-5.69(a, m, 2H), 5.90-6.03(b, m, 1H), 7.05-7.15, 7.19-7.38, 7.38-7.49(h and i, m, 5H) for CH₂^a=CH^bCH₂^cN(CH₃^d)(CH₂^jCH₂^k(CH₂^l)₁₃CH₃^m)CH₂^eCH₂^fCH₂^gOP(=O)(OH)(OC₆H₅^{h,i}); ¹³C-NMR (CDCl₃): δ 12.8(q), 22.7(p), 25.5(f and m), 29.5(n), 32.0(o), 39.2(d), 52.8(l), 58.4(e and g), 63.0(c), 120.2(i), 123.7(a), 126.0(k), 126.2(b), 129.4(j), 152.5(h) for

MS (m/z) (rel int): 510(100)[M-Br]⁺, 416(12)[M-Br-C₆H₅OH]⁺, 215(9)[CH₂CH₂CH₂OP(=O)(OH)(OC₆H₅)]⁺, 112(39) [CH₂=CHCH₂N(CH₃)CH₂]⁺, 84 (27) [CH₂=CHCH₂N(CH₃)CH₂]⁺, 41 (5) [CH₂=CHCH₂]⁺; HRMS (FAB+) [M-Br]⁺: obsd. 510.3708 [100.0, (calcd. 510.3710 for C₂₉H₅₃O₄N₁P₁)].

3.2.6 Synthesis of quaternary ammonium bromide salt of PPh-18-QAB

IR (cm⁻¹): 3415($\nu_{\text{O-H}}$), 2919 and 2850($\nu_{\text{C-H}}$), 1595 and 1492($\nu_{\text{C=C}}$), 1220($\nu_{\text{P=O}}$), 1220($\nu_{\text{C-O}}$), 1085($\nu_{\text{P-O}}$); ¹H-NMR (CD₃OD): δ 0.90(m, t, 3H; J = 13.14 Hz), 1.23-1.50 (l, m, 30H), 1.75(k, m, 2H), 2.08(f, m, 2H), 2.77(d, s, 3H), 3.15(j, m, 2H), 3.78(e, m, 2H),

4.09(g, m, 2H), 4.25(c, m, 2H), 5.57-5.70(a, m, 2H), 5.90-6.05(b, m, 1H), 7.05-7.15, 7.19-7.38, 7.38-7.49(h and i, m, 5H) for CH₂^a=CH^bCH₂^cN(CH₃^d)(CH₂^jCH₂^k(CH₂^l)₁₅CH₃^m)CH₂^eCH₂^fCH₂^gOP(=O)(OH)(OC₆H₅^{h,i}); ¹³C-NMR (CDCl₃): δ 14.1(q), 22.7(p), 25.4(f and m), 29.5(n), 31.9(o), 39.1(d), 52.8(l), 58.4(e and g), 63.0(c), 120.2(i), 123.6(a), 126.0(k), 126.4(b), 129.4(j), 152.7(h) for

MS (m/z) (rel int): 538(100)[M-Br]⁺, 444(44)[M-Br-C₆H₅OH]⁺, 215(10)[CH₂CH₂CH₂OP(=O)(OH)(OC₆H₅)]⁺, 112(35) [CH₂=CHCH₂N(CH₃)CH₂]⁺, 84 (44) [CH₂=CHCH₂N(CH₃)CH₂]⁺, 41 (4) [CH₂=CHCH₂]⁺; HRMS (FAB+) [M-Br]⁺: obsd. 538.4025 [100.0, (calcd. 538.4023 for C₃₁H₅₇O₄N₁P₁)].

3.3 Minimum inhibitory concentration (MIC) test on quaternary ammonium salts of alkylphosphates

The six synthesized compounds were antibacterial to varying degrees against all of the Gram-positive and Gram-negative bacteria, yeast, and fungi used in this work (Table 3). The mechanism of the antibacterial action of the synthesized compounds would be the same as that of quaternary ammonium salt-type antibacterial agents though the compounds are amphoteric surfactants with a phosphate group in their molecule. Many of quaternary ammonium salts are used as germicidal and disinfecting agent since they adsorb onto the negatively charged bacterium surface, thereby lowering the surface tension and the function of cell membrane⁵. The results of MIC test showed that PPh-10-QAB and PPh-12-QAB are highly antibacterial. The antibacterial spectrum was widest for PPh-8-QAB that has a moderate antibacterial activity. All compounds except PPh-16-QAB were effective against Gram-positive bacteria with MIC values ranging from 25 to 100 $\mu\text{g/mL}$, among which PPh-14-QAB was most effective against all Gram-positive bacteria with an MIC value of 25 $\mu\text{g/mL}$. The compounds having C₈, C₁₀, and C₁₂ hydrocarbon chains were effective against Gram-negative bacteria and the antibacterial activity decreased and the antibacterial spectrum widened with decreasing chain length. This would be due to the fact that the surface of Gram-negative bacteria is more hydrophilic than that of Gram-positive bacteria. Since the hydrophobicity of the compounds decreases with decreasing chain length, their interaction with the surface of Gram-negative bacterium surface would be stronger as the chain length decreases to affect the sensitivity of bacterium surface⁵. Similarly, the compounds were effective against yeast and fungi and the antibacterial spectrum widened with decreasing chain length though the antibacterial activity lowered. PPh-12-QAB was highly effective

Table 3 The MIC Values of PPh-*n*-QAB (*n*=8, 10, 12, 14, 16, 18).

Strain	MIC (μg/ml)					
	PPh-8-QAB	PPh-10-QAB	PPh-12-QAB	PPh-14-QAB	PPh-16-QAB	PPh-18-QAB
Gram-positive bacterium						
<i>S.aureus</i>	25	50	25	25	100	50
<i>B.subtilis</i>	50	50	50	25	>400	100
<i>M.luteus</i>	25	25	25	25	>400	50
Gram-negative bacterium						
<i>E.coli</i>	200	100	50	>400	>400	>400
<i>S.typhimurium</i>	400	>400	>400	>400	>400	>400
<i>Paeruginosa</i>	400	>400	>400	>400	>400	>400
Yeast						
<i>C.albicans</i>	400	200	200	>400	>400	>400
<i>S.cerevisiae</i>	>400	200	100	>400	>400	>400
Mold						
<i>T.mentagrophytes</i>	100	50	50	200	>400	>400
<i>M.gypseum</i>	100	50	50	200	>400	>400
<i>P.chrysogenum</i>	400	100	50	>400	>400	>400
<i>A.niger</i>	400	>400	>400	>400	>400	>400

against all microorganisms except *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Aspergillus niger*. Quaternary ammonium salt-type antibacterial agents are generally less active against yeast and fungi than against bacteria since the structure of the former is complex compared with that of the latter⁸⁻¹⁰. A reason why the compounds synthesized in this work showed a high antibacterial activity against yeast and fungi would be that the compounds have a quaternary ammonium group and a phosphate group in their molecule and are similar in structure to phospholipids, a constituent of cell membrane.

4 CONCLUSION

Six quaternary ammonium salt-type antibacterial agents were synthesized with the aim to develop oral antibacterial agents that are water-soluble and can keep their activity for long period of time in severe oral circumstances. Evaluations of their antibacterial activity in terms of minimum inhibitory concentration (MIC) test revealed that the activity depends on the hydrocarbon chain length in their molecule. This would be brought about by the fact that the structure of outer layer of microorganism differs from one species to another. PPh-12-QAB, among the six compounds synthesized, was shown to be highly effective against not only Gram-positive bacteria but also Gram-negative bacteria, yeast, and fungi. This agent was also effective against *Staphylococcus aureus* that causes aspiratory

pneumonia and *Escherichia coli*, an infectious bacterium inside the hospital, and hence, the agent is expected to be useful as oral antibacterial agent.

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