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Synthesis, characterization, and biological evaluation of new oxime-phosphazenes

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Abstract Hexachlorocylotriphosphazene (**1**) was reacted with 4-hydroxy-3-methoxybenzaldehyde to give hexakis[(4-formyl-2-methoxy)phenoxy]cyclotriphosphazene (**2**). Hexakis[(4-(hydroxyimino)2-methoxy)phenoxy]cyclotriphosphazene (**3**) was synthesized by reaction of **2** with hydroxlamine hydrochloride in pyridine. Compound **3** was reacted with benzyl chloride, acetyl chloride, allyl bromide, benzoyl chloride, propanoyl chloride, 4-methoxybenzoyl chloride, 2-chlorobenzoyl chloride, chloroacetyl chloride, methyl iodide, and thiophene-2-carbonyl chloride. From these reactions, full or partially substituted compounds were obtained, usually in high yields. Pure or defined products could not be obtained from reaction of **3** with methacryloyl chloride and *O*-acetylsalicyloyl chloride. The structures of the compounds were determined by elemental analysis, and IR, ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy. The synthesized compounds were screened for in-vitro antimicrobial activity against two Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*), two gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumonia*), and fungal strains (*Aspergillus niger*, and *Candida albicans*) by the agar well diffusion method. Few compounds had significant activity against both Gram-positive and Gram-negative bacteria. None of the compounds had antifungal activity except compounds **7** and **9**, which had moderate activity.

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Introduction

Phosphazenes are compounds that contain a framework of alternating phosphorus and nitrogen atoms, either in cyclic or linear form. Cyclophosphazenes are an important family of inorganic ring systems which have traditionally received attention for two main reasons:

1. to obtain small-molecule phosphazene derivatives; and
2. to produce polymeric phosphazene derivatives.

They are important in the chemistry of heteroatom compounds [1]. In recent years, phosphazene polymers have attracted substantial attention because they can be tailored to have a wide variety of physical and chemical properties by changing the side groups [2]. Phosphazenes have several biomedical properties and applications, for example strong antitumor activity [3–7]. Their antimicrobial and biological activity against bacterial and yeast cells have also been studied [8–10]. Other applications include model compounds for polyphosphazenes, starting materials for the preparation of cyclolinear and/or cyclomatrix phosphazene substrates, commercial polymers with carbon backbones containing pendant cyclophosphazene groups, inorganic hydraulic fluids and lubricants, biologically important substrates, for example anticancer agents, insect chemosterilants, pesticides and fertilizers, supports for catalysts, dyes, crown ether phase-transfer catalysts for nucleophilic substitution reactions, core substrates for dendrimers, thermal initiators for anionic polymerization reactions, and photosensitive materials [11].

Oximes are imines with the general formula $R^1R^2C{=}NOH$, where R^1 is an organic side chain and R^2 may be hydrogen, forming an aldoxime, or another organic group, forming a ketoxime. Oximes can be synthesized by condensation of an aldehyde or a ketone with hydroxylamine. Condensation of aldehydes with hydroxylamine gives aldoximes; ketoximes are produced from ketones and hydroxylamine. Oxime and dioxime derivatives are very important compounds in the chemical industry and in medicine [12]. For example, Oxime compounds are used as antidotes for nerve agents. A nerve agent inactivates acetylcholinesterase molecules by phosphorylation of the molecule. Oxime compounds can reactivate acetylcholinesterate by attaching to the phosphorus atom and forming an oxime-phosphonate which then splits away from the acetylcholinesterase molecule. Pralidoxime (also known as 2-PAM), obidoxime, and methoxime are examples for the most effective oxime nerve-agent antidotes [13]. Some oximes and their alkyl, oxyalkyl and amino derivatives have physiological and biological activity [14, 15].

The literature contains reports on the synthesis of different linear, cyclic, or polyphosphazenes [16–26]. There are also a large number of literature reports on the reactions of functional groups on phosphazene substituents [27]. Typical examples of these include coupling reactions of trimeric phosphazene azides with aryloxy, alkoxy, and dialkylamino cosubstituents [28], *N*-vinylic phosphazenes with

azodicarboxylic and acetylenic esters [29], oxime-phosphazene derivatives with alkyl and acyl substituents [30–33], and polymers from 4-formylphenoxy [34, 35], maleic [36], and 3,4-methylenedioxyphenoxy substituents [37].

Infections caused by multi-drug resistant bacteria are of major health concern worldwide. Particularly important are infections caused by the Gram-positive bacteria *Staphylococcus aureus* and species of the genus *Enterococcus*, because of increasing incidence of infections caused by these microorganisms in hospitals and communities, and their ability of developing antibiotic resistance to multiple antibiotics. Because of serious side effects of newly introduced antibacterial agents, for example the semi-synthetic streptogramins quinupristin/dalfopristin and daptomycin, development of diverse series of antimicrobials remains a necessity [38]. We planned the synthesis of some new phosphazene derivatives bearing different oximes, because phosphazene compounds and oxime derivatives have important biological properties as medical agents. Such derivatives could have interesting and useful biological properties. We recently synthesized oxime-phosphazene derivatives. For synthesis of these compounds, we used full and partially substituted products of hexachlorocyclotriphosphazene [30–33]. In this study we synthesized a new oxime-phosphazene, hexakis[(4-(hydroxyimino)2-methoxy)phenoxy]cyclotriphosphazene (**3**), by reaction of hexakis[(4-formyl-2-methoxy)phenoxy]cyclotriphosphazene (**2**) with hydroxylamine hydrochloride in pyridine. We also studied the reactions of **3** with different alkyl or acyl halides. All the synthesized compounds were tested for antibacterial and antifungal activity.

Experimental

General remarks

Solvents and other liquids used in the experimental work were dried by conventional methods. Hexachlorocyclotriphosphazene [$N_3P_3Cl_6$] (**1**) was recrystallized from hexane. Other chemicals were used as purchased. Hexakis[(4-formyl-2-methoxy)phenoxy]cyclotriphosphazene (**2**) was prepared as described by Tumer et al. [39]. Reaction of [$N_3P_3Cl_6$] with the phenol was performed under dry nitrogen. IR spectra were recorded on an Perkin Elmer FTIR spectrometer. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded by use of a Bruker DPX-300 spectrometer operating at 300.13, 75.46, and 121.49 MHz, respectively. The ^{1}H and ^{13}C NMR chemical shifts were measured by using $SiMe_4$ as internal standard whereas those for ^{31}P were measured by using 85 % H_3PO_4 as external standard. For the NMR studies, the DMSO-d was used as solvent for the compounds **2**, **5**, **7**, **8**, **9**, **10**, **11**, and **13**. Acetone-d_6 was used as solvent for the compounds **3**, **4**, **6**, and **12**. Chemical shifts downfield from the standard were assigned positive δ values. In the results of NMR spectra, s, d, t, and m indicate singlet, doublet, triplet, and multiplet, respectively. Microanalysis was performed with a Leco 932 CHNS-O instrument.

*Synthesis of compound **2***

A mixture of **1** (1.5 g, 4.31 mmol), 4-hydroxy-3-methoxy benzaldehyde (8.6 g, 51.80 mmol), and K_2CO_3 (7.16 g, 51.80 mmol) in THF (250 mL) was stirred at 0 °C and then reacted at ambient temperature for 48 h. The solvent was removed under vacuum. The residue was extracted with CH_2Cl_2 (4 × 25 mL). After removal of the solvent a white solid (**2**) was obtained (3.82 g, 85 %). Anal. Calcd. for $C_{48}H_{42}N_3O_{18}P_3$ (MW = 1041.78): C, 55.34; H, 4.06; N, 4.03. Found: C, 55.14; H, 3.98; N, 3.88 %. IR (cm^{-1}): 1693 $\nu_{C=O}$, 1187 $\nu_{P=N}$, 1285 ν_{P-N-P}, 950 ν_{P-O-C}. ^{31}P NMR: 7.82 (s). ^{1}H NMR: 9.82 (6 H, s, H(8)), 7.12–7.50 (18 H, m, H(3), H(5), H(6)), 3.80 (18 H, s, H(7)), 2.45 and 3.30 (DMSO-d). ^{13}C NMR: 190.18 C(8), 116.8 C(4), 115.25 C(2), 115.66 C(1), 113.22 C(5), 112.18 C(6), 111.62 C(3), 56.30 C(7), 39.98 (DMSO-d). 2.45 and 3.30 (DMSO-d).

*Synthesis of compound **3***

A mixture of **2** (5 g, 4.80 mmol) and hydroxylamine hydrochloride (2 g, 28.80 mmol) was heated under reflux in pyridine (7 mL) for 3 h. After the reaction was complete, the mixture was left to cool and was slowly poured into water (100 mL) and reprecipitated twice from water. The white solid **3** was washed with alcohol–*n*-hexane and dried at 50 °C under vacuum. After removal of the solvent a white solid (**3**) was obtained (4.34 g, 80 %). Anal. Calcd. for $C_{48}H_{48}N_9O_{18}P_3$ (MW = 1131.87): C, 50.93; H, 4.27; N, 11.14 %. Found: C, 50.52; H, 3.99; N, 10.97 %. IR (cm^{-1}): 3304 ν_{OH}, 1188 $\nu_{P=N}$, 1599, 1587, 1510 $\nu_{C=N,\ C=C}$, 951 ν_{P-O-C}. ^{31}P NMR: 8.71(s). ^{1}H NMR: 10.42 (6 H, s, H(9)), 8.10 (6 H, s, H(8)), 6.88–7.20 (18 H, m, H(3), H(5), H(6)), 3.76 (18 H, s, H(7)), 1.92 and 2.70 (acetone-d_6). ^{13}C NMR: 150.92 C(8), 147.80 C(1)·140.73 C(2), 130.49 C(4), 121.28 C(5), 119.25 C(6), 109.72 C(3), 55.00 C(7), 29.38 (acetone-d_6).

*Reaction of **3** with benzyl chloride*

A solution of benzyl chloride (0.35 mL, 0.34 g, 2.65 mmol) in acetone (10 mL) was slowly added dropwise to a stirred and cooled (0–5 °C) mixture of **3** (0.50 g, 0.44 mmol) and K_2CO_3 (0.74 g, 5.30 mmol) in acetone (30 mL). The reaction was conducted at room temperature for 1 h then the mixture was heated under reflux for 48 h. After the reaction was complete, the precipitate was isolated by filtration and the solvent was removed. The product was dissolved in a very small amount of acetone and was precipitated with hexane. The solvent was removed under vacuum over 96 h. The clear yellow solid **4** was obtained (0.37 g, 60 %). Anal. Calcd. for $C_{69}H_{66}O_{18}N_9P_3$, (MW = 1402.23). C, 59.10; H, 4.74; N, 8.99 %. Found: C, 58.88; H, 4.45; N, 8.48 %. IR (cm^{-1}): 3290 ν_{OH}, 3060, 3027 ν_{Ar-C-H}, 2934, 2868 $\nu_{C-H\ (Aliphatic)}$, 1509, 1584, 1598 cm^{-1} $\nu_{C=N,C=C}$, 1188 $\nu_{P=N}$, 950 ν_{P-O-C} .^{31}P NMR: 8.96(s). ^{1}H NMR: 10.40 (3 H, s, H(14)), 8.14 (3 H, s, H(8)), 8.11 (3 H, s, H(8′)), 6.82–7.57 (33 H, m, H(3), H(3′), H(5), H(5′), H(6), H(6′), H(11), H(12), H(13)), 5.25 (6 H, s, H(9)), 3,75 (9 H, s, H(7′)), 3.72 (9 H, s, H(7)), 1.91 and 2.60 (acetone-d_6). ^{13}C NMR: 150,90 C(8′), 150,87 C(8), 148,10 C(1), 148,04 C(1′), 137,89 C(2),

137,87 C(2′), 129,53 C(4), 129,49 C(4′), 128,27 C(13), 128,13 C(11), 128,08 C(10), 127,57 C(12), 121,22 C(5), 121,17 C(5′), 119,39 C(6), 119,35 C(6′), 109,99 C(3), 109,93 C(3′), 75.62 C(9), 55.16 C(7′), 55.12 C(7), 29.37 (acetone-d_6).

*Reaction of **3** with acetyl chloride*

A solution of acetyl chloride (0.40 mL, 0.45 g, 5.80 mmol) in THF (10 mL) was slowly added dropwise to a stirred and cooled (0–5 °C) mixture of **3** (0.80 g, 0.84 mmol) and triethylamine (1.55 g, 11.25 mmol) in THF (30 mL). The reaction was conducted at room temperature for 24 h. When reaction was complete, the mixture was slowly poured into water (50 mL) and reprecipitated twice from water. A fawn solid **5** was obtained (0.31 g, 86 %). Anal. Calcd. for $C_{60}H_{60}O_{24}N_9P_3$, (MW = 1384.09). C, 52.07; H, 4.37; N, 9.11 %. Found: C, 51.95; H, 3.97; N, 9.01 %. IR (cm^{-1}): 1766 $\nu_{C=O}$, 1193 $\nu_{P=N}$, 952 ν_{P-O-C}, 3000, 3071 ν_{ArC-H}, 2967, 2937 $\nu_{C-H\ (Aliphatic)}$, 1509, 1599, 1587, 1610 $\nu_{C=N,\ C=C}$. ^{31}P NMR: 8.46(s). ^{1}H NMR: 2.16 (18 H, s, H(10)), 8.53 (6 H, s, H(8)), 6.9–7.33 (18 H, m, H(3), H(5), H(6)), 3.73 (18 H, s, H(7)), 2.46 and 3.30 (DMSO-d). ^{13}C NMR: 168.56 C(9), 155.89 C(8), 150.75 C(1), 141.76 C(2)' 128.00 C(4), 121.40 C(5), 120.56 C(6), 111.32 C(3), 56.00 C(7), 19.70 C(10), 39.98 (DMSO-d).

*Reaction of **3** with allyl bromide*

A solution of allyl bromide (0.2 mL, 0.30 g, 2.30 mmol) in acetone (10 mL) was slowly added dropwise to a stirred and cooled (0–5 °C) mixture of **3** (0.42 g, 0.37 mmol) and K_2CO_3 (0.62 g, 4.50 mmol) in acetone (30 mL). The reaction was conducted at room temperature for 1 h then the mixture was heated under reflux for 24 h. When the reaction was complete, the precipitate formed was isolated by filtration and the solvent was removed. The product was dissolved in a very small amount of acetone and was precipitated with hexane. The solvent was removed under vacuum over 96 h. The yellow solid **6** was obtained (0.43 g, 84 %). Anal. Calcd. for $C_{66}H_{72}\ O_{18}N_9P_3$, (MW = 1372.25). C; 57.77; H, 5.29; N, 9.19 %. Found: C, 57.63; H, 5.14; N, 9.34 %. IR (cm^{-1}): 1188 $\nu_{P=N}$, 951 ν_{P-O-C}, 3010, 3079 ν_{ArC-H}, 2968, 2921, 2851 $\nu_{C-H\ (Aliphatic)}$, 1509, 1599, 1582, 1643 $\nu_{C=N,\ C=C}$. ^{31}P NMR: 8.99(s). ^{1}H NMR: 8.18 (6 H, s, H(8)), 6.8–7.38 (18 H, m, H(3), H(5), H(6)), 6.08 (6 H, m, H(10)), 5.35 (6 H, d, *J* 16.40, $H(11)_{trans,}$ 5.20 (6 H, d, *J* 10.35, $H(11)_{cis}$, 4.69 (12 H, d, J 5.64, H(9)) 3.81 (18 H, s, H(7)), 1.90 and 2.74 (acetone-d_6). ^{13}C NMR: 150.90 C(8), 147.84 C(1), 141.01 C(2), 134.50 C(10), 129.60 C(4), 121.26 C(5), 119.40 C(6), 116.40 C(11), 109.75 C(3), 74.50 C(9), 55.00 C(7), 29.37 (acetone-d_6).

*Reaction of **3** with benzoyl chloride*

A solution of benzoyl chloride (0.4 mL 0.44 g, 3.2 mmol) in THF (10 mL) was slowly added dropwise to a stirred and cooled (0–5 °C) mixture of **3** (0.3 g, 0.27 mmol) and triethylamine (0.66 mL, 0.48 g, 4.77 mmol) in THF (30 mL). The reaction was performed at room temperature for 24 h. When the reaction was

complete, the mixture was slowly poured into water (50 mL) and reprecipitated twice from water. A white solid **7** was obtained (0.34 g, 73 %). Anal. Calcd. for $C_{83}H_{68}O_{23}N_9P_3$, (MW = 1652.40). C, 60.33; H, 4.15; N, 7.63 %. Found: C, 60.02; H, 3.99; N, 7.45 %. IR (cm^{-1}): 3285 ν_{OH}, 1746 $\nu_{C=O}$, 1189 $\nu_{P=N}$, 3010, 3065 $\nu_{C-H(Ar)}$, 2972, 2938, 2603 $\nu_{C-H\ (Aliphatic)}$, 1508, 1599, 1583, 1610 $\nu_{C=N,\ C=C}$, 950 ν_{P-O-C}. ^{31}P NMR: 8.50(s). ^{1}H NMR: 10.61 (1H, s, H(14)), 7.15–7.99 (33 H, m, H(3), H(3′), H(5), H(5′), H(6), H(6′), H(11), H(12), H(13)), 3.76–3,78 (18 H, s, H(7), H(7′)), 8.82–8,79 (6 H, s, H(8), H(8′)), 2.45 and 3.32 (DMSO-d). ^{13}C NMR: 167.60 C(9), 157.46 C(8), 157.41 C(8′), 150.85 C(1), 150.82 C(1′), 142.77 C(2), 142.73 C(2′), 133.13 C(13), 131.02 C(10), 129.50 C(11), 129.18 C(12), 128.83 C(4), 128.80 C(4′), 121.72 C(5), 121.69 C(5′), 118.60 C(6), 118.58 C(6′), 111.47 C(3), 111.44 C(3′), 56.59 C(7), 56.09 C(7′), 39.97 (DMSO-d).

*Reaction of **3** with propanoyl chloride*

A solution of propanoyl chloride (0.35 mL, 0.35 g, 4.0 mmol) in THF (10 mL) was slowly added dropwise to a stirred and cooled (0–5 °C) mixture of **3** (0.4 g, 0.35 mmol) and triethylamine (0.60 mL, 0.42 g, 4.24 mmol) in THF (30 mL). The reaction was performed at room temperature for 48 h. When the reaction was complete, the mixture was slowly poured into water (50 mL) and reprecipitated twice from water. A white solid **8** was obtained (0.39 g, 76 %). Anal. Calcd. for $C_{66}H_{72}O_{24}N_9P_3$, (MW = 1468.24). Calcd.: C, 53.99; H, 4.94; N: 8.59 %. Found: C, 53.52; H, 4.61; N, 8.27 %. IR (cm^{-1}): 1764 $\nu_{C=O}$, 1190 $\nu_{P=N}$, 3000, 3070 $\nu_{C-H(Ar)}$, 2980, 2941 $\nu_{C-H\ (Aliphatic)}$, 1509, 1598, 1584 $\nu_{C=N,\ C=C}$, 950 ν_{P-O-C}. ^{31}P NMR: 8.55(s). ^{1}H NMR: 8.53 (6 H, s, H(8)), 6.95–7.30 (18 H, m, H(3), H(5), H(6)), 2.5 (12 H, m, H(10)), 2.13–3.81 (18 H, s, H(7)), 1.00–1.18 (18 H, t, H(11)), 2.47 and 3.32 (DMSO-d). ^{13}C NMR: 171.60 C(9), 155.99 C(8), 150.77 C(1), 141.72 C(2), 137.34 C(4), 128.05 C(5), 121.40 C(6), 111.33 C(3), 56.50 C(7), 25.70 C(10), 9.10 C(11), 39.97 (DMSO-d).

*Reaction of **3** with 4-methoxybenzoyl chloride*

A solution of 4-methoxybenzoyl chloride (0.45 g, 2.65 mmol) in acetone (10 mL) was slowly added dropwise to a stirred and cooled (0–5 °C) mixture of **3** (0.4 g, 0.35 mmol) and K_2CO_3 (0.58 g, 4.24 mmol) in acetone (30 mL). The reaction was performed at room temperature for 48 h. When the reaction was complete, the precipitate was isolated by filtration and the solvent was removed. The product was dissolved in a very small amount of acetone and was precipitated with hexane. The solvent was removed under vacuum over 96 h. A white solid **9** was obtained (0.54 g, 79 %). Anal. Calcd. for $C_{96}H_{84}O_{30}N_9P_3$, (MW = 1936.66). C, 59.54; H, 4.37; N, 6.51 %. Found: C, 59.06; H, 4.08; N, 6.12 %. IR (cm^{-1}): 1789 $\nu_{C=O}$, 1190 $\nu_{P=N}$, 3010, 3065 $\nu_{C-H(Ar)}$, 2930, 2845 $\nu_{C-H\ (Aliphatic)}$, 1606, 1579, 1510 $\nu_{C=N,\ C=C}$, 950 ν_{P-O-C}. ^{31}P NMR: 8.62(s). ^{1}H NMR: 8.74 (6 H, s, H(8)), 3.84 (18 H, s, H(7)), 3.78 (18 H, s, H(14)), 6.94–8.04 (42 H, m, H(3), H(5), H(6), H(11), H(12)), 2.44 and 3.33 (DMSO-d). ^{13}C NMR: 167.31 C(9), 163.25 C(13), 156.97 C(8), 150.82 C(1),

141.84 C(2)' 132.98 C(11), 131.62 C(4), 128.04 C(10), 121.55 C(5), 120.37 C(6), 114.40 C(12), 111.38 C(3), 57.00 C(7), 56.00 C(14), 39.98 (DMSO-d).

*Reaction of **3** with 2-chlorobenzoyl chloride*

A solution of 2-chlorobenzoyl chloride (0.40 mL, 0.56 g, 3.20 mmol) in THF (10 mL) was slowly added dropwise to a stirred and cooled (0–5 °C) mixture of **3** (0.3 g, 0.27 mmol) and triethylamine (0.66 mL, 0.48 g, 4.77 mmol) in THF (30 mL). The reaction was performed at room temperature for 24 h. When the reaction was complete, the mixture was slowly poured into water (50 mL) and reprecipitated twice from water. A white solid **10** was obtained (0.23 g, 45 %). Anal. Calcd. for $C_{76}H_{60}O_{22}N_9P_3Cl_4$, (MW = 1686.07). C, 54.1; H, 3.59; N, 7.48 %. Found: C, 54.01; H, 3.48; N, 7.32 %. IR (cm^{-1}): 3330 ν_{OH}, 1757 $\nu_{C=O}$, 1190 $\nu_{P=N}$, 3000, 3070 $\nu_{C–H(Ar)}$, 2938, 2976 $\nu_{C–H\ (Aliphatic)}$, 1591, 1508 $\nu_{C=N,\ C=C}$, 950 $\nu_{P–O–C}$. ^{31}P NMR: 8.37(s). ^{1}H NMR: 10.3 (2 H, s, H(16)), 7.38–7.76 (34 H, m, H(3), H(3′), H(5), H(5′), H(6), H(6′), H(12), H(13), H(14), H(15)), 3.76–3.78 (18 H, s, H(7), H(7′)), 8.77–8.50 (6 H, s, H(8), H(8′)), 2.45 and 3.30 (DMSO-d). ^{13}C NMR: 162.88 C(9), 157.86 C(8), 151.80 C(8′), 150.88 C(1), 149.91 C(1′), 142.90 C(2), 141.68 C(2′), 140.50 C(11), 138.16 C(13), 133.84 C(15), 131.60 C(12), 130.29 C(4′), 129.20 C(4), 127.60 C(14), 125.40 C(10), 121.30 C(5), 121.55 C(5′), 119.35 C(6′), 118.57 C(6), 111.58 C(3), 109.88 C(3′), 56.57 C(7), 56.12 C(7′), 39.98 (DMSO-d).

*Reaction of **3** with chloroacetyl chloride*

A solution of chloroacetyl chloride (0.30 mL, 0.46 g, 3.67 mmol) in THF(10 mL) was slowly added dropwise to a stirred and cooled (0–5 °C) mixture of **3** (0.3 g, 0.27 mmol) and triethylamine (0.66 mL, 0.48 g, 4.77 mmol) in THF (30 mL). The reaction was performed at room temperature for 24 h. When the reaction was complete, the mixture was slowly poured into water (50 mL) and reprecipitated twice from water. A brown solid **11** was obtained (0.34 g, 80 %). Anal. Calcd. for $C_{60}H_{54}O_{24}N_9P_3Cl_6$, (MW = 1590.75). C, 45.30; H, 3.42; N, 7.92 %. Found: C, 45.03; H, 3.13; N, 7.77 %. IR (cm^{-1}): 1778 $\nu_{C=O}$, 1190 $\nu_{P=N}$, 3000, 3076 $\nu_{C–H(Ar)}$, 2938, 2976 $\nu_{C–H\ (Aliphatic)}$, 1598, 1587, 1509 $\nu_{C=N,\ C=C}$, 952 $\nu_{P–O–C}$. ^{31}P NMR: 8.17(s). ^{1}H NMR: 8.65 (6 H, s, H(8)), 6.83–8.15 (18 H, m, H(6), H(3), H(5)) 4.67 (12 H, s, H(10))' 3.75 (18 H, s, H(7)), 2.46 and 3.34 (DMSO-d). ^{13}C NMR: 168.88 C(9), 157.16 C(8), 150.90 C(1), 142.96 C(2), 130.61 C(4), 121.56 C(5), 118.89 C(6), 111.69 C(3), 56.09 C(7), 40.76 C(10), 39.97 (DMSO-d).

*Reaction of **3** with methyl Iodide*

A solution of methyl iodide (0.25 mL, 0.60 g, 4.18 mmol) in acetone (10 mL) was slowly added dropwise to a stirred and cooled (0–5 °C) mixture of **3** (0.5 g, 0.44 mmol) and K_2CO_3 (0.74 g, 5.30 mmol) in acetone (30 mL). The reaction was performed at room temperature for 1 h and was then heated under reflux for 24 h. When the reaction was complete, the precipitate was isolated by filtration and the solvent was removed. It was dissolved in a very small amount of acetone and was

precipitated with hexane. The solvent was removed under vacuum over 96 h. A clear yellow solid **12** was obtained (0.5 g, 93 %). Anal. Calcd. for $C_{54}H_{60}O_{18}N_9P_3$, (MW = 1216.02). C, 53.34; H, 4.97; N, 10.37 %. Found: C, 53.08; H, 4.73; N, 10.23 %. IR (cm^{-1}): 1188 $\nu_{P=N}$, 3000, 3080 $\nu_{C-H(Ar)}$, 2936, 2960 $\nu_{C-H\ (Aliphatic)}$, 1599, 1581, 1509 $\nu_{C=N,\ C=C}$, 951 ν_{P-O-C}. ^{31}P NMR: 8.94(s). ^{1}H NMR: 8.08 (6 H, s, H(8)), 6.90–7.24 (18 H, m, H(6), H(3), H(5)), 3.90 (18 H, s, H(9)), 3.80 (18 H, s, H(7)), 1.92 and 2.71 (acetone-d_6). ^{13}C NMR: 150.91 C(8), 147.49 C(1), 140.98 C(2), 129.62 C(4), 121.23 C(5), 119.65 C(6), 109.84 C(3), 61.00 C(9), 55.18 C(7), 29.38 (acetone-d_6).

*Reaction of **3** with thiophene-2-carbonyl chloride*

A solution of thiophene-2-carbonyl chloride (0.30 mL, 0.31 g, 2.12 mmol) in THF (10 mL) was slowly added dropwise to a stirred and cooled (0–5 °C) mixture of **3** (0.4 g, 0.35 mmol) and triethylamine (0.60 mL, 0.42 g, 4.24 mmol) in THF (30 mL). The reaction was performed at room temperature for 24 h. When the reaction was complete, the mixture was slowly poured into water (50 mL) and reprecipitated twice from water. A clear yellow solid **13** was obtained (0.49 g, 77 %). Anal. Calcd. for $C_{68}H_{56}O_{22}N_9P_3S_4$, (MW = 1572.41). C, 51.94; H, 3.59; N, 8.02 %. Found: C, 51.75; H, 3.37; N, 7.53 %. IR (cm^{-1}): 3290 ν_{OH}, 1736 $\nu_{C=O}$, 1186 $\nu_{P=N}$, 3010, 3087 $\nu_{C-H(Ar)}$, 2936, 2850 $\nu_{C-H\ (Aliphatic)}$, 1626, 1597, 1511 $\nu_{C=N,C=C}$, 952 ν_{P-O-C}. ^{31}P NMR: 8.14(s). ^{1}H NMR: 10.60 (2 H, s, H(14)), 8.80 (6 H, s, H(8)· H(8′)), 6.88–8.02 (30 H, m, H(3), H(5), H(6), H(3′), H(5′), H(6′), H(11), H(12), H(13)), 3.75 (18 H, s, H(7), H(7′)), 2.46 and 3.35 (DMSO-d). ^{13}C NMR: 163.19 C(9), 158.91 C(8), 151.02 C(8′), 150.93 C(1), 148.93 C(1′), 142.64 C(2), 141.94 C(2′), 134.82 C(10), 133.51 C(11), 131.23 C(4), 130.40 C(4′), 121.42 C(5), 121.30 C(5′), 128.72 C(13), 125.42 C(12), 119.20 C(6′), 118.51 C(6), 109.65 C(3′), 109.09 C(3), 56.58 C(7), 56.11 C(7′), 39.98 (DMSO-d).

In-vitro antimicrobial assay

The bacterial pathogens *S. aureus*, *E. faecalis*, *Escherichia coli*, and *Klebsiella pneumonia* and the fungi *A. niger* and *C. albicans* were used for antimicrobial activity studies. The synthesized compounds were evaluated for antimicrobial activity by the agar well diffusion method [40, 41]. The bacteria and fungi were tested on nutrient agar and Sabouraud dextrose agar media, respectively. The medium was sterilized by autoclaving at 120 °C. Approximately 30 ml agar medium with the respective strains of bacteria and fungi was transferred aseptically into each sterilized Petri dish. The dishes were left at room temperature for solidification. Wells of diameter 6 mm were made by use of a sterile cork borer. The test drugs were added at concentrations of 0 (control), 25, 50, 75, and 100 μg/mL. Ciprofloxacin (30 μg/mL) and fluconazole (30 μg/mL) were used as positive controls. The antibacterial assay plates were incubated at 37 ± 2 °C for 24 h, antifungal assay plates were incubated at 28 ± 2 °C for 48 h. After the incubation period, the diameter of the inhibition zone was measured as an indicator of the

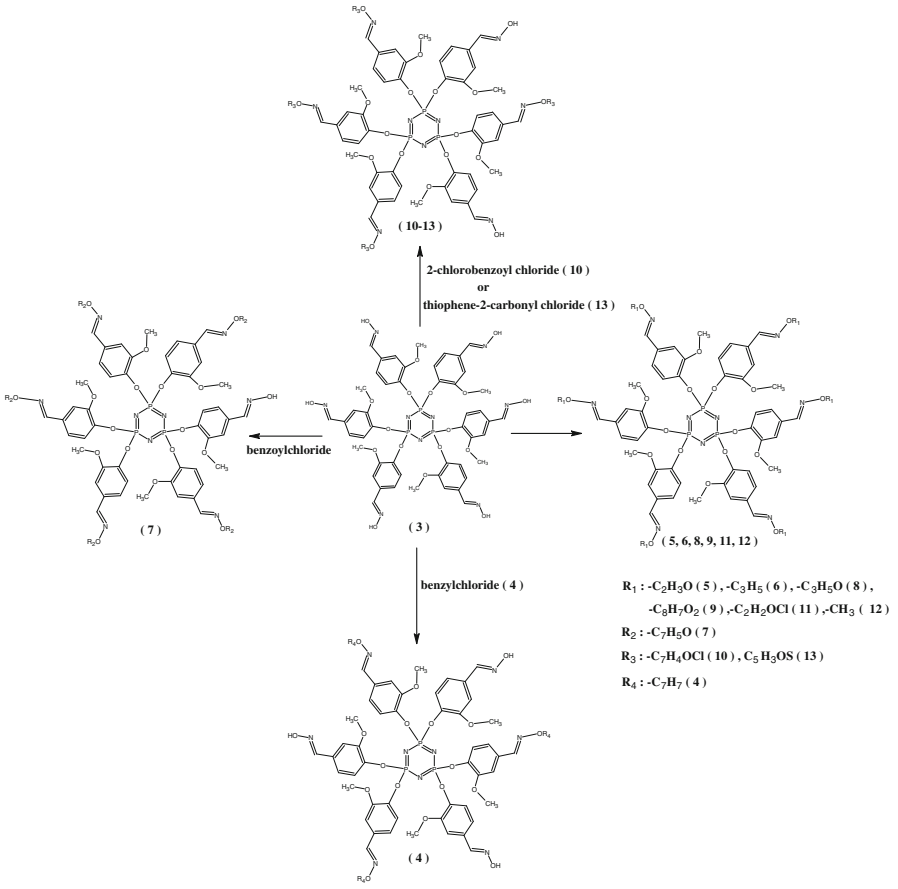
activity of the compounds. Dimethyl sulfoxide was used both as a solvent and as a negative control. No inhibition zone was observed in the control (i.e. for DMSO).

Results and discussion

Reaction of **1** with 6 equiv. 4-hydroxy-3-methoxybenzaldehyde in the presence of K_2CO_3 in THF gave hexakis[(4-formyl-2-methoxy)phenoxy]cyclotriphosphazene (**2**) [39]. New oxime-phosphazene hexakis[(4-(hydroxyimino)2-methoxy)phenoxy]cyclotriphos-phazene (**3**) was synthesized by reaction of **2** with hydroxylamine hydrochloride in pyridine. Hexasubstituted compounds were obtained by reaction of **3** with allyl bromide, 4-methoxybenzoyl chloride, and methyl iodide in acetone in the presence of K_2CO_3 via replacement of all the oxime protons with alkyl or acyl groups. Hexasubstituted compounds were obtained by reaction of **3** with acetyl chloride, propanoyl chloride, and chloroacetyl chloride in tetrahydrofuran in the presence of triethylamine, via replacement of all the oxime protons with acyl groups. However, reaction with **3** also furnished a trisubstituted compound (with benzyl chloride), tetrasubstituted compounds (with 2-chlorobenzoyl chloride and thiophene-2-carbonyl chloride) and a pentasubstituted compound (with benzoyl chloride). Pure and defined products could not be obtained by reaction of **3** with methacryloyl chloride and *O*-acetylsalicyloyl chloride. All products were generally in high yields. The structures of the compounds were determined by elemental analysis, and IR and ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy. A general presentation of the reactions is shown in Scheme 1, and the structures of the compounds **3–13** are shown in Scheme 2.

The characteristic stretching peaks in the IR spectra of the phosphazenes have been assigned in the Experimental section. The P=N stretching vibrations, observed between 1,186 and 1,193 cm^{-1}, are characteristic of cyclophosphazenes. Compared with **1**, which appeared at 1,218 cm^{-1}, these peaks are shifted to longer wavelengths for **2–13**. The presence of OH stretching vibration and the absence of carbonyl bands in the IR spectra of **3** indicates the oxime compound. The absence of the OH stretching vibration in the IR spectra of **5**, **6**, **8**, **9**, **11**, and **12** indicates that all the hydrogen atoms of the OH groups have been replaced by alkyl or acyl substituents. However; the presence of OH stretching vibration in the IR spectra of **4**, **7**, **10**, and **13** indicates that not all the hydrogen atoms of the OH groups could be replaced by benzyl, benzoyl, 2-chlorobenzoyl, and thiophene-2-carbonyl substituents respectively. C=N, C=C stretching vibrations were observed between 1508 and 1643 cm^{-1} in the spectra of **3–13**. The carbonyl bands for **2**, **5**, **7**, **8**, **9**, **10**, **11**, and **13** were observed at 1693, 1766, 1746, 1764, 1789, 1757, 1778, and 1736 cm^{-1} respectively.

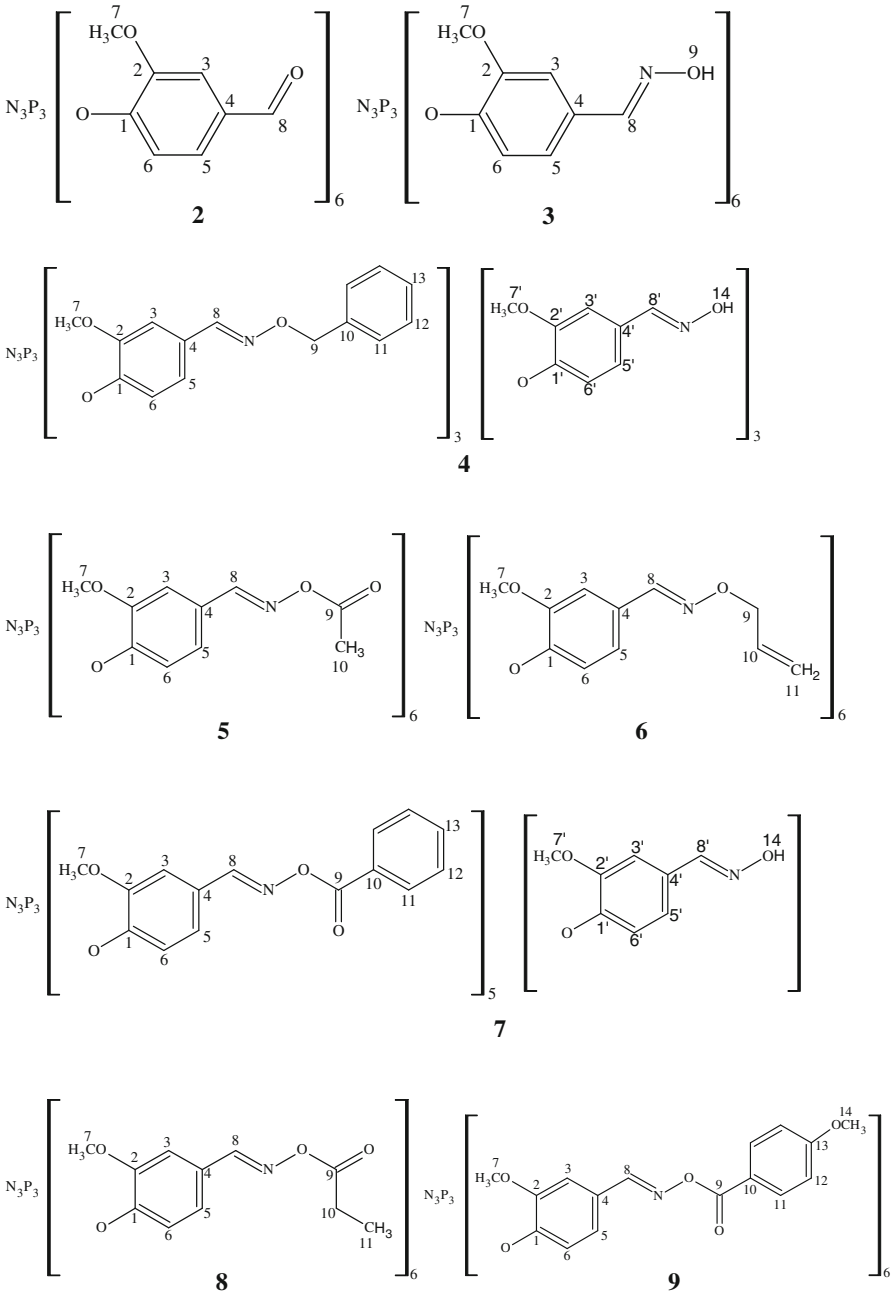
The NMR data of compounds **2–13** are given in Experimental section. Typical spectra (^{13}C and ^{31}P NMR spectra of compound **3**) are presented in Figs. 1 and 2. In the ^{31}P NMR spectra of **2–13**, one signal was observed as singlet. Although there are different phosphorus environments in the molecules of **4**, **7**, **10**, and **13**, the main peak is observed as a singlet. It is understood that the phosphorus peaks are not affected by these changes because the substituted groups are far from the



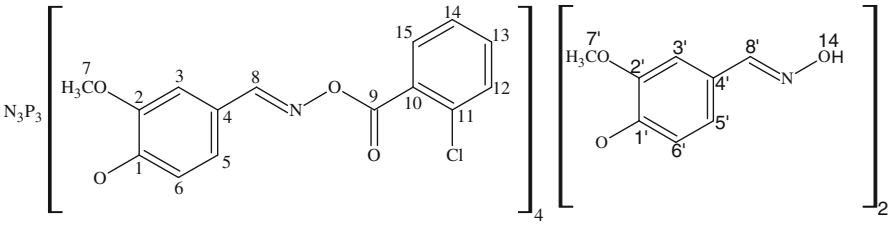
Scheme 1 General presentation of the reactions

phosphorus atoms in these compounds. The ^{31}P NMR shifts of **2–13** change between 7.82 and 8.99 ppm as a singlet. These data indicate that compounds **2–13** have one isomer. However, in our similar published studies, we also observed weak peaks arising because of the *syn* and *anti* isomerism of the –C=N groups, so we obtained compounds that are mixtures of *syn* and *anti* isomers from the reactions of hexakis(4-[(hydroxyimino)methyl]phenoxy)cyclotriphosphazene and hexakis(4-[(1)-*N*-hydroxyethanimidoyl] phenoxy)cyclotriphosphazene with different alkyl and acyl halogens [30, 31]. It may be that minor isomers were removed during purification, so we observed one isomer only in this study.

The ^{1}H and ^{13}C NMR data also confirm the structures of **3–13** (Scheme 2). Detailed ^{1}H NMR spectral data are given in the Experimental section. In the ^{1}H NMR spectra, the aldehyde proton for **2** was observed at 9.86 ppm. The azomethine protons for **3–13** were observed between 8.02 and 8.82 ppm. In the ^{1}H NMR spectra, OH protons were observed at 10.42, 10.40, 10.61, 10.30, and 10.60 ppm for **3**, **4**, **7**, **10**, and **13**, respectively. It was apparent from the integral intensities that there are six OH protons in **3**, which is the original oxime-



Scheme 2 The structures of compounds **2–13**



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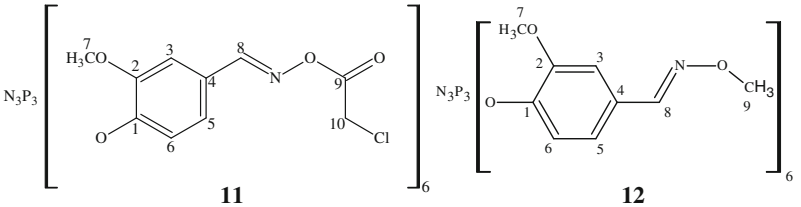
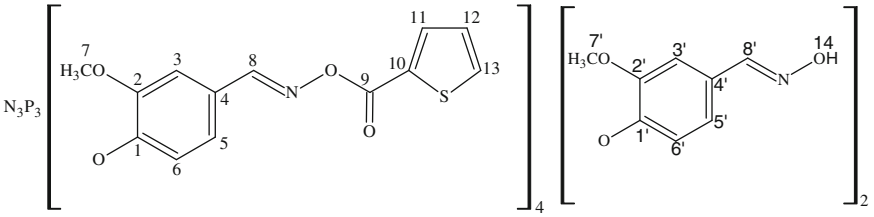


11 **12**



13

Scheme 2 continued

phosphazene, three OH protons in **4**, one OH proton in **7**, two OH protons in **10** and two OH protons in **13**. These observations for **4**, **7**, **10**, and **13** indicate that the benzyl, benzoyl, 2-chlorobenzoyl, and thiophene-2-carbonyl groups have not replaced all the OH protons in **4**, **7**, **10**, and **13** respectively.

The detailed ^{13}C NMR spectral data are given in the Experimental section. All of the carbon peaks in the compounds are visible in the ^{13}C NMR spectra, as expected. The aldehyde carbon atom was observed at 191.2 ppm for **2**. The azomethine protons for **3–13** were observed at between 150.87 and 158.91 ppm. The carbonyl carbon for **5**, **7**, **8**, **9**, **10**, **11**, and **13** were observed 168.56, 167.60, 171.60, 167.31, 162.88, 168.88, and 163.19 ppm respectively.

The newly synthesized phosphazenes were screened for their in-vitro antimicrobial activity against two Gram-positive bacteria, *S. aureus* and *Enterococcus faecalis*, two Gram-negative bacteria, *E. coli* and *K. pneumoniae*, and the fungal

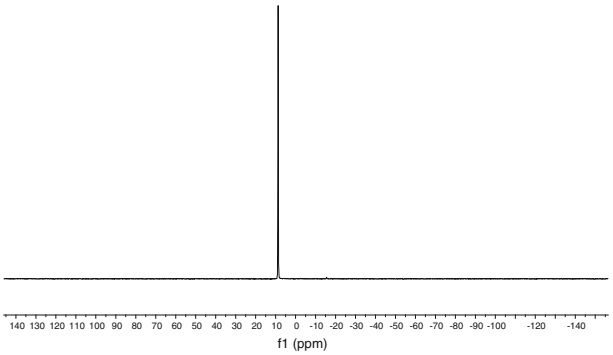


Fig. 1 ^{31}P NMR spectrum of compound **3**

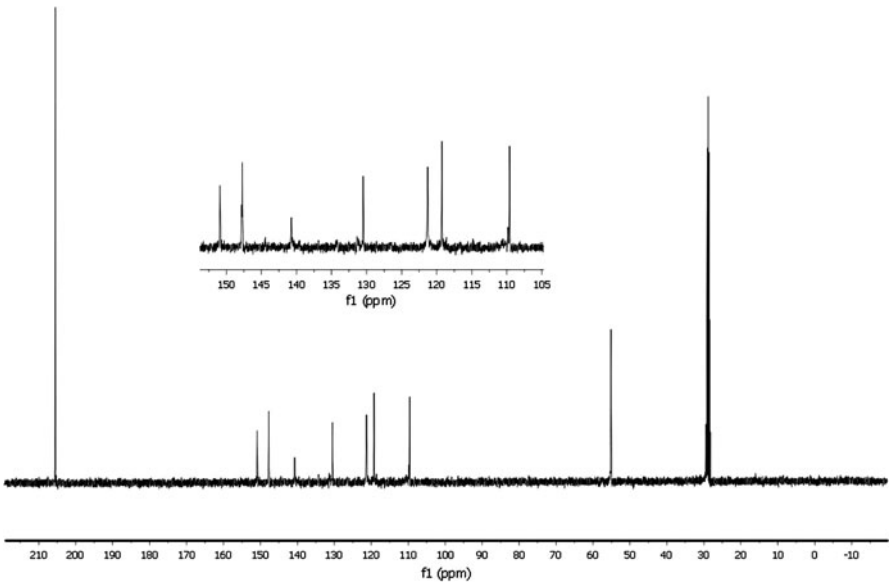


Fig. 2 ^{13}C NMR spectrum of compound **3**

strains *Aspergillus niger* and *Candida albicans*, by the agar well diffusion method using ciprofloxacin and fluconazole as control drugs for antibacterial and antifungal activity, respectively. The pathogens were cultured on the nutrient agar medium (antibacterial activity) and Sabouraud dextrose agar (antifungal activity). DMSO was used as solvent and as negative control. The susceptibility was assessed on the basis of the diameters of zones of inhibition against the organisms. Inhibition zones were measured and compared with those for ciprofloxacin for antibacterial activity and fluconazole for antifungal activity. The diameters of zones of inhibition, measured in mm, for antibacterial and antifungal tests are listed in Table 1. Minimum inhibitory concentrations (MIC) are listed in Table 2. It is apparent the compounds had different inhibitory activity against these microorganisms. The

Table 1 Antibacterial and antifungal activity of the synthesized compounds (in-vitro activity zone of inhibition in mm)

Compd. no.	Gram-positive		Gram-negative		Fungi	
	S. aureus	*E. faecalis*	*E. coli*	*K. pneumonia*	*A. niger*	*C. albicans*
2	13	15	17	16	5	4
3	25	24	23	26	7	8
4	18	20	18	19	6	10
5	21	21	20	19	4	6
6	26	25	24	26	7	11
7	27	30	28	29	16	18
8	15	16	14	17	5	3
9	24	23	22	26	6	8
10	22	19	20	23	8	6
11	20	18	21	22	5	10
12	26	25	24	27	8	12
13	28	29	27	30	18	19
Ciprofloxacin	29	30	28	31	–	–
Fluconazole	–	–	–	–	20	23
Control	–	–	–	–	–	–

– no activity

Table 2 Minimum inhibitory concentration (μg/mL)

Compd. no.	Gram-positive		Gram-negative		Fungi	
	S. aureus	*E. faecalis*	*E. coli*	*K. pneumonia*	*A. niger*	*C. albicans*
2	100	100	100	100	>100	>100
3	25	25	25	25	>100	>100
4	50	50	50	50	>100	>100
5	50	50	50	50	>100	>100
6	25	25	25	25	>100	>100
7	25	25	25	25	75	75
8	100	100	100	100	>100	>100
9	25	25	25	25	>100	>100
10	50	50	50	50	>100	>100
11	50	50	50	50	>100	>100
12	25	25	25	25	>100	>100
13	25	25	25	25	75	75
Ciprofloxacin	12.5	12.5	12.5	12.5	–[a]	–
Fluconazole	–	–	–	–	12.5	12.5

[a] No activity

efficacy of compounds **7** and **13** was almost equal to that of the standard drug ciprofloxacin against *E. faecalis* and *E. coli*, respectively; they also had good activity against all the other microorganisms, with inhibition zones 27–30 mm at 25 μg/mL. Compounds **3**, **6**, **9**, and **12** had significant activity against all four microorganisms, with inhibition zones ranging between 22 and 27 mm at 25 μg/mL. Compounds **4**, **5**, **10**, and **11** had moderate activity, with inhibition zones ranging between 18 and 23 mm at 50 μg/mL. Compounds **2** and **8** had least activity, with inhibition zones of 13–17 mm at 100 μg/mL. The in-vitro antifungal evaluation revealed that the activity of all the compounds was relatively weak compared to their antibacterial activity. Unexpectedly, all target compounds had negative efficacy except compounds **7** and **13** which had moderate activity. In general, the compounds had better antibacterial than antifungal activity.

Conclusion

For preparation of phosphazenes containing oxime groups (in high yield), this method is very suitable. Compounds **2–13** had different antibacterial activity. The thiophene ring in compound **13** had a positive effect on antibacterial effectiveness which resulted in better antibacterial activity against all four bacterial pathogens than all the other compounds except compound **7**, the efficacy of which was almost equal. Compound **2**, which does not contain an oxime group, and compound **8**, which contains a propanoyloxime group, were least active (active at 100 μg/mL only). We can make no generalizations about the antibacterial and antifungal activity of oxime phosphazenes because the literature does not contain sufficient basic information on the subject. We hope this original work is a potentially useful addition to the literature and can lead to other high-polymer and biological work.

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