MARMARA
PHARMACEUTICAL JOURNAL

Research Article

www.marmarapharmj.com

Synthesis and antimicrobial effects of cyclotriphosphazenes containing monocarbonyl curcumin analogs

Mine GÜL ŞEKER [1]*, Tuğçe AKBAL [2], Devrim ATİLLA [2], Neslihan AVŞAR[2], Hanife İBİŞOĞLU [2]*

[1] Gebze Technical University, Faculty of Science, Department of Molecular Biology and Genetics, 41400, Gebze, Kocaeli, Turkey.
[2] Gebze Technical University, Faculty of Science, Department of Chemistry, 41400, Gebze, Kocaeli, Turkey.
* Corresponding Authors. E-mail gul@gtu.edu.tr (M.G.Ş.), ibisoglu@gtu.edu.tr (H.İ.); Tel .+90 262 605 25 96, +90 262 6053012; ORCID No: 0000-0002-6226-7507 (M.G.Ş.), 0000-0003-3570-1264 (H.İ.).

Received: 08 January 2018/ Revised: 12 February 2018/ Accepted: 18 February 2018

ABSTRACT: Six novel bridged structure cyclotriphosphazenes (**4a-f**) were synthesized from the reactions of aryloxycyclotriphosphazenes [aryloxy= phenoxy (**2a**) and (2-naphthoxy) (**2b**)] with monocarbonyl curcumin derivatives [acetone (**3a**), cyclopentanone (**3b**) and cyclohexanone (**3c**)] for the first time. The structures of the compounds (**4a-f**) were defined by elemental analysis, FT-IR, mass and NMR (^{1}H and ^{31}P) spectroscopies. The antimicrobial properties of the compounds (**2a**, **2b**, **3a-c** and **4a-f**) were screened *in vitro* against *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* DSMZ 4312 and *Candida albicans* ATCC 10231. In addition, effective substance of **3b** and **4c** were evaluated Minimal Inhibition Concentration.

KEYWORDS: **:** Antimicrobial activity; mininimal inhibition concentration; phosphazene; curcumin.

1. INTRODUCTION

Curcumin [1, 7-*bis*-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], a natural polyphenolic compound isolated from *Curcuma longa L.* (Zingiberaceae family) is nontoxic and possesses a variety of pharmacological activities and therapeutic properties [1-4]. However, the poor absorption and low bioavailability of curcumin limits its clinical application [4-7]. To improve these properties, a number of curcumin derivatives have been chemically synthesized [2,8]. Among these derivatives, monocarbonyl curcumin analogs, {1,5-*bis*(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one (**3a**), 2,5-*bis*-(4-hydroxy-3-methoxybenzylidene)- cyclopentanone (**3b**), 2,6-*bis*-(4-hydroxy-3-methoxy-benzylidene)-cyclohexanone(**3c**)} exhibited better biological activities including antitumor [9-16], antioxidant [17,18], antiinflammation [17,19]. 1,5-*bis*(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one(**3a**) was used on allergic diseases [20]. Compounds **3a-c** also exhibited enhanced antibacterial activities [1,7,21].

Cyclotriphosphazenes are a class of inorganic heterocyclic rings containing phosphorous-nitrogen double bounds [22-24]. These molecules have reactive phosphorus halogen (e.g. chlorine atom) bonds that can give easy substitution reactions with a wide variety of organic groups and prepared new derivatives having different properties can be obtained depending on the characteristics of the substituted groups [22-27]. For example, they have been extensively used such as anticancer agents [26-30] and antimicrobial agents [28,33-39].

Although there have been two studies about the reactions of curcumin {1,7-*bis*(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene- 3,5-dione} with hexachlorocyclotriphosphazatriene (trimer, **1**) in the literature [40,41] and obtained phosphazene polymer containing curcumin from these reactions, according to best of our knowledge, there is no report so far about monocarbonyl curcumin derivatives substituted cyclotriphosphazenes.

Curcumins are candidate of a superb compound for a new drug design, because they have characteristics of non-toxicity against human cells, highly bioactivities and easy synthesis [4, 6]. Antibacterial properties of curcumin compounds have reported in several studies. Especially curcumins, with the aims of

How to cite this article: Gül Şeker, M, Akbal, T, Atilla, D, Avşar, N, İbişoğlu, H. Synthesis and antimicrobial effects of cyclotriphosphazenes containing monocarbonyl curcumin analogs. Marmara Pharm J. 2018; 22 (4): 536-546.

https://doi.org/10.12991/jrp.2018.95
ISSN: 1309-0801

improving biological activities, modified by new conjugates such as palmitic acid [1, 42]. Therefore, in the present work, three series of monocarbonyl curcumin analogues (**4a-f**) (Figure 1) by presenting with cyclotriphosphazenes derivatives to enhance antimicrobial activity of the molecule were prepared and their antimicrobial properties *in vitro* were evaluated by using strains of *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* DSMZ 4312 and *Candida albicans* ATCC 10231.

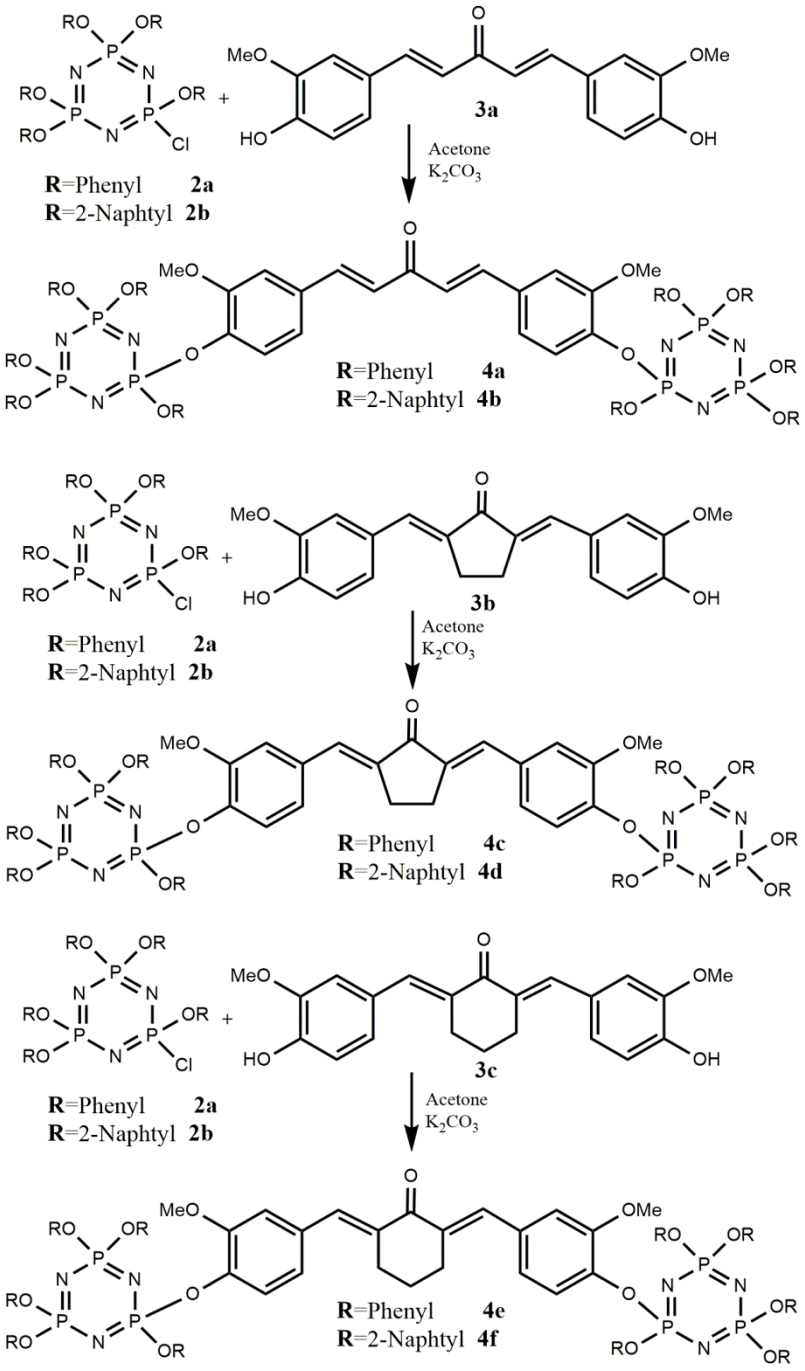

Figure 1. The synthesis scheme of compounds **4a-4f**.

https://doi.org/10.12991/jrp.2018.95
Marmara Pharm J 2018; 22(4): 536-546

2. RESULT AND DISCUSSION

2.1. Syntheses and characterizations of the compounds

Monocarbonyl curcumin derivatives substituted cyclotriphosphazenes (**4a-f**) were synthesized from the reactions of chloropenta(phenoxy)cyclotriphosphazatriene (**2a**) and chloropenta(2-naphthoxy)cyclotriphosphazatriene (**2b**) with compounds **3a-c** in the presence of K_2CO_3 at the boiling point of dry acetone under argon atmosphere for 48h, respectively (Figure 1). The products (**4a-f**) were isolated by column chromatography. Each of the compounds (**4a-f**) was characterized by using elemental analysis, FT-IR, mass, NMR (^{1}H and ^{31}P) spectroscopy techniques. The elemental and mass analyses, FT-IR and ^{1}H NMR results for each new compound were provided as part of the analytical data in the synthesis section.

The results of elemental and mass of bridged cyclotriphosphazenes (**4a-f**) confirmed that the dipotassium salt of monocarbonyl curcumin derivatives (**3a-c**) replaced one chlorine atom in compounds **2a** and **2b**, respectively. The MALDI-TOF spectrum of **4f** was depicted as an example in Figure 2.

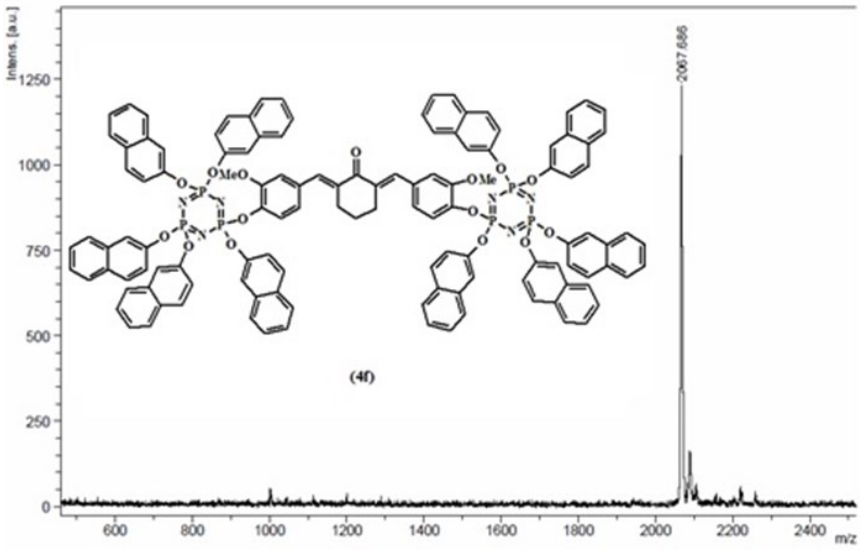

Figure 2. The MALDI-TOF spectrum of **4f.**

Although, the -OH stretching vibration in the structures of compounds **3a-c** was observed between at 3488 and 3276 cm^{-1}, this vibration was not displayed in the FT-IR spectra of compounds **4a-f**. Furthermore, these spectra shown characteristic stretching band at around 1147-1263($\nu_{P=N}$), 941-969 ($\nu_{P\text{-}O\text{-}Phenyl}$ or $_{P\text{-}O\text{-}2\text{-}Naphthyl}$), 1024-1035($\nu_{P\text{-}O\text{-}C}$) and 1651-1695 cm^{-1} ($\nu_{C=O}$), as expected [43].

^{31}P{^{1}H} NMR results of **4a-f** confirmed the proposed structures and were summarized in Table 1. The ^{31}P{^{1}H} NMR spectra of **4a-f** were observed as AB_2 spin systems (see Figure 3 as an example for **4a**) due to two different phosphorus environments within the molecules. The signals of for P(O-Phenyl)$_2$ (at ca. δ = 8.33-8.37 ppm), P(O-2-Naphthyl)$_2$ (at ca. δ = 8.99-9.10 ppm), P(O)(O-Phenyl) (at ca. δ = 9.16-9.25 ppm) and P(O)(O-2-Naphthyl) (at ca. δ = 9.38-9.51 ppm) groups were observed, respectively (as shown in Table 1).

Table 1. ^{31} P NMR parameters of cyclotriphosphazenes (**4a-f**).

		δ (^{31}P NMR) [ppm]				^{2}J(PNP) [Hz]	
Compound	**Spin System**	P(O)(O-Phenyl) (1)	P(O-Phenyl)$_2$ (2)	P(O)(O-2-Naphthyl) (3)	P(O-2-Naphthyl)$_2$ (4)	1,2	3,4
4a	AB_2	9.16	8.33	-	-	90.5	-
4b	AB_2	-	-	9.38	9.03	-	92.6
4c	AB_2	9.21	8.33	-	-	90.3	-
4d	AB_2	-	-	9.58	8.99	-	89.1
4e	AB_2	9.25	8.37	-	-	90.4	-
4f	AB_2	-	-	9.51	9.10	-	89.1

[a]202.38 MHz ^{31}P NMR chemical shifts (ppm) in $CDCl_3$ with respect to external 85 % H_3PO_4.

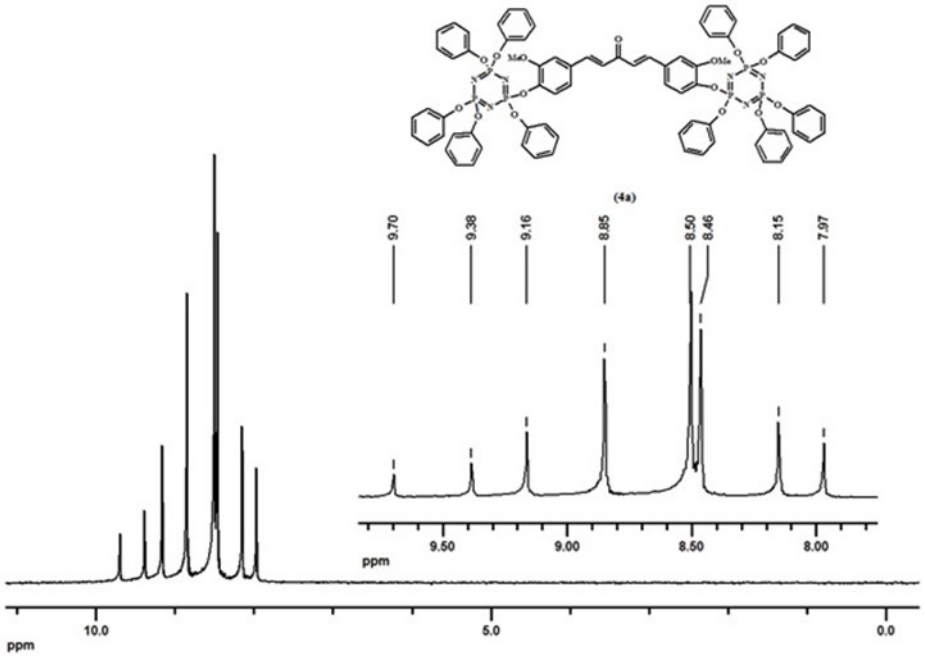

Figure 3. ^{31}P {^{1}H} NMR spectrum of compound **4a** in $CDCl_3$ solution.

The ^{1}H NMR spectra of compounds **4a-f** were similar. The methoxy (at ca. δ = 3.62-3.77 ppm) and -CH= (at ca. δ = 6.82-6.76 ppm) protons were observed in the ^{1}H NMR spectra of compounds **4a-f**, respectively. The chemical shifts for -CH_2 protons were also observed at around 1.77-3.04 ppm in the ^{1}H NMR spectra of compounds **4c-f**.

2.2. Antimicrobial activity and minimal inhibition concentration

Antimicrobial test results were shown that compounds **3b** and **4c** had influence against Gram-positive bacteria *B. cereus* and *B. subtilis*. All tested curcumin compounds had not affect against the other evaluated standard microorganisms. Antimicrobial effect was determined as an inhibition percentage as showed Figure 4. According to antimicrobial test results, it was shown that the most effective curcumin compound was **4c** with 52% inhibition rate compared to control (chloramphenicol inhibition zone diameter) against *B. subtilis*. That was followed by compound **3b** with 48% against same microorganism. According to *B. cereus* test results, compounds **3b** and **4c** were found less effective against *B. cereus* with 40% and 36% inhibition rate, respectively. These indicated that the cyclotriphosphazene substitution may enhance the bioactivities the curcumin analog (**3b**) and of cyclopentanone analogues (**3b** and **4c**) was more active than the acetone (**3a** and **4a**) and cyclohexanone analogues (**3c** and **4e**).

MIC value of compounds **3b** and **4c**, which have antibacterial effect were determined and given in Table 2. Their MIC value were observed against *B. subtilis* as 9.76 µg/mL, 19.53 µg/mL and against *B. cereus* as <625 µg/mL and 1250 µg/mL, respectively.

Table 2. Final results of MIC values (µg/mL).

Control strains	Effective Curcumin Compounds (µg/mL)	
	3b	**4c**
Bacillus subtilis	9.76	19.53
Bacillus cereus	<625	1250

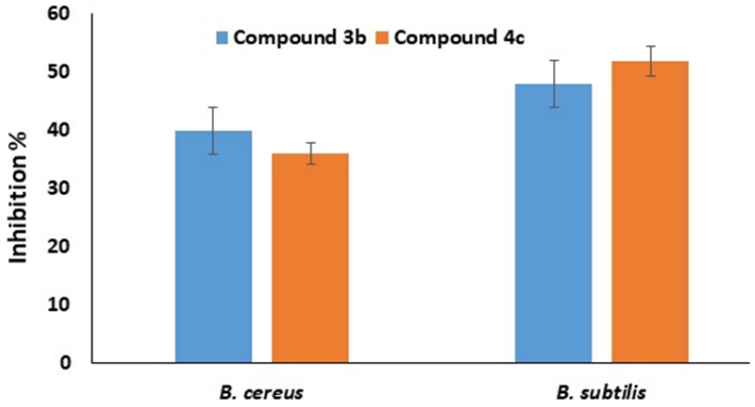

Figure 4. Representation of bactericidal effect of curcumin compounds (**3b** and **4c**) as inhibition percentage. P<0.05 is considered as significant.

When examining antibacterial features of **3b** and **4c**, it can be seen that having some structural features of these compounds. Molecules have two domains within two aromatic or Michael acceptor and two cyclotriphosphazene rings with aromatic rings within unsaturated C=C bonds flanking the carbonyl groups. These characteristics may be gained an antibacterial features to **3b** and **4c**. This is likely to play a role compound's antimicrobial activity [44].

3. CONCLUSION

In this paper, three series of monocarbonyl analogues of curcumin (**3a-c** and **4a-f**) were synthesized and evaluated for antimicrobial activities against five microorganisms. It was observed that heterocycle substituents may enhance the activity of curcumin analogues and Gram positive spore forming bacteria *B. cereus* and *B. subtilis* as antibacterial were sensitive to cyclopentanone curcumin analogues (**3b** and **4c**). Future work will be designed on reason why same compounds have different behaviors of antimicrobial effect against Gram positive spore forming bacteria of *B. cereus* and *B. subtilis*, which belongs to same genus *Bacillus*.

4. MATERIALS AND METHODS

4. 1. Materials

Hexachlorocyclotriphosphazatriene (**1**) (Aldrich) was purified by fractional crystallization from *n*-hexane. The following chemicals were obtained from Merck: tetrahydrofurane (THF), dichloromethane (DCM), sodium hydride (NaH) (60 % dispersion in mineral oil), acetone, vanillin, *n*-hexane, petroleum ether (bp: 60-80 °C), 2-naphthol, cyclopentanone, cyclohexanone, methanol, ethanol, 2,5-dihydroxybenzoic acid, ethyl acetate, H_2SO_4, K_2CO_3, Na_2SO_4, phenol. $CDCl_3$ for NMR spectroscopy was also obtained from Merck. 1,8,9-anthrasenetriol (DIT) was also obtained from Alfa aesar. Acetone (Merck) dried over 3A molecular sieves. All solvents used in this work were purified by conventional methods.

4.2. Measurements

Thin layer chromatography (TLC) was performed on Merck Silica gel plates (Merck 60, 0.25 mm thickness) with F_{254} indicator. Column chromatography was performed on silica gel (Merck 60, 0.063–0.200 mm; for 3 g crude mixture, 120 g silica gel was used in a column of 3 cm in diameter and 110 cm in length). The melting point was measured with a Gallenkamp apparatus using a capillary tube. Elemental analyses were obtained using a Thermo Finnigan Flash 1112 Instrument. Mass analyses were recorded on a Bruker MALDI–TOF (Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight mass, Rheinstetten, Germany) spectrometer using 2, 5-dihydroxybenzoic acid (for **2a**, **2b**, **3b**, **3c**, **4a**, **4c**, **4d**, **4e**, **4f**) and 1,8,9-anthrasenetriol (DIT) (for **3a** and **4b**) as a matrix. Infrared spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrophotometer. The ^1H and ^{31}P NMR spectra for compounds in $CDCl_3$ solutions were recorded on a Varian INOVA 500 MHz spectrometer using $SiMe_4$ as an internal reference for ^1H, and 85% H_3PO_4 as an external reference for ^{31}P NMR measurements.

4.3. Syntheses

4.3.1. General synthetic procedures for the compounds

2-chloro-2,4,4,6,6-pentaphenoxy-1,3,5,2λ⁵,4λ⁵,6λ⁵-triazatriphosphinine (**2a**) [45,46] and 2-chloro-2,4,4,6,6-pentakis(naphthalen-2-yloxy)-1,3,5,2λ⁵,4λ⁵,6λ⁵-triazatriphosphinine (**2b**) [47] were synthesized and purified according to the literature procedures. The synthetic pathway and structure of **2a** and **2b** were given in Figure 5.

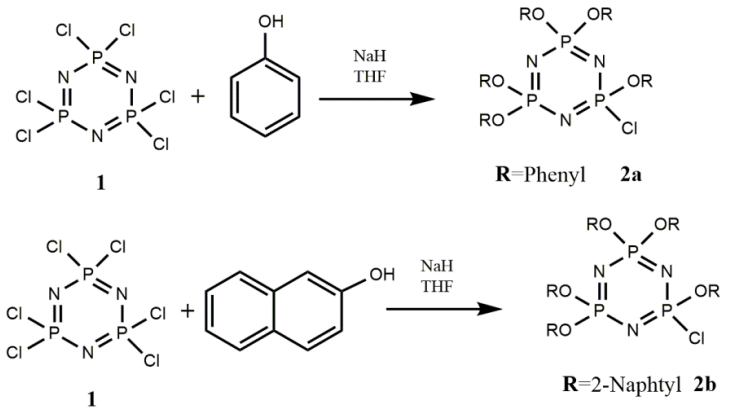

Figure 5. The synthesis scheme of compounds **2a** and **2b**.

The melting points of these compounds were 111 °C and 135 °C, respectively and consistent with the literature. Monocarbonyl curcumin analogues (**3a-c**) were also synthesized according to literatures [21, 48]. The synthetic pathway and structure of **3a-c** were given in Figure 6. The melting points of the curcumin derivatives were 69 °C, 212 °C and 179 °C according to the literature, respectively.

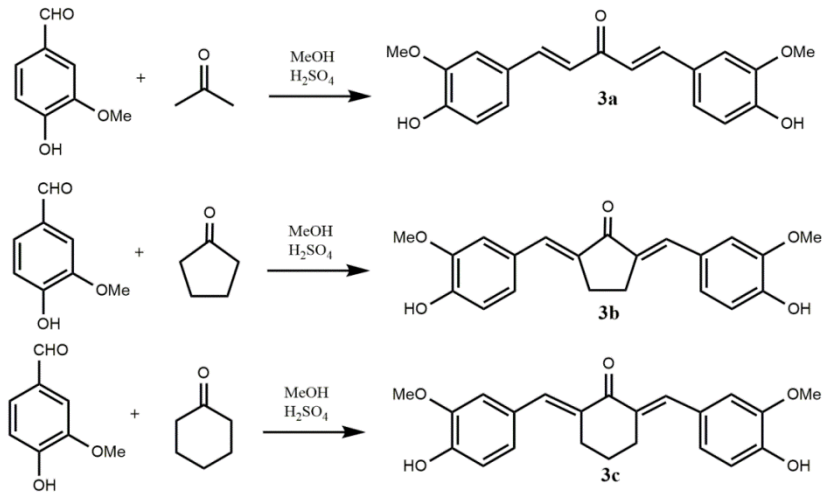

Figure 6. The synthesis scheme of compounds **3a-c**.

The general method for the synthesis monocarbonyl curcumin analogues substituted cyclotriphosphazenes (**4a-f**) was stated as below:

The compounds **2a** and **2b** with monocarbonyl curcumin derivatives (**3a-c**) were reacted using K_2CO_3 in dry acetone by refluxing for 48 h, respectively. The reactions were followed on TLC silicagel plates using *n*-hexane: ethyl acetate (3:2) as the eluent. The reactions mixture was allowed to cool to room temperature. Potassium chloride and any other insoluble materials were filtered off and the solvent was removed under reduced pressure at 30 °C. The crude products were isolated by column chromatography with *n*-hexane: ethyl

acetate (3:2) as the mobile phase. Generally, the products (**4b**, **4c**, **4d**, **4f**) were dissolved in DCM and obtained as yellow solid by precipitation with petroleum ether.

The same general procedure gave compounds (**4a-f**), with scale (gram, mol), yield, and spectroscopy data given below. Synthetic pathway and molecular structures of the compounds were given in Figure 1.

4.3.2. Synthesis of 4a

K_2CO_3 (1.497 g, 0.011 mol), compounds **2a** (0,5 g, 7.86x10^{-4} mol) and **3a** (0.26 g, 7.86x10^{-4} mol) were used. The product **4a,** (1*E*,4*E*)-1,5-*bis*(3-methoxy-4-((2,4,4,6,6-pentaphenoxy-1,3,5,2λ^5,4λ^5,6λ^5-triazatriphosphinin-2-yl)oxy)phenyl)penta-1,4-dien-3-one, (0.2 g, 80 %) was isolated as an oil. Anal. Calc. for $C_{79}H_{66}N_6O_{15}P_6$: C, 62.21; H, 4.36; N, 5.51. Found: C 62.22; H 4.34; N 5.50%. MALDI-TOF-MS (m/z): $[M+H]^+$, 1525.845 (calcd. 1525.28). FT-IR (ATR, cm $^{-1}$) ν 3060 (C-H aromatic), 2931 and 2843 (C-H Aliphatic), 1651(C=O), 1621,1590 and 1510(C=C), 1260 and 1157 (P=N), 1025 (P-O-C) and 968(P-O-Ph).^{1}H NMR (500 MHz, $CDCl_3$, 298 K); δ(ppm): 6.88-7.68 (m, 60H, -CH=), 3.78 (s, 6H, -OCH_3).

4.3.3. Synthesis of 4b

K_2CO_3 (0.84 g, 6.093x10^{-3} mol), compounds **2b** (0,45 g, 5.07x10^{-4} mol) and **3a** (0.1986 g, 6.073x10^{-4} mol) were used. The product 4b, (1E,4E)-1,5-bis(3-methoxy-4-((2,4,4,6,6-pentakis(naphthalen-2-yloxy)-1,3,5,2λ^5,4λ^5,6λ^5-triazatriphosphinin-2-yl)oxy)phenyl)penta-1,4-dien-3-one, (mp: 81 ^{o}C, 0.08 g, 40 %) was obtained as a yellow solid. Anal. Calc. for $C_{119}H86N_6O_{15}P_6$: C, 70.55; H, 4.28; N, 4.15. Found: C 70.56; H 4.26; N 4.14%.MALDI-TOF-MS (m/z): $[M+H]^+$, 2025.111 (calcd. 2025.89). FT-IR (ATR, cm $^{-1}$) ν 3056(C-H aromatic), 2923 (C-H Aliphatic), 1655 (C=O), 1627, 1598 and 1510 (C=C), 1195 and 1149(P=N), 1035 (P-O-C) and 968 (P-O-$C_{10}H_7$). ^{1}H NMR (500 MHz, $CDCl_3$, 298 K); δ(ppm): 6.93-7.76 (m, 80H, -CH=), 3.64 (s, 6H, -OCH_3).

4.3.4. Synthesis of 4c

K_2CO_3 (1.44 g, 0.01 mol), compounds **2a** (0.46 g, 7.2x10^{-4} mol) and **3b** (0.25 g, 7.2x10^{-4} mol) were used. The product **4c**, 2,5-*bis*((*E*)-3-methoxy-4-((2,4,4,6,6-pentaphenoxy-1,3,5,2λ^5,4λ^5,6λ^5-triazatriphosphinin-2-yl)oxy)benzylidene)cyclopentan-1-one, (mp: 68 ^{o}C, 0.09 g, 35%) was isolated as a yellow solid. Anal. Calc. for $C_{81}H_{68}N_6O_{15}P_6$: C, 62.71; H, 4.42; N, 5.42. Found: C 62.72; H 4.43; N 5.41%. MALDI-TOF-MS (m/z): $[M+H]^+$, 1552.828 (calcd. 1551.32). FT-IR (ATR, cm $^{-1}$) ν 3068 (C–H aromatic), 2920 and 2830 (C-H Aliphatic), 1695 (C=O), 1590, 1509, and 1487(C=C), 1263 and 1173 (P=N), 1024 (P-O-C) and 944 (P-O-Ph).^{1}H NMR (500 MHz, $CDCl_3$, 298 K); δ(ppm): 6.89-7.52 (m, 58H, -CH=), 3.77 (s, 6H, -OCH_3), 3.04 (s, 4H, -H_2C-CH_2).

4.3.5. Synthesis of 4d

K_2CO_3 (1.07 g, 0.0078 mol), compounds **2b** (0.5 g, 5.4 x10^{-4} mol) and **3b** (0.197 g, 5.6x10^{-4} mol) were used. The product **4d**, 2,5-*bis*((*E*)-3-methoxy-4-((2,4,4,6,6-pentakis(naphthalen-2-yloxy)-1,3,5,2λ^5,4λ^5,6λ^5-triazatriphosphinin-2-yl)oxy)benzylidene)cyclopentan-1-one, (mp: 83 ^{o}C, 0.06 g, 30%) was obtained as a yellow solid. Anal. Calc. for $C_{121}H_{88}N_6O_{15}P_6$: C, 70.83; H, 4.10; N, 5.42. Found: C 70.84; H 4.31; N 4.09%. MALDI-TOF-MS (m/z): $[M+H]^+$, 2052.166 (calcd. 2051.92). FT-IR (ATR, cm $^{-1}$) ν 3056(C–H aromatic), 2956 and 2933 (C-H Aliphatic), 1693 (C=O), 1630, 1598 and 1508 (C=C), 1209 and 1196(P=N), 1034 (P-O-C) and 967 (P-O-$C_{10}H_7$). ^{1}H NMR (500 MHz, $CDCl_3$, 298 K); δ(ppm): 6.84-7.74 (m, 78H, -CH=), 3.64 (s, 6H, OCH_3), 2.65 (s, 4H, -H_2C-CH_2).

4.3.6. Synthesis of 4e

K_2CO_3 (0.456 g, 3.29x10^{-3} mol), compounds **2a** (0.25 g, 3.93x10^{-4} mol) and **3c** (0.173 g, 4.72x10^{-4} mol) were used. The product **4e**, 2,6-*bis*((*E*)-3-methoxy-4-((2,4,4,6,6-pentaphenoxy-1,3,5,2λ^5,4λ^5,6λ^5-triazatriphosphinin-2-yl)oxy)benzylidene)cyclohexan-1-one, (0.08 g, %13) was isolated as an oil. Anal. Calc. for $C_{82}H_{70}N_6O_{15}P_6$: C, 62.92; H, 4.51; N, 5.37. Found: C 62.93; H 4.53; N 5.36%. MALDI-TOF-MS (m/z): $[M+H]^+$, 1566.058 (calcd. 1565.35).FT-IR (ATR, cm $^{-1}$) ν 3068 (C-H aromatic), 2923 (C-H Aliphatic), 1663 (C=O), 1594, 1508, and 1486(C=C), 1262 and 1155 (P=N), 1025 (P-O-C) and 941 (P-O-Ph). ^{1}H NMR (500 MHz, $CDCl_3$, 298 K); δ(ppm): 6.82-7.72 (m, 58H, -CH=), 3.75 (s, 6H, OCH_3), 2.86 (t, J= 5.2 Hz, 4H, H_2C-C-CH_2), 1.77 (quintet, J= 5.5 Hz, 2H, C-H_2C-C).

4.3.7. Synthesis of 4f

K_2CO_3 (1.804 g, 3.29x10^{-3} mol), compounds **2b** (0.638 g, 7.2x10^{-4} mol) and **3c** (0.344 g, 9.4x10-4 mol) were used. The product **4f**, 2,6-*bis*((*E*)-3-methoxy-4-((2,4,4,6,6-pentakis(naphthalen-2-yloxy)-1,3,5,2λ^5,4λ^5,6λ^5-triazatriphosphinin-2-yl)oxy)benzylidene) cyclohexan-1-one, (mp: 81 ^{o}C, 0.23 g, 89%) was obtained as a

yellow solid. Anal. Calc. for $C_{122}H_{90}N_6O_{15}P_6$: C, 70.93; H, 4.39; N, 4.07. Found: C 70.94; H 4.38; N 4.08%. MALDI-TOF-MS (m/z): $[M+H]^+$, 2067.689 (calcd. 2065.95). FT-IR (ATR, cm^{-1}) ν 3052(C-H aromatic), 2927 (C-H Aliphatic), 1669 (C=O), 1631, 1609 and 1510 (C=C), 1195 and 1147 (P=N), 1030 (P-O-C) and 969 (P-O-$C_{10}H_7$). 1H NMR (500 MHz, $CDCl_3$, 298 K); δ(ppm): 6.82-7.72 (m, 78H, -CH=), 3.62 (s, 6H, OCH_3), 2.62(br, 4H, H_2C-C-CH_2), 2.12 (br, 2H, C-H_2C-C).

4.4. Antimicrobial Assay

4.4.1. Antimicrobial activity

All of the compounds (**2a**, **2b**, **3a-c** and **4a-f**) were screened by agar well method on Mueller Hinton Agar (MHA) [49]. Prepared fresh microorganism cultures (*E. coli* ATCC 8739, *S. aureus* ATCC 29213, *B. subtilis* ATCC 6633, *B. cereus* DSMZ 4312 and *C. albicans* ATCC 10231) in Mueller Hinton Broth (MHB) were adjusted to 0.5 McFarland [10^8 colony forming unit (cfu)/ mL for *E. coli*, *S. aureus* and 10^5 cfu/mL for *B. cereus, B. subtilis* and *C. albicans*] in sterile phosphate buffer saline (PBS 0,85%) by using nephelometer (Crystal SpecTM, Becton Dickenson, USA). They were inoculated with sterile swab on MHA Petri dishes. Then, all of compounds solved in dimethyl sulfoxide (DMSO) (20 mg/mL) were added in wells on agar. Antimicrobial screening test were carried out two times. DMSO were used as negative control, Chloramphenicol disk (30 mg) (HIMEDIA) for bacteria and Nystatin (10000 u/mL) for fungi were used as positive controls in all experiments. Finally, all Petri dishes were incubated for 24 h at 30 °C and 37 °C for bacteria and fungi, respectively. The results of antibacterial activity were evaluated as percentage inhibition term. It was determined by inhibition zone diameter on test plates against compound and compared with control antibiotic inhibition zone.

Inhibition percentage was calculated as given below:

% inhibition = (C-T)/C x100

C: Control antibiotics zone diameter

T: Substances zone diameter

4.4.2. Minimum inhibitory concentration (MIC) of the curcumin compounds

Associatively the result of antimicrobial activity test, MIC values of efficient curcumin compounds on test microorganisms were revealed. MIC assays were carried out by 96 well plate in MHB using of two folds serial dilution way [50, 51]. They were set from 10 000 µg/mL to 4.89 µg/mL with 12 serial dilutions (10 000 µg/mL; 5000 µg/ml; 2500 µg/mL; 1250 µg/mL; 625 µg/mL; 312.5 µg/mL; 156.25 µg/mlL; 78.125 µg/mL; 39.062 µg/mL; 19.53 µg/mL; 9.76 µg/mL; 4.89 µg/mL) in microplaque with using by MHB. Fresh bacterial cultures were adjusted 0.5 McFarland using sterile MHB. 100 µL of bacterial suspensions were inoculated in dilutions of antibacterial compounds on microplaques. All microplaques were incubated at 30 °C for 24 h. After incubation period, absorbance value (OD600) of mixtures of bacterial culture and curcumin compounds dilutions were screened by micro plaque reader (BMG LABTECH, FLUOstar Omega, USA). Chloramphenicol and fresh bacteria culture were used as a positive control and negative control, respectively. The half of absorbance of fresh bacteria culture were accepted as a MIC value (µg/mL) of compounds [50]. MIC tests were realized duplicate.

Acknowledgements: The authors would like to thank the Scientific and Technical Research Council of Turkey (TÜBITAK) for financial support (grant no: 215Z089).

Author contributions: Concept – M.G.Ş.,D.A.,H.İ.; Design – D.A,H.İ.; Supervision – M.G.Ş.,H.İ.; Materials –N.A.,D.A; Data Collection and/or Processing – M.G.Ş., T.A.,D.A.,N.A.,H.İ; Analysis and/or Interpretation – M.G.Ş., T.A.,D.A.,N.A.,H.İ; Literature Search – M.G.Ş., D.A.,H.İ.; Writing – M.G.Ş., T.A.,D.A.,N.A.,H.İ.; Critical Reviews – M.G.Ş., D.A.,H.İ.

Conflict of interest statement: "The authors declared no conflict of interest" in the manuscript.

REFERENCES

[1] Liang G, Yang S, Jiang L, Zhao Y, Shao L, Xiao J, Ye F, Li Y, Li X. Synthesis and antibacterial properties of mono-carbonyl analogues of curcumin. Chem Pharm Bull 2008; 56(2): 162-167. [CrossRef]

[2] Cao J, Liu Y, Jia L, Zhou H-M, Kong Y, Yang G, Jiang L-P, Li Q-J, Zhong L-F. Curcumin induces apoptosis through mitochondrial hyperpolarization and mtDNA damage in human hepatoma G2 cells. Free Radical Bio Med 2007; 43(6): 968-975. [CrossRef]

[3] Altunatmaz SS, Aksu FY, Issa G, Kahraman BB, Altiner DD, Buyukunal SK. Antimicrobial effects of curcumin against *L. monocytogenes, S. aureus, S. Typhimurium* and *E. coli* O157:H7 pathogens in minced meat. Vet Med-Czech 2016; 61(5): 256-262.

[4] Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: A short review. Life Sci 2006; 78 (18): 2081-2087. [CrossRef]

[5] Sharma RA, Steward WP, Gescher AJ. Pharmacokinetics and pharmacodynamics of curcumin. Adv Exp Med Biol 2007; 595: 453-470. [CrossRef]

[6] Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of Curcumin: problems and promises. Mol Pharmacol 2007; 4(6): 807-818.

[7] Sanabria-Ríos DJ, Rivera-Torres Y, Rosario J, Gutierrez R, Torres-García Y, Montano N, Ortíz-Soto G, Ríos-Olivares E, Rodríguez JW, Carballeira NM. Chemical conjugation of 2-hexadecynoic acid to C5-curcumin enhances its antibacterial activity against multi-drug resistant bacteria. Bioorg Med Chem Lett 2015; 25(22): 5067–5071. [CrossRef]

[8] Zambre AP, Kulkarni VM, Padhye S, Sandur SK, Aggarwal BB. Novel curcumin analogs targeting TNF-induced NF-κB activation and proliferation in human leukemic KBM-5 cells. Bioorg Med Chem 2006; 14(21): 7196-7204. [CrossRef]

[9] Yerdelen KO, Gul HI, Sakagami H, Umemura N. Synthesis and biological evaluation of 1,5-*bis*(4-hydroxy-3-methoxyphenyl) penta-1,4-dien-3-one and its aminomethyl derivatives. J Enzyme Inhib Med Chem 2015; 30(3): 383-388. [CrossRef]

[10] Kohyama A, Yamakoshi H, Hongo S, Kanoh N, Shibata H, Iwabuchi Y. Structure activity relationships of the antitumor C_5-Curcuminoid GO-Y030. Molecules 2015; 20(8): 15374-15391. [CrossRef]

[11] Putri H, Jenie RI, Handayani S, Kastian RF, Meiyanto E. Combination of potassium pentagamavunon-0 and doxorubicin induces apoptosis and cell cycle arrest and inhibits metastasis in breast cancer cells. Asian Pac J Cancer Prev 2016; 17(5): 2683-2688.

[12] Chang JY, Hsieh HP, Pan WY, Liou JP, Bey SJ, Chen LT, Liua JF, Song JS. Dual inhibition of topoisomerase I and tubulin polymerization by BPR0Y007, a novel cytotoxicagent. Biochem Pharmacol 2003; 65(12): 2009-2019.

[13] Juang SH, Pana WY, Kuoa CC, Lioub JP, Hung YM, Chen LT, Hsieh HP, Chang JY. A novel *bis*-benzylidenecyclopentanone derivative, BPR0Y007, inducing a rapid caspase activation involving upregulation of Fas (CD95/APO-1) and wild-type p53 in human oral epidermoid carcinoma cells. Biochem Pharmacol. 2004; 68(2): 293-303. [CrossRef]

[14] Mapoung S, Pitchakarn P, Yodkeeree S, Ovatlarnporn C, Sakorn N, Limtrakul P. Chemosensitizing effects of synthetic curcumin analogs on human multi-drug resistance leukemic cells. Chem Biol Interact. 2016; 244: 140-148. [CrossRef]

[15] Markaverich BM, Schauweker TH, Gregory RR, Varma M, Kittrell FS, Medina D, Varma RS. Nuclear Type II sites and malignant cell proliferation: inhibition by 2,6-*bis*benzylidenecyclohexanones. Cancer Res. 1992; 52(9): 2482-2488.

[16] Khazaei A, Sarmasti N, Seyf JY. Quantitative structure-eactivity relationship of the curcumin-related compounds using various regression methods. J Mol Struct. 2016; 1108: 168-178. [CrossRef]

[17] Mousa MN. Synthesis, characterization and *in vitro* antioxidant activity of (1*E*, 4*E*)-1,5- *bis*(4- hydroxyl-3-methoxyphenyl) penta- 1,4-dien-3-one. J Pharm Res. 2012; 5(2): 913-914.

[18] Chen B, Zhu Z, Chen M, Dong W, Li Z. Three-dimensional quantitative structure–activity relationship study on antioxidant capacity of curcumin analogues. J Mol Struct. 2014; 1061:134-139. [CrossRef]

[19] Nurfina AN, Reksohadiprodjo MS, Timmerman H, Jenie UA, Sugiyanto D, van der Goot H. Synthesis of some symmetrical curcumin derivatives and their anti-inflammatory activity. Eur J Med Chem. 1997; 321-328. [CrossRef]

[20] Nugroho AE, Yuniarti N, Istyastono EP, Maeyama SK, Hakim L. Anti-allergic effects of 1,5-*bis*(4′-hydroxy-3′-methoxyphenyl)-1,4-pentadiene-3-one on mast cell-mediated allergy model. Malay J Pharm Sci. 2009; 7 (1): 51-71.

[21] Kannapan V, Jonathan DR. A study on the synthesis and bactericidal efficacy of certain poly(ester-amides) containing 2,5-*bis*(benzylidene)cyclopentanone moiety in the main chain. J Chem Pharm Res. 2013; 5(4): 382-386.

[22] Davarcı D, Zorlu Y. Group 12 metal coordination polymers built on a flexible hexakis –(3-pyridyloxy) cyclotriphosphazene ligand: effectof the central metal ions on the construction of coordination polymers. Polyhedron 2017; 127: 1-8. [CrossRef]

[23] Şenkuytu E, Eçik ET. Octa-BODIPY derivative dendrimeric cyclotetraphosphazenes;photophysical properties and fluorescent chemosensor for Co^{2+} ions. Spectrochim Acta. 2017; 173: 863-870. [CrossRef]

[24] İbişoğlu H, Güzel AM, Yuksel F. The reaction of hexachlorocyclotriphosphazatriene with p-aminophenol. Phosphorus Sulfur Silicon Relat Elem. 2017; 192(1): 92-97. [CrossRef]

[25] Uslu A, Balcı CM, Yuksel F, Özcan E, Dural S, Beşli S. The investigation of thermosensitive properties of phosphazene derivatives bearing amino acid ester groups. J Mol Struct. 2017; 1136: 90-99. [CrossRef]

[26] Gleria M, De Jaeger R, Phosphazenes: a Worldwide Insight, Nova Science Publishers, New York, USA 2004.

[27] Steiner A, Supramolecular Structures of Cyclotriphosphazenes. In: Adrianov AK. (Eds). Polyphosphazenes for Biomedical Applications, Wiley, New Jersey, 2009, pp. 411-455.

[28] Akbaş H, Okumuş A, Karadağ A, Kılıç Z, Hökelek T, Koç LY, Açık L, Aydın B, Türk M. Structural and thermal characterizations, antimicrobial and cytotoxic activities, and *in vitro* DNA binding of the phosphazenium salts. J Therm Anal Calorim. 2016; 123(2): 1627-1641.

[29] Yıldırım T, Bilgin K, Çiftçi GY, Eçik ET, Şenkuytu E, Uludağ Y, Tomak L, Kılıç A. Synthesis, cytotoxicity and apoptosis of cyclotriphosphazene compounds asanti-cancer agents. Eur J Med Chem. 2012; 52 (1): 213-220. [CrossRef]

[30] Çiftçi GY, Eçik ET, Yıldırım T, Bilgin K, Şenkuytu E, Yuksel F, Uludağ Y, Kılıç A. Synthesis and characterization of new cyclotriphosphazene compounds. Tetrahedron 2013; 69: 1454-1461. [CrossRef]

[31] Akbaş H, Karadağ A, Aydın A, Destegül A, Kılıç Z. Synthesis, structural and thermal properties of the hexapyrrolidinocyclotriphosphazenes-based protic molten salts: Antiproliferative effects against HT29, HeLa, and C6 cancer cell lines. J Mol Liq. 2017; 230: 482-495. [CrossRef]

[32] Koran K, Tekin Ç, Çalışkan E, Tekin S, Sandal S, Görgülü AO. Synthesis, structural and thermal characterizations and *in vitro* cytotoxic activities of new cyclotriphosphazene derivatives. Phosphorus Sulfur Silicon Relat Elem. 2017; 192(9): 1002-1011.

[33] Elmas G, Okumuş A, Cemaloğlu R, Kılıç Z, Çelik SP, Açık L, Tunalı BÇ, Türk M, Çerçi NA, Güzel R, Hökelek T. Phosphorus-nitrogen compounds. Syntheses,characterizations, cytotoxic, antituberculosis and antimicrobial activities and DNA interactions of spirocyclotetraphosphazenes with bis-ferrocenyl pendant arms J Organomet Chem. 2017; 853: 93-106. [CrossRef]

[34] Okumuş A, Akbaş H , Kılıç Z , Koç LY, Açık L, Aydın B, Türk M, Hökelek T, Dal H. Phosphorus–nitrogen compounds: *in vitro* cytotoxic and antimicrobial activities, DNA interactions, syntheses, and structural investigations of new mono(4-nitrobenzyl)spirocyclotriphosphazenes. Res Chem Intermed. 2016; 42(5): 4221-4251.

[35] Asmafiliz N, Kılıç Z, Civan M, Avcı O, Gönder LY, Açık L, Aydın B, Türk M, Hökelek T. Phosphorus–nitrogen compounds. Part 36. Syntheses, Langmuir–Blodgett thin films and biological activities of spiro-bino-spiro trimericphosphazenes. New J Chem. 2016; 40: 9609-9626.

[36] Çıralı DE, Uyar Z, Koyuncu İ, Hacıoğlu N. Synthesis, characterization and catalytic,cytotoxic and antimicrobial activities of two novel cyclotriphosphazene-based multisite ligands and their Ru(II) complexes. Appl Organometal Chem. 2015; 29: 536-542.

[37] Tümer Y, Koç LY, Asmafiliz N, Kılıç Z, Hökelek T, Soltanzade H, Açık L, Yola ML, Solak AO. Phosphorus–nitrogen compounds: part 30. Syntheses and structural investigations, antimicrobial and cytotoxicactivities and DNA interactions of vanillinato-substituted NN or NO spirocyclic monoferrocenyl cyclotriphosphazenes. J Biol Inorg Chem. 2015; 20: 165-178.

[38] Çıralı DE, Dayan O, Özdemir N, Hacıoğlu N. A new phosphazene derivative, spiro-$N_3P_3[(O_2C_{12}H_8)_2(OC_6H_6N\text{-}3)_2]$, and its Ru(II) complex: Syntheses, crystal structure, catalytic activity and antimicrobial activity studies. Polyhedron 2015; 88: 170-175. [CrossRef]

[39] Koçak SB, Koçoğlu S, Okumuş A, Kılıç Z, Öztürk A, Hökelek T, Öner Y, Açık L. Syntheses, spectroscopic properties, crystal structures, biological activities, and DNA interactions of heterocyclic amine substituted spiro-ansa-spiro- and spiro-bino-spiro-phosphazenes. Inorg Chim Acta. 2013; 406: 160-170. [CrossRef]

[40] Wei W, Lu R, Tang S, Liu X. Highly cross-linked fluorescentpoly(cyclotriphosphazene-co-curcumin) microspheres for the selective detection of picric acid in solution phase. J Mater Chem. 2015; A3: 4604- 4611.

[41] Wang H, Wang Y, Lee PPS, Chen Y, Huang C, Xin JH, Cheuka KKL. Syntheses, characterization, and photophysical properties of new type of curcumın-containing hyperbranched polymer. Poly Prepr. 2010; 51(2): 499-500.

[42] Yamakoshi H, Ohori H, Kudo C, Sato A, Kanoh N, Ishioka C, Shibata H, Iwabuchi Y. Structure–activity relationship of C5-curcuminoids and synthesis of their molecular probes thereof. Bioorg Med Chem. 2010; 18: 1083-1092.

[43] Görgülü AO, Koran K, Özen F, Tekin S, Sandal S. Synthesis, structural characterization and anti-carcinogenic activity of new cyclotriphosphazenes containing dioxybiphenyl and chalcone groups. J Mol Struct. 2015; 1087: 1-10. [CrossRef]

[44] Baldwin PR, Reeves AZ, Powell KR, Napier RJ, Swimm AI, Sun A, Giesler K, Bommarius B, Shinnick TM, Snyder JP, Liotta DC, Kalman D. Monocarbonyl analogs of curcumin inhibit growth of antibiotic sensitive and resistant strains of *Mycobacterium tuberculosis*. Eur J Med Chem. 2015; 92: 693-699. [CrossRef]

[45] Fitzsimmons BW, Shaw RA. Phosphorus-Nitrogen Compounds: Allcoxy and Aryloxy- cyclophosphaxenes. J Chem Soc. 1964; 1735-1741.

[46] Santos JSO, Bauchart D, Besset C, Dez I. 1-Chloro-1,3,3,5,5-pentaphenoxy-cyclotriphosphazene: a precursor of functionalized cyclophosphazene derivatives. Acta Cryst. 2004; C60(10): o751-o753. [CrossRef]

[47] Çoşut B, Yeşilot S. Synthesis, thermal and photophysical properties ofnaphthoxycyclotriphosphazenyl-substituted dendrimeric cyclic phosphazenes. Polyhedron 2012; 35(1): 101-107. [CrossRef]

[48] Sardjiman SS, Reksohadiprodjo MS, Hakim L, van der Goot H, Timmerman H. l,5-Diphenyl-1,4-pentadiene-3-ones and cyclic analogues as antioxidative agents. Synthesis and structure-activity relationship. Eur J Med Chem. 1997; 32(7-8): 625-630. [CrossRef]

[49] Valgas C, Souza SM, Smânia EFA, Jr AS. Screening methods to determine antibacterial activity of natural products. Braz J Microbiol. 2007; 38(2): 369-380.

[50] Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc. 2008; 3(2): 163-167.

[51] CLSI/NCCLS Guidelines: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; 7th Ed. Approved Standard document M-7: A5, Villanova, PA, NCCLS, 2006.