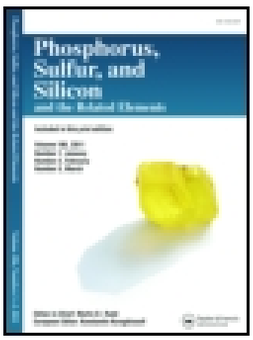

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Synthesis, Structural and Thermal Characterizations and In vitro Cytotoxic Activities of New Cyclotriphosphazene Derivatives

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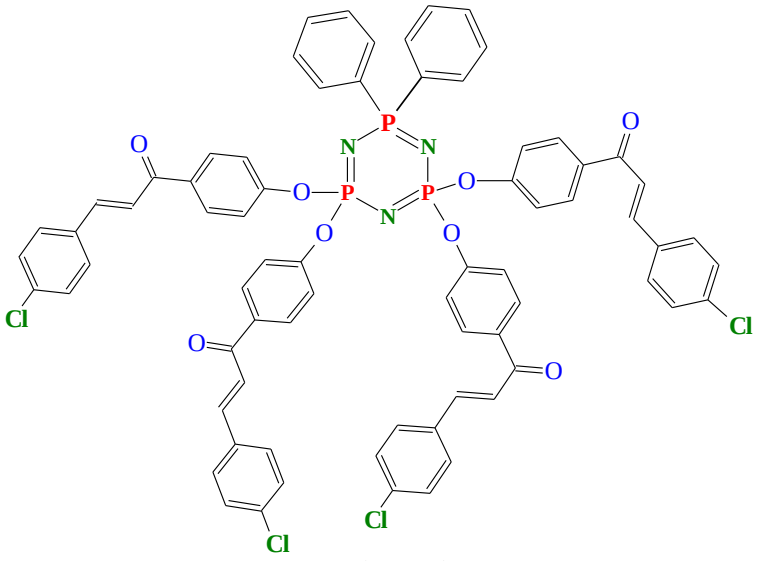
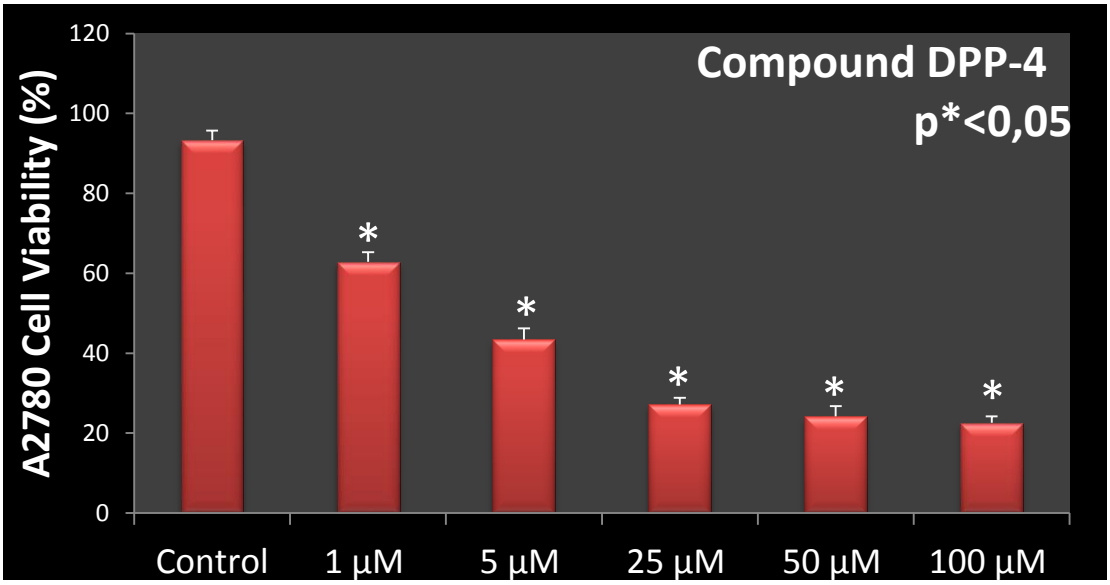
Abstract

We investigated the cytotoxic effects of the newly synthesized cyclotriphosphazene derivatives on A2780 (ovarian), PC-3 and LNCaP (prostate) cancer cell lines. 4'-hydroxy-substituted-chalcone compounds **(2-8)** were reacted with diphenyl-cyclotriphosphazene **(DPP)** in the presence of acetone/K_2CO_3 in order to obtain novel cyclotriphosphazene compounds **(DPP 2-8)**. The structures of **DPP 2-8** were characterized by MALDI-TOF mass spectrometry, FT-IR, elemental analysis, ^{1}H, ^{13}C-APT and ^{31}P NMR measurements. The thermal properties of all phosphazene compounds have been studied after synthesis and characterization procedure. The cytotoxic effects of **DPP 2-8** were examined primarily by applying the MTT method based on the measurement of mitochondrial activity. In this regard, several phosphazene compounds have shown high chemotherapeutic effect at low dose ($p<0.05$). When the cytotoxic effects of **DPP 2-8** at doses of 1, 5, 25, 50 and 100 μM on A2780 cells were examined, it was observed that **DPP-3**, **DPP-4**, **DPP-5** and **DPP-7** were more effective than other derivatives suggested by their high Log IC_{50} values ($p<0.05$). The compounds **DPP 2-8** possess cytotoxic activity against PC-3 and LNCaP cells (especially compounds **DPP-4** and **DPP-5,** $p<0.05$).



(DPP4)



Keywords

cyclotriphosphazene; cytotoxic activities; chalcone-phosphazene; cancer cell lines

Introduction

Phosphazenes are a class of inorganic compounds that gain noteworthy attention because of possessing various physical and biological properties including anti-cancer, anti-bacterial, anti-HIV, photodynamic therapy and anti-microbial activity.[1-13] The phosphazene chemistry enables substitution reactions with different types of inorganic and organic functional groups bound to phosphorus atoms which make these compounds potential for application included organic light emitting diodes and fluorescence sensors,[14-18] dielectric behaviors,[19,20] cathode material for rechargeable lithium batteries[21] and flame retardants.[22,23]

Chalcones are a class of flavonoids found in various plants and gain considerable attention due to their physical and biological properties.[24-30] Chalcones derivatives have been used as medicine that was extracted from plants and several pure chalcones were approved for clinical use some of which include metochalcone and sofalcone.[31] Chalcones are synthesized by acid or base catalyzed reaction of aldehyde and ketone via Claisen-Schimdt condensation, followed by a dehydration step.[32,33] The structure of chalcone bears two aromatic rings linked to each other by three carbons, α-β-unsaturated system, that possesses completely delocalized π electron system and have a greater probability of undergoing electron transfer interaction.[20] In addition, chalcones have various potential applications in optical and fluorescence materials, dielectric devices in addition to their use in anti-HIV and anti-cancer studies.[34-45]

In this work, an investigation of the cytotoxic effects of newly synthesized chalcone substituted cyclotriphosphazenes at different concentrations (1, 5, 25, 50, and 100 µM) on A2780, PC-3 and LNCaP cancer lines was performed. The cytotoxic effects of the compounds were analyzed by the MTT method, a colorimetric method based on the reduction of methyl

thiazole tetrazolium salts via mitochondria of living cells. The logIC_{50} values of the derivatives were determined by the GraphPad Prism software. The compounds were confirmed to be highly effective against A2780 cancer cells ($p<0.05$). Cytotoxic effect has been associated with the dose applied to the phosphazene compounds and the organic side group bound to the *ortho, meta* or *para* position of the chalcone compounds linked to phosphazene ring.

Results and discussion

Chemistry

In the first step of this work, 2,2,4,4-tetrachloro-6,6-diphenylcyclotriphosphazene **(DPP)** was obtained by Friedel-Crafts alkylation in the presence of hexacyclotriphosphazene (trimer, HCCP), benzene, triethylamine and anhydrous $AlCl_3$.[46] In the second step, substituted hydroxyl-chalcone compounds **(2-8)** were synthesized from reaction of substituted hydroxy benzaldehydes and 4'-hydroxyacetophenone via Claisen-Schimdt condensation.[32,33] The obtained compounds **2-8** and **DPP** were interacted in the presence of potassium carbonate and acetone to synthesize novel cyclotriphosphazene compounds **(DPP 2-8)** that bear a phenyl and chalcone ring. The reactions were monitored by thin layer chromatography and ^{31}P-NMR spectroscopy. The original synthesized compounds were characterized using melting points, elemental analysis, mass and FT-IR, ^{1}H, ^{13}C-APT, ^{31}P-NMR spectroscopy methods. The structures of compounds are shown in Scheme 1. The thermal behaviors of compounds were observed as single peak in DSC curves (Figure 1 (A)). The stability and purity of the compounds were supported by the previously mentioned techniques and the analyses of the compounds were elucidated through these methods. At the temperature up to 500 ^{0}C, the structure of the compounds were not degraded by more than 50% while at a temperature of 900 ^{0}C almost all compounds were not degraded (Table

S 1 Supplemental Materials). According to obtained results, it is obvious that synthesized compounds possess high thermal stability (Figure 1 (B)).

When the MALDI-TOF MS spectra of **DPP 2-8** compounds are examined, it appears they have almost the same molecular weights with the theoretically calculated masses. For example, the molecular ion peak of **DPP-5** was displayed at 1303.31 (Figure 2).

Carbonyl stretching vibrations (-C=O) observed at 1645, 1649, 1643, 1640, 1645, 1641 and 1690 cm^{-1} respectively shift to 1660, 1662, 1660, 1660, 1660, 1658 and 1660 cm^{-1} because of the interaction DPP and chalcone compounds which proves the reaction occurred. Another evidence of the binding is that the -OH peaks observed between 3120 and 3430 cm^{-1} in the chalcone compound did not occur in the FT-IR spectrum of compounds **DPP 2-8**. In addition to this evidence, -OH protons observed at 10.40 to 10.56 ppm in the ^{1}H-NMR spectra of **2-8** compounds were not observed in the ^{1}H-NMR spectra of the **DPP 2-8** compounds. For example, the ^{1}H-NMR spectra of compounds **5** and **DPP-5** was given in Figure 3. Moreover, the integral fit in the ^{1}H-NMR spectra is exactly consistent with the structure. The -P=N stretching vibrations in the FT-IR spectrum of the **DPP** compound were observed at 1172 to 1221 cm^{-1}. In the case of chalcone phosphazene compounds, -P=N vibrations at 1170 to 1210 $cm^{-1}$and P-O-Ph vibrations were observed between 920 and 940 cm^{-1}.

[Insert Figure 3]

A doublet peak at 16.2 ppm and a triplet peak of 20.4 ppm for two equivalent phosphorus environments were monitored in ^{31}P-NMR spectrum of **DPP** compound. Shifting of the doublet peaks of an equivalent phosphorus to the range of 6.9-7.1 ppm, and the shift of the peaks of single phosphorus to the range of 22.1-22.2 ppm after binding of chalcones to the phosphazene

ring and the disappearance of peaks belonging to the **DPP** compound is the most important proof that the reactions took place. For example, the ^{31}P-NMR spectra of compounds **DPP** and **DPP-5** were given in Figure 4. When the FT-IR spectra of the chalcone-phosphazene compounds are examined, the -OH functional group of chalcone is not observed and the disappearance of the -OH proton belonging to the chalcone group, as well as the integral heights, are well consistent with structure according to the ^{1}H-NMR spectrum. ^{13}C-NMR results support the formation of these compounds. In addition, ^{13}C APT-NMR technique was used to determine the chemical shift values of the primary, secondary and tertiary carbon atoms. The ^{1}H-NMR and ^{13}C APT-NMR evaluations are given in detail in the experimental section. The ^{13}C APT-NMR spectrum of **DPP-5** was given in Figure 5.

In vitro cytotoxic activity

The percent change in cell viability rates at concentrations of 1, 5, 25, 50 and 100 µM of 2,2,4,4-tetra(substituted-chalcone)-6,6-diphenylcyclotriphosphazene **(DPP 2-8)** and docetaxel and cisplatin, which is the most effective anticancer agents were used as the reference drugs, were investigated by MTT assay in order to determine the effective dose in the cell line of both androgen-independent (negative) PC-3 and androgen-dependent (positive) prostate cancer cell line (LNCaP) and human ovarian cancer (A2780). The $LogIC_{50}$ values of compounds and docetaxel and cisplatin (reference drugs, positive control) for the corresponding experiments are given in Table S 1 (Supplemental Materials). Anti-cancer experiments were carried out in three phases. Comparisons of the compounds were made based on solvent control.

In recent years, the effects of phosphazene compounds on several cancer cells have been studied and relevant works are still continuing with this regard. It is determined that phosphazene

compounds are nonhazardous when degraded in body. According to relevant studies, they have shown anti-cancer activity and studies are being kept up in this field.[47-52] **DPP 2-8** showed distinct cytotoxic effect based on substituent position on phenyl ring in the structure of the chalcone group ($p<0.05$). In general, all compounds exhibited cytotoxic effects again three cell types studied in this work. However, the compounds particularly **DPP-3, DPP-4, DPP-5** and **DPP-7** are highly effective against A2780 when examined in terms of structure activity in comparison to control group ($p<0.05$, Figure S 6 (A), Figure S 7 (A), Supplemental Materials). It was monitored that all doses of **DPP-3** and **DPP-4** compounds reduced cancer cells ($p<0.05$, Figure S 6 (A)).

When the effects of methoxy group or groups substituted **DPP-5, DPP-6** and **DPP-7** compounds were examined against A2780 cell line, the most significant effect was observed at ortho-methoxy substituted **DPP-5** compound ($p<0.05$, Figure S 7 (A)). **DPP-6** compound, which bears a methoxy group in the para position ($p<0.05$, Table S 1), have generally weak effect despite its cytotoxicity in all cells. Compounds **DPP-2**, **DPP-4**, **DPP-5** and **DPP-7** also showed significant cytotoxic effects in three cell types ($p<0.05$, Figure S 6 and Figure S 7 (A), (B) and (C)). Among these compounds, chlorine-substituted **DPP-4** exhibited has dramatic effect on all cells ($p<0.05$). It is observed that doses of these compounds, particularly 100 µM, significantly reduce PC-3 and LNCaP cancer cells (($p<0.05$, Table S 1; Figure S 6 and Figure S 7 (B) and (C)). **DPP-8** compound bearing pyridine ring generally showed cytotoxic effect at high doses, but its effect was less than other derivatives.

Although all compounds exhibit different effects at different doses for three cell types, they all show cytotoxic effect at higher doses which is verified by statistical analysis. The dose

dependent effect of compounds on the % cell viability is given separately in Figure S 6 and also Table S 1 pointing out the $logIC_{50}$ values of compounds.

Conclusion

In this work, the structures of newly synthesized 2,4,4-tetra(4'-oxy-substituted-chalcone)-6,6-diphenylcyclotriphosphazene **(DPP 2-8)** derivatives were elucidated by various methods including melting point, MALDI-TOF mass spectrometry, ^{1}H, ^{13}C-APT, ^{31}P NMR spectroscopy. The cytotoxic effects of **DPP 2-8** were analyzed by MTT assay. $LogIC_{50}$ values of compounds were calculated by Graphpad 6. According to obtained results, all doses (1, 5, 25, 50 and 100 µM) of some of the phosphazene compounds have strong chemotherapeutic effect against A2780 cancer cells. Additionally, **DPP 2-8** have cytotoxic effect against PC-3 and LNCaP cells (particularly **DPP-4**, **DPP-5**). When the cytotoxic effects of the compounds at doses of 1, 5, 25, 50 and 100 µM applied on A2780 cells were examined, **DPP-3** ($LogIC_{50}$; 1.72 µM), **DPP-4** ($LogIC_{50}$; 0.62 µM), **DPP-5** ($LogIC_{50}$; 1.41 µM) and **DPP-7** ($LogIC_{50}$; 1.75 µM) were observed to be more effective than other compounds on the cells. Based on these results, the effects of different side group on the activity of the compounds were determined to be significant which was as much as the dose applied in cancer research.

The cytotoxic effect of phosphazene compounds on various cancer cells have been studied in recent years, and relevant studies are increasingly continuing. Increasing the variety of different organic side groups increases the cytotoxic effect. Applied chemotherapeutic doses on different cancer cells are expected to have an important effect on cancer research of such compounds.

Experimental

Materials used for synthesis and purification

Phosphonitrilic chloride trimer (HCP, Alfa Aesar) was recrystallized from n-hexane. Tetrahydrofuran (THF, Sigma-Aldrich), acetone (Merck), chloroform (Sigma-Aldrich), ethanol (Merck), dichloromethane (Sigma-Aldrich) were purified by standard procedures. All the aldehyde compounds and K_2CO_3, NaOH, sodium metabisulfite ($NaHSO_3$) and the deuterated chloroform-d and dimethylsulfoxide-d_6 for the NMR analysis were procured from Merck. The human ovarian cancer cell lines (A2780) and prostate cancer cells lines (PC-3 and LNCaP) have been retrieved from the American Type Culture Collection (ATCC). Calf serum, penicillin, streptomycin and trypsin were purchased from Hyclone (Waltham, MA, USA).

Equipment used for structural characterizations

FT-IR spectroscopy (Perkin Elmer FT-IR spectrometer), microanalysis (LECO 932 CHNS-O apparatus), ^{1}H, ^{13}C-APT and ^{31}P NMR spectroscopy (Bruker DPX-400 spectrometer) and mass spectroscopy (MALDI-TOF Bruker Daltonics microflex mass spectrometer) methods were used for the structural characterization of compounds. The differential scanning calorimetry (DSC, SHIMADZU (10 $^{0}C\ min^{-1}$)) and thermogravimetric analysis (TGA, TGA-50 thermobalance (20 $^{0}C\ min^{-1}$)) methods were used for thermal characterizations. The structural of compounds were confirmed with using of these spectroscopy methods.

Synthesis

General reaction method for the synthesis of chalcones

Substituted hydroxyl-chalcone compounds were synthesized according to Claisen-Schmidt condensation protocol.[32,33]

4'-Hydroxyacetophenone (**1**) (3.0 g; 22.02 mmol) was dissolved in absolute ethyl alcohol (50 mL) and added to a 250 mL reaction flask. The reaction medium was brought to 0 ^{0}C, then a

30% NaOH solution was added to the reaction medium. After stirring for 30 min, the substituted aldehyde (22.02 mmol) was added drop wise. The remainder of the reaction was carried out at room temperature for 24 h. Subsequently, the materials were precipitated in sodium bisulphite solution. It was washed with excess amount of water, then the product was dried and compounds were obtained as solids.

The synthesis of 2,2,4,4-tetrachloro-6,6-dihenylcyclotriphosphazene (DPP)

2,2,4,4-tetrachloro-6,6-diphenylcyclotriphosphazane **(DPP)** compound was obtained through Friedel-Crafts alkylation protocol (Scheme 1).[46]

Benzene (200 mL) was added in a three necked reaction flask under argon atmosphere, then anhydrous $AlCl_3$ (26.56 g; 0.2 mol) and triethylamine (7.91 g; 0.078 mol) were added into the flask. The reaction was stirred at refluxed for 30 min then hexachlorocyclotriphosphazene (HCP, 10 g; 0.029 mol) was slowly added as solid and refluxed for 48 h. The reaction was terminated after 48 h and allowed to cool. The solution was added to of 15% HCl solution (200 mL) and stirred. The mixture was taken up in a separation funnel and benzene extract was separated. Extraction was performed by adding benzene (3 x 15 mL) to the aqueous solution, and the benzene solvents were combined, and dried with anhydrous $MgSO_4$, then filtered. Evaporation was carried out until 15 mL of benzene remained in the flask, then hexane was added into solution and precipitation was filtered off. The solvent of the remaining filtrate was re-evaporated and the residue solid was crystallized by re-dissolving in benzene (5-10 mL). The obtained white solid was 7.40 g, yield is 60%. Melting point: 93-96 ^{0}C, molecular weight is 430.96 g/mol. FT-IR (KBr) cm^{-1}: 3011 and 3069 (C-H_{Ar}), 1438, 1483 and 1590 (C=C_{Ar}), 1172 and 1221(P=N). ^{31}P-NMR ($CDCl_3$-d, ppm): 20.4 (1P, t, P_A ($C_{12}H_{10}$)), 16.2 (2P, d, P_B). ^{1}H-NMR

($CDCl_3$-d, ppm): 7.52-7.55 (2H, H^4, Ar-H), 7.58-7.60 (4H, H^3, Ar-H), 7.81 (4H, H^2, Ar-H). ^{13}C-NMR ($CDCl_3$-d, ppm): 132.0 and 133.3 (C^1, Ar-C), 128.7-128.9 (C^2, Ar-CH), 130.6-130.7 (C^3, Ar-CH), 132.5-132.6 (C^4, Ar-CH). MALDI-MS *m/z*: 431 $(M+H)^+$. Anal. Calc. for $C_{12}H_{10}N_3P_3Cl_4$: C, 33.44; H, 2.34; N, 9.75. Found: C, 33.48; H, 2.36; N, 9.79.

General reaction method for the synthesis of 2,2,4,4-tetra(4'-oxy-substituted-chalcone)-6,6-diphenylcyclotriphosphazene compounds

Characterization and synthesis of chalcone substituted cyclophosphazene derivatives were also carried out in a similar manner. In addition, detailed synthesis and characterization procedure was given below for only compound **DPP-2**.

2,2,4,4-tetra(4'-oxychalcone)-6,6-diphenylcyclotriphosphazene (DPP-2)

50 mL of acetone was added to three-necked reaction flask and 2,2,4,4-tetrechloro-6,6-diphenylcyclotriphosphazene **(DPP)** (1 g; 2.32 mmol), 4'-hydroxycarboxylate **(2)** (3.12 g; 13.92 mmol), K_2CO_3 (2.25 g; 16.24 mmol) were added sequentially under argon atmosphere at 0 ^{0}C. The reaction condition was maintained for 30 minutes, and refluxed for 12 hours then terminated and then the reaction mixture was filtered. The filtrate was precipitated in 5% of 250 mL NaOH. The precipitate was filtered and washed with excess amount of water until pH is approximately 7 and dried. The dried solid was dissolved in chloroform and re-precipitated in n-hexane and washed 3 times with ethyl alcohol. Compound **DPP-2** was obtained in pure form as white solid (1.97 g, 72% yield). The molecular weight of the compound is 1182.14 g/mol, melting point is 185-186 ^{0}C. FT-IR (KBr) cm^{-1}: 3027 and 3058 (C-H_{Ar}), 2925 and 2956 (C-$H_{Alp.}$), 1660 (C=O), 1503, 1576, and 1595 (C=C), 1181 and 1200 (P=N), 932 (P-O-Ph). ^{31}P-NMR (DMSO-d_6, ppm): 22.1 (1P, t, P_A ($C_{12}H_{10}$)), 6.8-7.0 (2P, d, P_B ($C_{15}H_{11}O_2$)). ^{1}H-NMR (DMSO-d_6, ppm): 7.24-7.26

(8H, d, J=8.8 Hz, H^6, Ar-H), 7.29-7.35 (4H, H^3, Ar-H), 7.40-7.48 (20H, m, H^4, H^{13}, H^{14}, Ar-H), 7.69-7.73 (4H, d, J=15.6 Hz, H^{10}, -CH=), 7.80-7.86 (12H, m, H^2, H^{15}, (Ar-H), H^{11}, =CH-), 8.02-8.04 (8H, d, J=8.8 Hz, H^7, Ar-H). ^{13}C-NMR (DMSO-d_6, ppm): 135.0 (C^1, Ar-C), 128.9-129.0 (C^2, Ar-CH), 130.2-130.3 (C^3, Ar-CH), 132.2 (C^4, Ar-CH), 154.0 (C^5, Ar-C), 121.5 (C^6, Ar-CH), 131.2 (C^7, Ar-CH), 135.2 (C^8, Ar-CH), 188.3 (C^9, -C=O), 122.2 (C^{10}, -CH=), 144.6 (C^{11}, =CH-), 135.0 (C^{12}, Ar-C), 129.4 (C^{13}, Ar-CH), 129.4 (C^{14}, Ar-CH), 131.0 (C^{15}, Ar-CH). MALDI-MS *m/z*: 1183 $(M+H)^+$. Anal. Calc. for $C_{72}H_{54}O_8N_3P_3$: C, 73.15; H, 4.60; N, 3.55. Found: C, 73.19; H, 4.66; N, 3.58.

2,2,4,4-Tetra(4'-oxy-3-bromochalcone)-6,6-diphenylcyclotriphosphazene (DPP-3)

Yield: 70 %. M.p. 133-134 ^{0}C. FT-IR (KBr) cm^{-1}: 3017 and 3057 (C-H_{Ar}), 2935 and 2964 (C-$H_{Alp.}$), 1662 (C=O), 1501, 1558, 1579 and 1600 (C=C), 1179 and 1203 (P=N), 931 (P-O-Ph). ^{31}P-NMR (DMSO-d_6, ppm): 22.1 (1P, t, P_A ($C_{12}H_{10}$)), 6.7-6.9 (2P, d, P_B ($C_{15}H_{10}O_2Br$)). ^{1}H-NMR (DMSO-d_6, ppm): 7.24-7.26 (8H, d, J=8.4 Hz, H^6, Ar-H), 7.34-7.37 (6H, m, H^3, H^4, Ar-H), 7.39-7.42 (4H, t, H^{16}, Ar-H), 7.46-7.47 (4H, d, J=6.8 Hz, H^{15}, Ar-H), 7.62-7.64 (4H, d, H^{17}, Ar-H), 7.64-7.68 (4H, d, J=15.6 Hz, H^{10}, -CH=), 7.80-7.82 (4H, d, H^2, Ar-H), 7.92-7.95 (4H, d, J=15.6 Hz, H^{11}, =CH-), 8.04-8.07 (8H, d, J=8.4 Hz, H^7, Ar-H), 8.16 (4H, s, H^{13}, Ar-H). ^{13}C-NMR (DMSO-d_6, ppm): 134.9 (C^1, Ar-C), 128.9-129.0 (C^2, Ar-CH), 130.2-130.3 (C^3, Ar-CH), 131.3-131.4 (C^4, Ar-CH), 154.1 (C^5, Ar-C), 121.4 (C^6, Ar-CH), 131.1 (C^7, Ar-CH), 137.5 (C^8, Ar-CH), 188.0 (C^9, -C=O), 123.5 (C^{10}, -CH=), 142.8 (C^{11}, =CH-), 137.5 (C^{12}, Ar-C), 133.5 (C^{13}, Ar-CH), 122.8 (C^{14}, Ar-C), 128.7 (C^{15}, Ar-CH), 131.1 (C^{16}, Ar-CH), 131.1 (C^{17}, Ar-CH). MALDI-MS *m/z*: 1498.12 $(M+H)^+$. Anal. Calc. for $C_{72}H_{50}O_8N_3P_3Br_4$: C, 57.74; H, 3.36; N, 2.81. Found: C, 57.79; H, 3.41; N, 2.88.

2,2,4,4-Tetra(4'-oxy-4-chlorochalcone)-6,6-diphenylcyclotriphosphazene (DPP-4)

Yield: 36 %. M.p. 172-173 ^{0}C. FT-IR (KBr) cm^{-1}: 3002 and 3062 (C-H_{Ar}), 2838 and 2930 (C-$H_{Alp.}$), 1660 (C=O), 1502, 1579 and 1596 (C=C), 1175 and 1202 (P=N), 929 (P-O-Ph). ^{31}P-NMR (DMSO-d_6, ppm): 22.1 (1P, t, P_A ($C_{12}H_{10}$)), 6.7-6.9 (2P, d, P_B ($C_{15}H_{10}O_2Cl$)). ^{1}H-NMR (DMSO-d_6, ppm): 7.23-7.25 (8H, d, J = 8.8 Hz, H^6, Ar-H), 7.33-7.47 (10H, m, H^2, H^3, H^4, Ar-H), 7.50-7.52 (8H, d, J = 8.8 Hz, H^{14}, Ar-H), 7.65-7.69 (4H, d, J=15.6 Hz, H^{10}, -CH=), 7.85-7.89 (4H, d, J=15.6 Hz, H^{11}, =CH-), 7.87-7.89 (8H, d, J=8.8 Hz, H^{13}, Ar-H), 8.01-8.03 (8H, d, J=8.8 Hz, H^7, Ar-H). ^{13}C-NMR (DMSO-d_6, ppm): 134.9 (C^1, Ar-C), 128.8-128.9 (C^2, Ar-CH), 130.2-130.3 (C^3, Ar-CH), 130.6-130.7 (C^4, Ar-CH), 154.0 (C^5, Ar-C), 121.4 (C^6, Ar-CH), 131.0 (C^7, Ar-CH), 135.6 (C^8, Ar-CH), 188.0 (C^9, -C=O), 122.8 (C^{10}, -CH=), 143.1 (C^{11}, =CH-), 135.6 (C^{12}, Ar-C), 131.0 (C^{13}, Ar-CH), 129.4 (C^{14}, Ar-C), 134.0 (C^{15}, Ar-CH). MALDI-MS *m/z*: 1320.75 $[M+H]^+$. Anal. Calc. for $C_{72}H_{50}O_8N_3P_3Cl_4$: C, 65.52; H, 3.82; N, 3.18. Found: C, 65.59; H, 3.87; N, 3.24.

2,2,4,4-Tetra(4'-oxy-2-methoxychalcone)-6,6-diphenylcyclotriphosphazene (DPP-5)

Yield: 65 %. M.p. 129-130 ^{0}C. FT-IR (KBr) cm^{-1}: 3003 and 3064 (C-H_{Ar}), 2836 and 2938 (C-$H_{Alp.}$), 1660 (C=O), 1489, 1501 and 1595 (C=C), 1176 and 1199 (P=N), 926 (P-O-Ph). ^{31}P-NMR (DMSO-d_6, ppm): 22.1 (1P, t, P_A ($C_{12}H_{10}$)), 6.9-7.1 (2P, d, P_B ($C_{16}H_{13}O_3$)). ^{1}H-NMR (DMSO-d_6, ppm): 3.89 (12H, s, H^{18}, -OCH_3), 7.02-7.05 (4H, t, H^{15}, Ar-H), 7.10-7.12 (4H, d, J = 8.4 Hz, H^{14}, Ar-H), 7.22-7.25 (8H, d, J = 8.4 Hz, H^6, Ar-H), 7.30-7.34 (4H, m, H^3, Ar-H), 7.38-7.49 (10H, m, H^4, H^{16}, H^{17}, Ar-H), 7.78-7.82 (4H, d, J = 15.6 Hz, H^{10}, -CH=), 7.95-8.01 (12H, m, H^2, H^7, Ar-H), 8.02-8.06 (4H, d, J = 15.6 Hz, H^{11}, =CH-). ^{13}C-NMR (DMSO-d_6, ppm): 135.3 (C^1, Ar-C), 128.9-129.0 (C^2, Ar-CH), 130.2-130.3 (C^3, Ar-CH), 132.9 (C^4, Ar-CH), 153.9 (C^5, Ar-C), 121.5 (C^6, Ar-CH), 130.8 (C^7, Ar-CH), 135.2 (C^8, Ar-C), 188.3 (C^9, -C=O), 121.8 (C^{10}, -CH=), 139.2

(C^{11}, =CH-), 123.3 (C^{12}, Ar-C), 158.8 (C^{13}, Ar-C), 112.2 (C^{14}, Ar-CH), 128.8 (C^{15}, Ar-CH), 121.1 (C^{16}, Ar-CH), 130.8 (C^{17}, Ar-CH), 56.2 (C^{18}, -OCH_3). MALDI-MS *m/z*: 1303.31 $[M+H]^+$. Anal. Calc. for $C_{76}H_{62}O_{12}N_3P_3$: C, 70.10; H, 4.80; N, 3.23. Found: C, 70.22; H, 4.87; N, 3.27.

2,2,4,4-Tetra(4'-oxy-4-methoxychalcone)-6,6-diphenylcyclotriphosphazene (DPP-6)

Yield: 82 %. M.p. 152-153 ^{0}C. FT-IR (KBr) cm^{-1}: 3007 and 3065 (C-H_{Ar}), 2839 and 2964 (C-$H_{Alp.}$), 1660 (C=O), 1503, 1510, 1571, 1593 and 1628 (C=C), 1174 and 1201 (P=N), 931 (P-O-Ph). ^{31}P-NMR (DMSO-d_6, ppm): 22.0 (1P, t, P_A ($C_{12}H_{10}$)), 6.9-7.1 (2P, d, P_B ($C_{16}H_{13}O_3$)). ^{1}H-NMR (DMSO-d_6, ppm): 3.83 (12H, s, H^{18}, -OCH_3), 7.01-7.03 (8H, d, J = 8.4 Hz, H^{14}, Ar-H), 7.22-7.24 (8H, d, J = 8.4 Hz, H^6, Ar-H), 7.32-7.34 (4H, H^3, Ar-H), 7.39-7.44 (6H, m, H^2, H^4, Ar-H), 7.81-7.83 (8H, d, J = 8.8 Hz, H^{13}, Ar-H), 7.70 (8H, H^{10}, -CH=, H^{11}, =CH-), 8.0-8.02 (8H, d, J = 8.8 Hz, H^7, Ar-H). ^{13}C-NMR (DMSO-d_6, ppm): 133.8-135.1 (C^1, Ar-C), 128.8-128.9 (C^2, Ar-CH), 130.2-130.3 (C^3, Ar-CH), 132.2 (C^4, Ar-CH), 153.8-153.9 (C^5, Ar-C), 119.5 (C^6, Ar-CH), 130.8 (C^7, Ar-CH), 135.4 (C^8, Ar-C), 188.0 (C^9, -C=O), 121.4 (C^{10}, -CH=), 144.6 (C^{11}, =CH-), 127.7 (C^{12}, Ar-C), 131.3 (C^{13}, Ar-CH), 114.9 (C^{14}, Ar-C), 161.9 (C^{15}, Ar-CH), 55.9 (C^{16}, -OCH_3). MALDI-MS *m/z*: 1303 $[M+H]^+$. Anal. Calc. for $C_{76}H_{62}O_{12}N_3P_3$: C, 70.10; H, 4.80; N, 3.23. Found: C, 70.16; H, 4.85; N, 3.21.

2,2,4,4-Tetra(4'-oxy-2,3,4-trimethoxychalcone)-6,6-diphenylcyclotriphosphazene (DPP-7)

Yield: 65 %. M.p. 167-168 ^{0}C. FT-IR (KBr) cm^{-1}: 3001 and 3060 (C-H_{Ar}), 2834 and 2937 (C-$H_{Alp.}$), 1658 (C=O), 1494, 1575 and 1584 (C=C), 1175 and 1196 (P=N), 921 (P-O-Ph). ^{31}P-NMR (DMSO-d_6, ppm): 22.1 (1P, t, P_A ($C_{12}H_{10}$)), 6.9-7.1 (2P, d, P_B ($C_{18}H_8O_5$)). ^{1}H-NMR (DMSO-d_6, ppm): 3.57 (12H, s, H^{18}, -OCH_3), 3.77 (12H, s, H^{20}, -OCH_3), 3.84 (12H, s, H^{19}, -OCH_3), 6.90-6.93 (4H, d, J = 9.2 Hz, H^{16}, Ar-H), 7.21-7.24 (8H, d, J = 8.4 Hz, H^6, Ar-H), 7.32-7.35 (4H, H^3,

Ar-H), 7.39-7.46 (6H, m, H^2, H^4, Ar-H), 7.71-7.75 (4H, d, J = 15.6 Hz, H^{10}, -CH=), 7.76-7.78 (4H, d, J = 9.2 Hz, H^{17}, Ar-H), 7.89-7.92 (4H, d, J = 15.6 Hz, H^{11}, =CH-), 7.97-7.99 (8H, d, J = 8.4 Hz, H^7, Ar-H). ^{13}C-NMR (DMSO-d_6, ppm): 133.8-135.1 (C^1, Ar-C), 128.8-128.9 (C^2, Ar-CH), 130.2-130.4 (C^3, Ar-CH), 132.2 (C^4, Ar-CH), 153.8 (C^5, Ar-C), 121.5 (C^6, Ar-CH), 130.8 (C^7, Ar-CH), 135.4 (C^8, Ar-C), 188.1 (C^9, -C=O), 120.4 (C^{10}, -CH=), 139.1 (C^{11}, =CH-), 121.4 (C^{12}, Ar-C), 153.6 (C^{13}, Ar-C), 142.2 (C^{14}, Ar-C), 156.3 (C^{15}, Ar-C), 108.9 (C^{16}, Ar-CH), 123.9 (C^{17}, Ar-CH), 61.9 (C^{18}, -OCH_3), 60.9 (C^{19}, -OCH_3), 56.5 (C^{20}, -OCH_3). MALDI-MS *m/z*: 1543.9 $[M+H]^+$. Anal. Calc. for $C_{84}H_{78}O_{20}N_3P_3$: C, 65.41; H, 5.10; N, 2.72. Found: C, 65.44; H, 5.17; N, 2.77.

2,2,4,4-Tetra(1-(4'-oxyphenyl)-3-(4-pyridine)-2-propen-1-one)-6,6-diphenylcyclotriphosphazene (DPP-8).

Yield: 78 %. M.p. 166-167 ^{0}C. FT-IR (KBr) cm^{-1}: 3006 and 3063 (C-H_{Ar}), 2925 and 2952 (C-$H_{Alp.}$), 1660 (C=O), 1504, 1580 and 1605 (C=C), 1179 and 1204 (P=N), 930 (P-O-Ph). ^{31}P-NMR (DMSO-d_6, ppm): 22.1 (1P, t, P_A ($C_{12}H_{10}$)), 6.8-7.0 (2P, d, P_B ($C_{14}H_{10}O_2N$)). ^{1}H-NMR (DMSO-d_6, ppm): 7.23-7.25 (8H, d, J = 8.4 Hz, H^6, Ar-H), 7.32-7.47 (10H, H^2, H^3, H^4, Ar-H), 7.50-7.52 (8H, d, H^{13}, Ar-H), 7.65-7.69 (4H, d, J = 15.6 Hz, H^{10}, -CH=), 7.85-7.89 (12H, m, H^{11}, (=CH-), H^{14}, (Ar-H)), 8.01-8.03 (8H, d, J = 8.4 Hz, H^7, Ar-H). ^{13}C-NMR (DMSO-d_6, ppm): 133.6-135.0 (C^1, Ar-C), 128.8-129.0 (C^2, Ar-CH), 130.3-130.4 (C^3, Ar-CH), 132.3 (C^4, Ar-CH), 150.5 (C^5, Ar-C), 121.6 (C^6, Ar-CH), 131.0 (C^7, Ar-CH), 134.8 (C^8, Ar-C), 188.5 (C^9, -C=O), 125.2 (C^{10}, -CH=), 143.6 (C^{11}, =CH-), 154.0 (C^{12}, Ar-C), 137.6 (C^{13}, Ar-CH), 150.5 (C^{14}, Ar-C). MALDI-MS *m/z*: 1187.12 $[M+H]^+$. Anal. Calc. for $C_{68}H_{50}O_8N_7P_3$: C, 68.86; H, 4.25; N, 8.27. Found: C, 68.91; H, 4.28; N, 8.22.

Thermal behaviors of compounds DPP 2-8

The melting points of synthesized cyclotriphosphazane **DPP 2-8** compounds were obtained from DSC thermograms with a heating rate of 10 ^{0}C min^{-1} to 250 ^{0}C and recorded DSC curves (Figure 1 (A)). The thermal degradation of **DPP 2-8** compounds were obtained by heating at 900 ^{0}C with a heating rate of 20 ^{0}C min^{-1} and recorded TGA curves (Figure 1 (B)).

The temperatures at which degradation begins until 900 ^{0}C, temperature where the degradation is 50 % and the percentages of waste at 900 ^{0}C are given in Table 1 as a result of TGA measurements. The temperature which the compounds first begin to decompose is higher than 250 ^{0}C. Besides, half of the materials degrade above 500 ^{0}C. This approved that the compounds are quite stable.

In vitro cytotoxic activity

The percentage changes in the cell viability rates in the presence of synthesized compounds and docetaxel and cisplatin, which are the most effective anticancer agents, were used as the reference drugs, at the concentrations of 1, 5, 25, 50 and 100 µM were determined by MTT assay. This method is based on the principle that MTT dye can decompose tetrazolium ring. In this method, MTT is actively absorbed to living cells and reaction is catalyzed by mitochondrial succinate dehydrogenase and by the activity of this enzyme it is reduced to a blue-violet, water-insoluble formazan. Formazan formation is observed only in living cell with active mitochondria. This is regarded as an indicator of cell viability and spectrophotometrically determined value is associated with the number of living cells. 0.5 mg/mL MTT solution was prepared from the stock MTT solution in sterile PBS and added to 96-well plates. After 3 hours in the incubator, optical densities of the cells in plates were monitored at 550 nm in the ELISA instrument

(Synergy HT USA). The control wells were read and obtained absorbance values were averaged and this value was accepted as 100% live cell. The absorbance values obtained from the solvent and agent-applied wells were proportional to the control absorbance value and accepted as percent liveliness.[53-56] In our study, human prostate cancer cell line (LNC, PPC-3 and human ovarian cancer cell lineA2780) was used as cell type. All cells were fed with RPMI-1640 medium (prepared in 10% FCS, 100 U/Ml penicillin and 0.1 mg/mL streptomycin) in 25 cm^2 culture flask.

In carbon dioxide (5% CO_2) incubator, the media of the cells, kept at 37 ^{0}C in humidified environment, were changed twice a week. When cells were confluent, trypsin-EDTA solution were used to remove them from the flask and transfer to 96-well plates to operate 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide (MTT) analyzes. The solutions of materials in the DMSO (negative control) were used in cell culture. The effects of the substances against DMSO in comparison to the results were determined by statistical analysis. The toxic effect of DMSO in the cell was determined and found not to be statistically significant despite its known toxic property. The solvent (DMSO) was added to wells that contain cells in the same amounts as the concentration of tested compounds **(DPP 2-8**) and docetaxel and cisplatin (positive control) at 1, 5, 25, 50 and 100 µM and incubated at 37 ^{0}C for 24 h in a CO_2 incubator (Panasonic/Japan). The viability of the cells was determined by using 0.4% trypan blue in a hemocytometer after incubation. Normal distribution suitability of the groups was assessed by the Kolmogorov Smirnov tests. One-way variance analysis was used to compare groups among each other. Homogeneity of variance was analyzed by Levene test. After one-way variance analysis, it was monitored that variances were not homogenous that's why TAMHANE T2 test

was used for multiple comparisons. $P<0.05$ was considered to be statistically meaningful. Data were expressed as mean ± standard error. $LogIC_{50}$ values were calculated by Graphpad Prism.

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REFERENCES

1. Allcock, H. R. *Phosphorus-Nitrogen Compounds,* 5rd ed.; Academic Press: New York and London, **1972**; pp. 3-13.

2. Gleria, M.; Jaeger D. R. *Polyphosphazenes,* Nova Science Publishers: New York, **2004**; pp. 1-15.

3. Allen, C. W.; MacKay, J. A. *Inorg. Chim. Acta,* **1982,** 67, L17-L18.

4. Labarre, J-F.; Faucher, J-P.; Levy, G.; Sournies, F.; Cros, S.; François, G. *Eur. J. Cancer,* **1979**, 15, 637-643.

5. Dabrzalsaka, M.; Zablocka, M.; Mignani, S.; Majoral, J. P.; Maculewicz, B. K. *Intl. J. Pharma.,* **2015,** 492, 266-274.

6. Işıklan, M.; Sayın, L.; Sonkaya, Ö.; Hökelek, T.; Türk, M.; Oğuztüzün, S. *Phosphorus Sulfur Silicon Relat. Elem.,* **2016,** 191, 1216-1222.

7. Çıralı, D. E.; Uyar, Z.; Koyuncu, İ.; Hacıoğlu, N. *Appl. Organomet. Chem.,* **2015,** 29, 536-542.

8. Ozay, O.; Yildirim, M.; Ozay, H. *Phosphorus Sulfur Silicon Relat. Elem.,* **2016,** 20, 1–9.

9. Ozay, H; Yildiz, M; Dulger, B; Unver, H. *Asian J. Chem.,* **2010**, 22, 3813-3823.

10. Brandt, K.; Kruszynski, R.; Bartzak, T. J.; Czomperlik, I. P. *Inorg. Chim. Acta,* **2001,** 322, 138-144.

11. Akbas¸ H.; Okumus, A.; Karadağ, A.; Kılıç, Z.; Hökelek, T.; Koç, L. Y.; Açık, L.; Aydın, B.; Türk, M. *J. Therm. Anal. Calorim.,* **2016,** 123, 1627-1641.

12. Siwy, Mariola.; Seük, D.; Kaczmarczyk, B.; Jaroszewicz, I.; Nasulewicz, A.; Pelczynska, M.; Nevozhay, D.; Opolski, A. *J. Med. Chem.*, **2006,** 49, 806-810.

13. Asmafiliz, N.; Kılıç, Z.; Öztürk, A.; Süzen, Y.; Hökelek, T.; Açık, L.; Çelik, Z. B.; Koç, L. Y.; Yola, M. L.; Üstündağ, Z. *Phosphorus Sulfur Silicon Relat. Elem.*, **2013,**188, 1723-1742.

14. Franc, G.; Mazeres, S.; Turrin, C.-O.; Vendier, L.; Duhayon, C.; Caminade, A. M.; Majoral, J. P. *J. Org. Chem.*, **2007**, 72, 8707-8715.

15. Xu, J.; Toh, C. L.; Ke, K. L.; Li, J. J.; Cho, C. M.; Lu, X.; Tan, E. W.; He, C. *Macromolecules*, **2008**, 41, 9624-9636.

16. Şenkuytu, E.; Eçik, E. T.; Durmuş, M.; Çiftçi, G. Y. *Polyhedron*, **2015**, 101, 223-229.

17. Kecin, İ.; Elmali, F. T.; Demirhan, N. *Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry*, **2011,** 41, 1-7.<AQ>Incorrect abbreviation</AQ>

18. Bolink, H. J.; Barea, E.; Costa, R. D.; Coronado, E.; Sudhakar, S.; Zhen, C.; Sellinger, A. *Org. Electron.*. **2008,** 9, 155–163.

19. Koran, K.; Ozen, F.; Biryan, F.; Görgülü, A. O. *J. Mol. Struct.*, **2016,** 1105, 135-141.

20. Koran, K.; Ozen, F.; Biryan, F.; Demirelli, K.; Görgülü, A. O. *Inorg. Chim. Acta*, **2016,** 450, 162-169.

21. Xu, G. X.; Lu, Q.; Yu, B. T.; Wen, L. *Solid State Ionics*, **2006**, 177, 305-309.

22. Kuan, J. F.; Lin, K. F.; *J. Appl. Polym. Sci.*, **2004,** 91, 697–702.

23. Jiang, P.; Gu, X.; Zhanga, S.; Sun, J.; Wua, S.; Zhao, Q. *Phosphorus Sulfur Silicon Relat. Elem.*, **2014,** 189, 1811–1822.

24. Kaymakçıoğlu, B. K.; Beyhan, N.; Tabanca, N.; Ali, A.; Wedge, D. E.; Duke, S. O.; Bernier, U. R.; Khan, I. A. *Med. Chem. Res.*, **2015**, 24, 3632-3644.

25. Makhlouf, M. M.; Radwan, A. S.; Aly, M. R. E. *J Photochem. Photobiol A: Chem,* **2017**, 332, 465-474.

26. Opletalova, V.; Hartl, J.; Palat, J. K.; Patel, A. *J. Pharm. Biomed. Anal.,* **2000,** 23, 55-59.

27. Harborne, J. B.; Marby, H.; Marby, T. J. The Flavonoids, *Springer US,* **1975,** 1-1204.<AQ>What is the publication city?</AQ>

28. Asiri, A. M.; Khan, S. A. *Mater. Lett.,* **2011,** 65, 1749–1752.

29. Niu, C. G.; Guan, A. L.; Zeng, G. M.; Liu, Y. G.; Li, Z. W. *Anal. Chim. Acta*, **2006,** 577, 264–270.

30. Pandey, V. S.; Dhar, R.; Singh, A. K.; Achalkumar, A. S.; Yelamaggad, C. V. *Phase Trans.,* **2010,** 83, 1049–1058.

31. Tanaka, H.; Nakamura, S.; Onda, K.; Tazaki, T.; Hirano, T. *Biochem Biophys Res. Commun.*, **2009,** 381, 566-571.

32. Hwang, D.; Huyn, J.; Jo, G.; Koh, D.; Lim, Y. *Magn. Reson. Chem.*, **2011,** 49, 41-45.

33. Modzelewska, A.; Pettit, C.; Achanta, G.; Davidson, N. E.; Huang, P.; Khan, S. R. *Bioorg. Med. Chem.*, **2006,** 14, 3491-3495.

34. D'silva, E. D.; Rao, D. N.; Philip, R.; Butcher, R. J.; Rajnikant, Dharmaprakash, S. M. *J. Phys. Chem. Solids,* **2011,** 72, 824–830.

35. Detsi, A.; Majdalani, M.; Kontogiorgis, C. A.; Litina, D. H.; Kefalas, P. *Bioorg. Med. Chem.*, **2009,** 17, 8073–8085.

36. Bondock, S.; Naser, T.; Ammar, Y. A. *Eur. J. Med. Chem.,* **2013,** 62, 270–279.

37. Mishra, L.; Itokawa, H.; Bastow, K. F.; Tachibana, Y.; Nakanishi, Y.; Kilgore, N.; Lee, K. H.; Sinha, R. *Bioorg. Med. Chem.* **2001,** 9, 1667–1671.

38. Kaveri, M. V.; Prabhakaran, R.; Karvembu, R.; Natarajan, K. *Spectrochim. Acta Part: A,* **2005,** 61, 2915–2918.

39. Herencia, F.; Ferrandiz, M. L.; Ubeda, A.; Dominguez, J. N.; Charris, J. E.; Lobob, G. M.; Alcaraz, M. *J. Bioorg. Med. Chem. Lett.,* **1998,** 8, 1169–1174.

40. Hayat, F.; Moseley, E.; Salahuddin, A.; Zyl, R. L. V.; Azam, A. *Eur. J. Med. Chem.*, 2011, 46, 1897–1905.

41. Kim, S. H.; Lee, E.; Baek, K. H.; Kwon, H. B.; Woo, H.; Lee, E. S.; Kwon, Y.; Na, Y. *Bioorg. Med. Chem. Lett.,* **2013**, 23, 3320–3324.

42. Jin, C.; Liang, Y. J.; He, H.; Fu, L. *Biomed. Pharmacother.*, **2013,** 67, 215–217.

43. Sabzevari, O.; Galati, G.; Moridani, M. Y.; Siraki, A.; O'Brien, P. J. *Chem. Biol. Interact.*, **2004,** 148, 57–67.

44. Kamal, A.; Ramakrishna, G.; Raju, P.; Viswanath, A.; Ramaiah, M. J.; Balakishan, G.; Bhadra, M. P. *Bioorg. Med. Chem. Lett.*, **2010,** 20, 4865–4869.

45. Gul, H. I.; Yerdelen, K. O.; Gul, M.; Das, U.; Pandit, B.; Li, P. K.; Secen, H.; Sahin, F. *Arch. Pharm. Chem. Life Sci*., **2007,** 340, 195–201.

46. McBee, E. T.; Okuhara, K.; Morton, C. J. *Inorganic Chemistry,* **1965,** 4, 1672-1673.

47. Tumer, Y.; Koc, L. Y.; Asmafiliz, N.; Kılıc, Z.; Hokelek, T.; Soltanzade, H.; Acık, L.; Yola, M. L.; Solak, A. O. *J. Biol. Inorg. Chem.*, **2015,** 20, 165–178.

48. Görgülü, A. O.; Koran, K.; Özen, F.; Tekin, S.; Sandal, S. *J. Mol Struct.*, **2015,** 1087, 1-10.

49. Machakanur, S. S.; Patil, B. R.; Naik, G. N.; Bakale, R. P.; Bligh, S. W. A.; Gudasi, K. B. *Inorg. Chim. Acta,* **2014,** 421, 459-464.

50. Yıldırım T.; Bilgin, K.; Çiftçi, G. Y.; Eçik, E. T.; Şenkuytu, E.; Uludağ, Y.; Tomak, L.; Kılıç, A. *Eur. J. Med. Chem.,* **2012**, 52, 213-220.

51. Akram, M.; Li, W.; Yu, H.; Amer, W. A.; Khalid, H.; Abbasi, N. M.; Chen, Y.; Abdin, Z.; Saleem, M.; Tong, R. *Prog. Polym. Sci.,* **2014,** 39, 1987-2009.

52. Xu, J.; Zhu, C.; Qiu, L. *Int. J. Pharm.,* **2016,** 498, 70-81.

53. Mosmann, T. R.; Cherwinski, H.; Bond, M. V.; Giedlin, M. A.; Coffmann, R. L. *J. Immunol.,* **1986,** 136, 2348-2357.

54. Kucukbay, H.; Mumcu, A.; Tekin, S.; Sandal, S. *Turk. J. Chem.,* **2016,** 40, 393-401.

55. Singh, N. K.; Singh, S. B. *Synth. React. Inorg. Met-Org. Chem.,* **2002,** 32, 25-47.

56. Özen, F.; Tekin, S.; Koran, K.; Sandal, S.; Görgülü, A. O. *Appl. Bio. Chem.,* **2016,** 59:239-248.

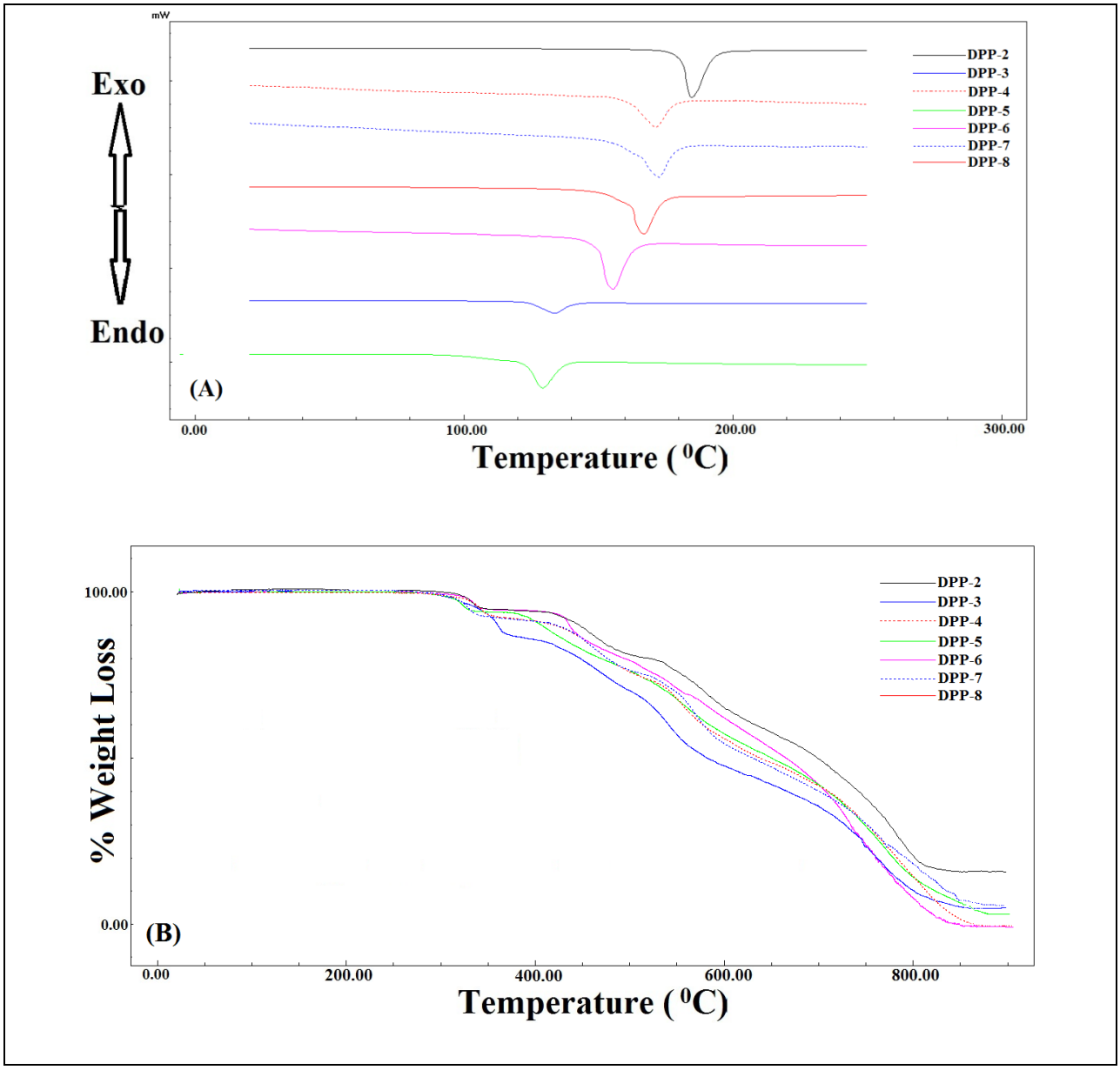


Figure 1. (A) The DSC curves heated under nitrogen to 250 ^{0}C at a heating rate 10 ^{0}C min^{-1} and (B) the TG curves of compounds **DPP 2-8** heated under nitrogen to 900 ^{0}C at a heating rate 20 ^{0}C min^{-1}.

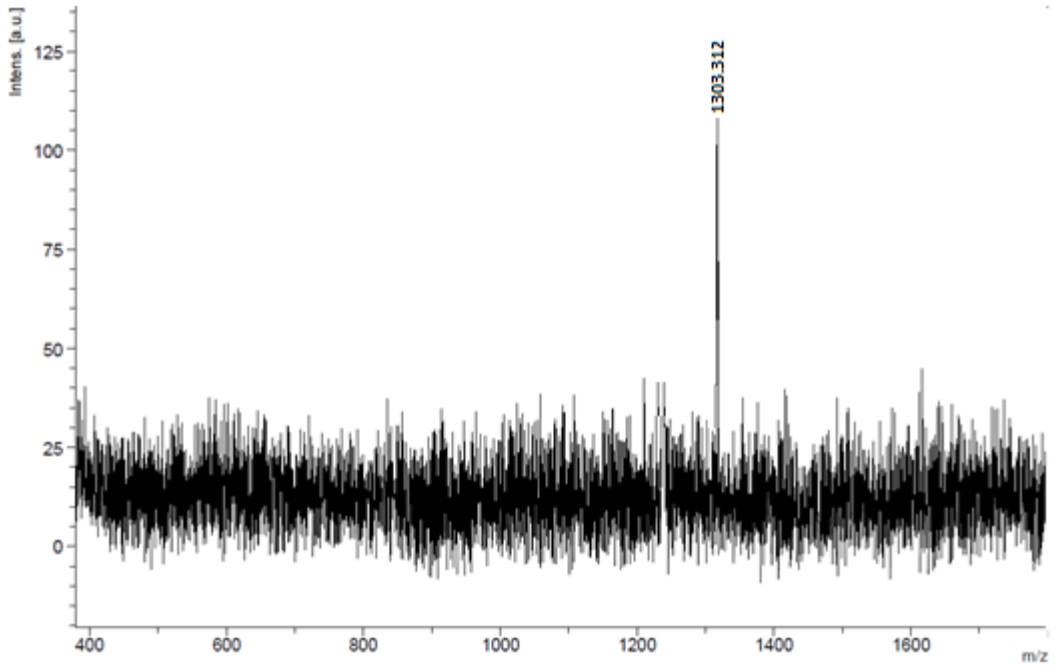


Figure 2. Positive ion and linear mode MALDI TOF-MS spectrum of compound **DPP-5** was obtained in 1,8,9-anthracenetriol (20 mg/mL THF) MALDI matrix using nitrogen laser accumulating 50 laser shots.

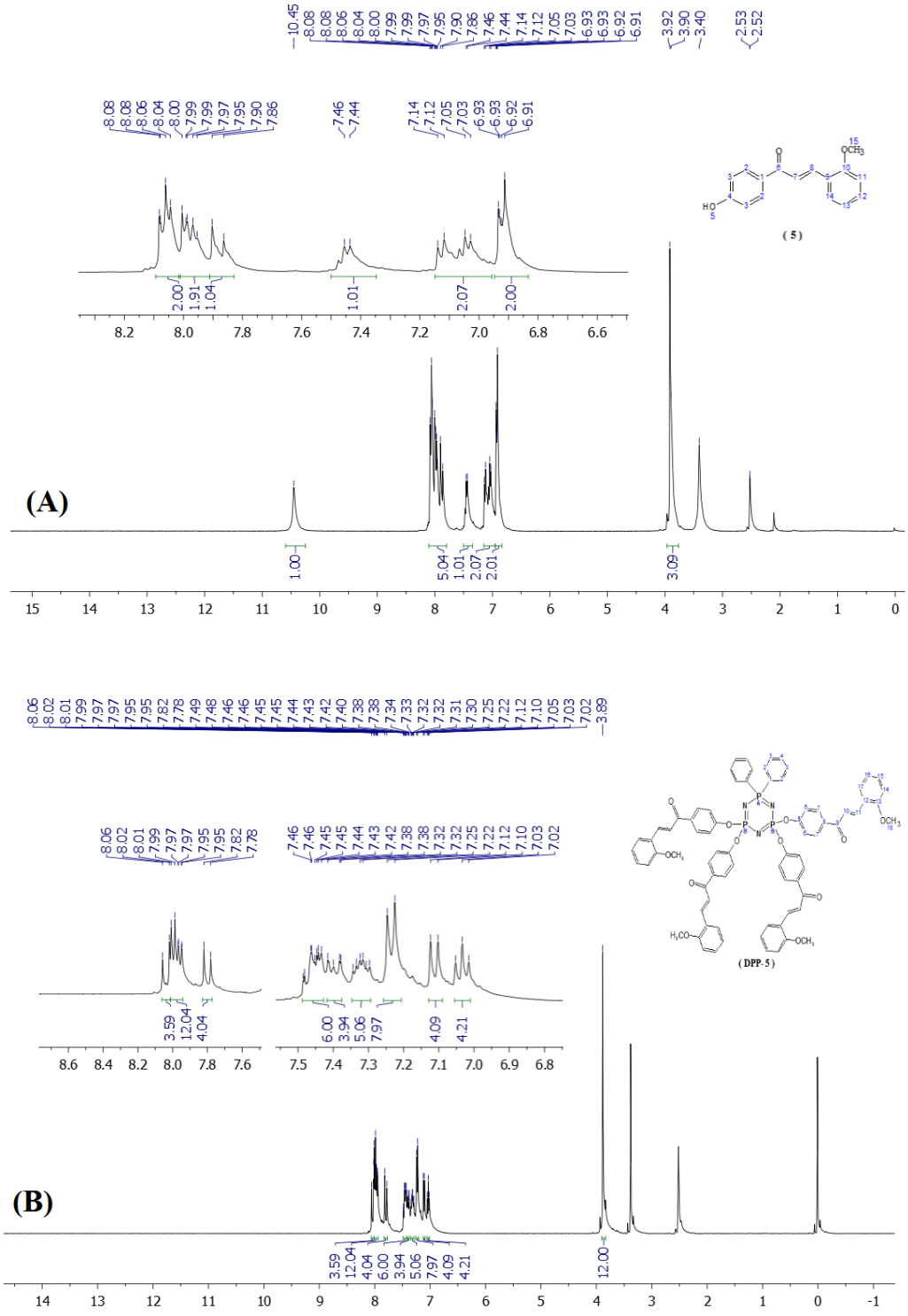
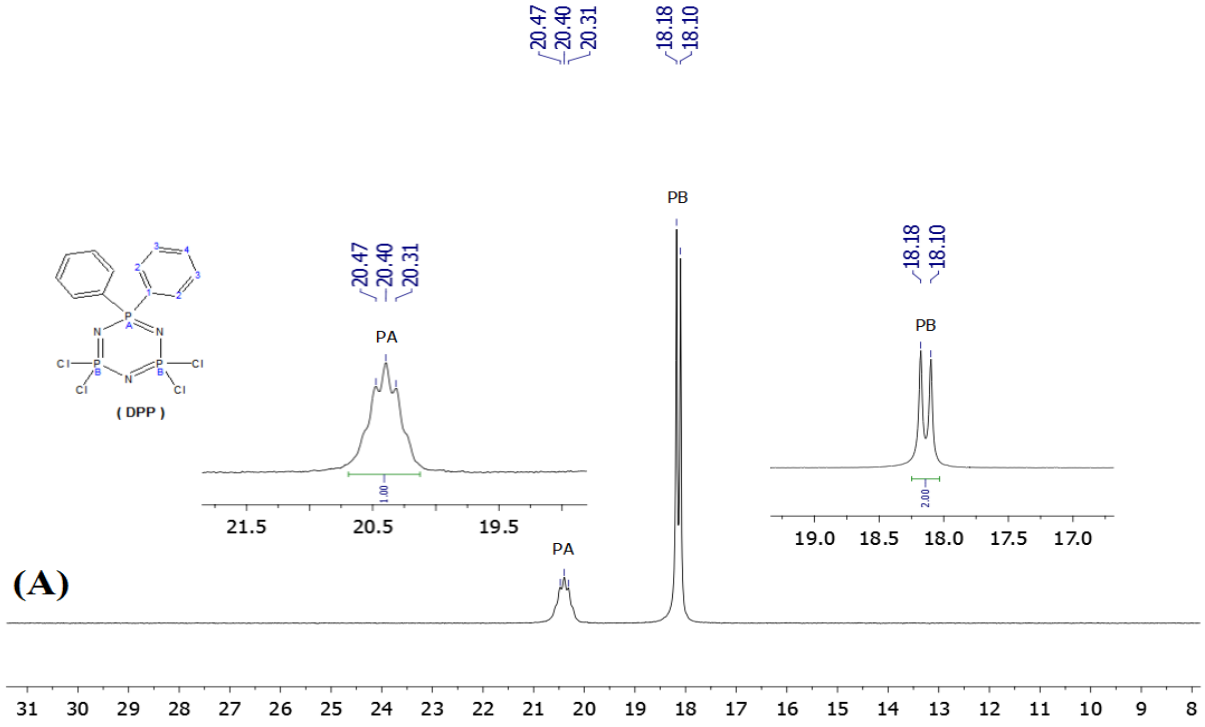


Figure 3. ^{1}H-NMR spectrum of compounds **5 (A)** and **DPP-5 (B)** (DMSO-d_6).



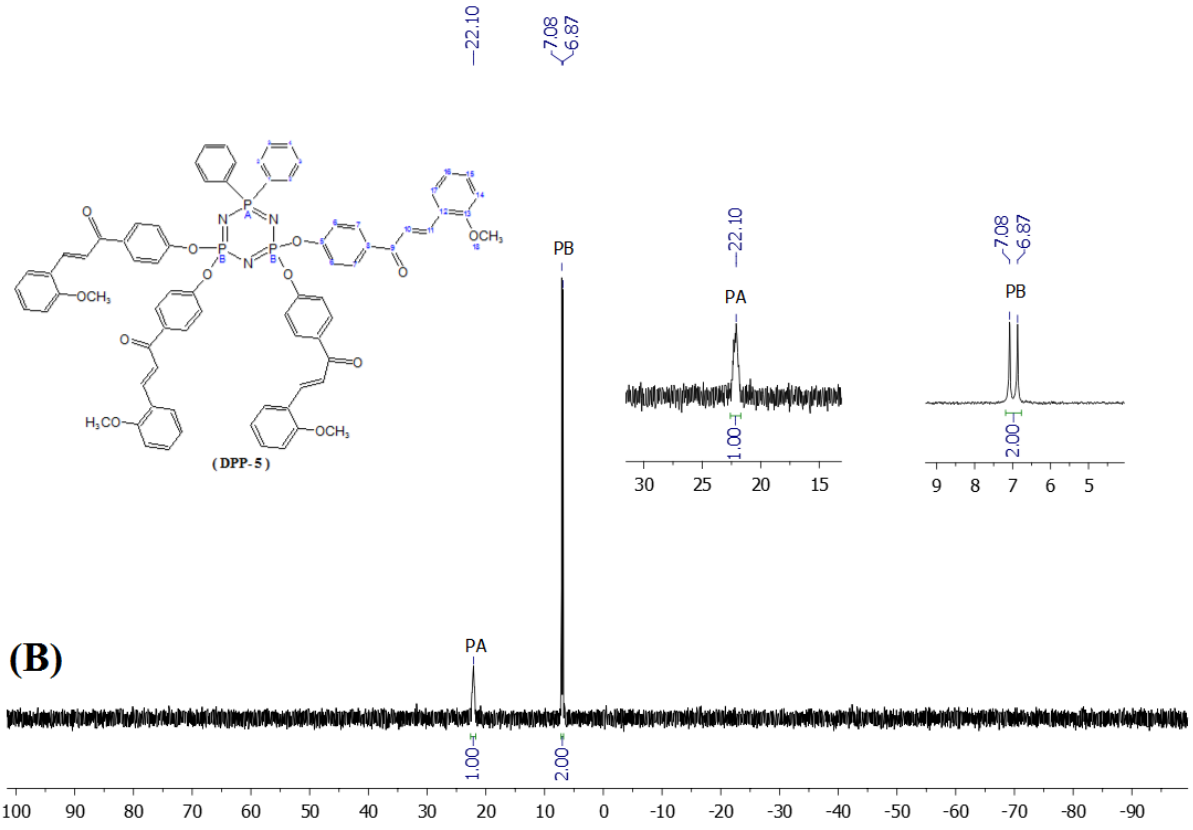


Figure 4. ^{31}P-NMR spectrum of compounds **5 (A)** and **DPP-5 (B)**.

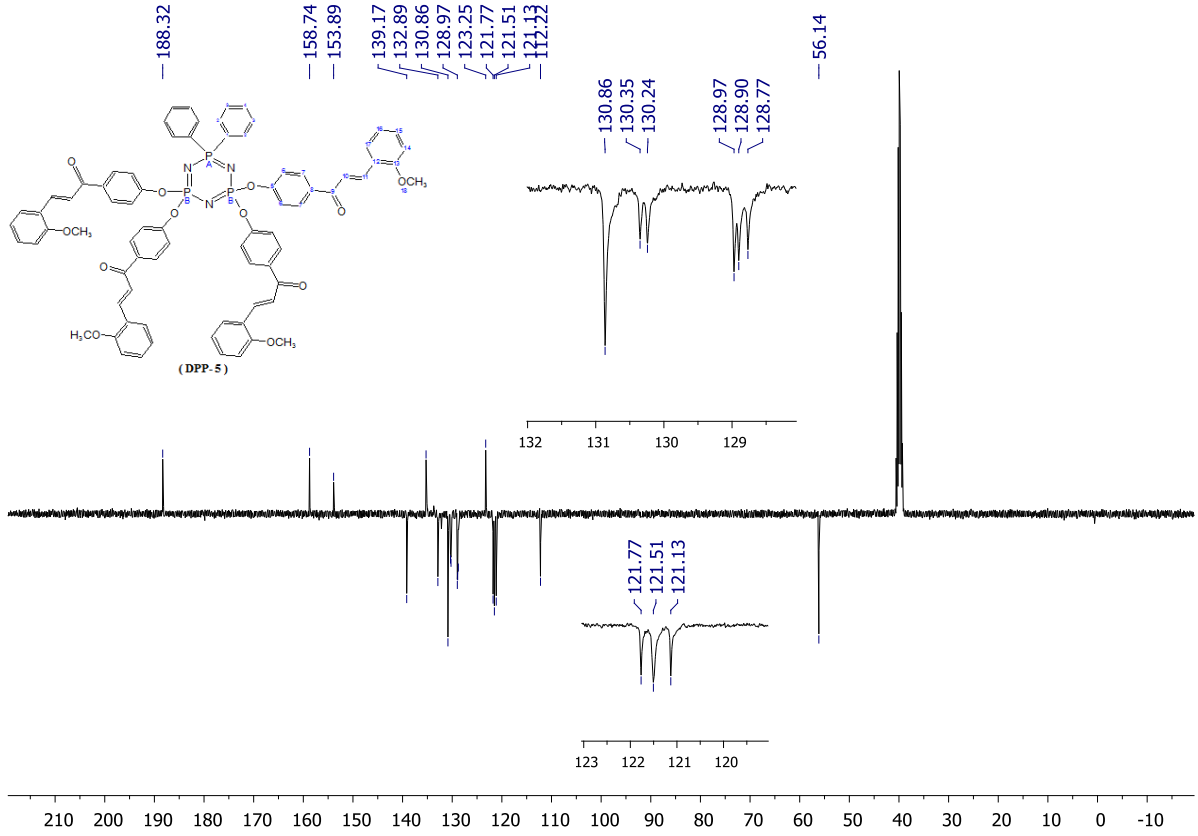
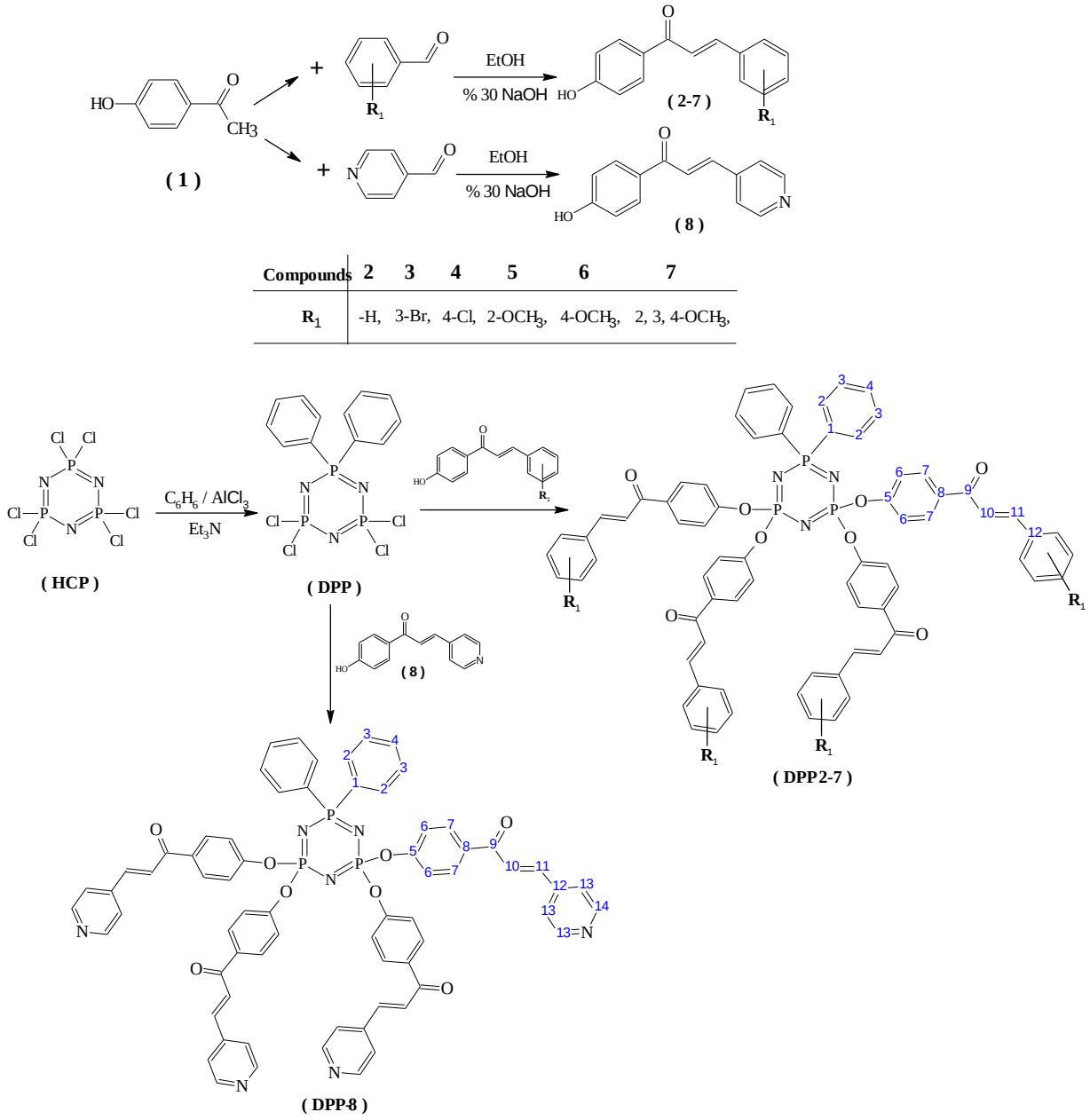


Figure 5. ^{13}C APT-NMR spectrum of **DPP-5** (DMSO-d_6).



Compounds	2	3	4	5	6	7
R_1	-H,	3-Br,	4-Cl,	2-OCH_3,	4-OCH_3,	2, 3, 4-OCH_3,

Scheme 1. Chemical structure and synthetic pathway of compounds **2-8** and **DPP 2-8**