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Synthesis, structural characterization and anti-carcinogenic activity of new cyclotriphosphazenes containing dioxybiphenyl and chalcone groups

Ahmet Orhan Görgülü [a], Kenan Koran [a,*], Furkan Özen [a], Suat Tekin [b], Süleyman Sandal [b]

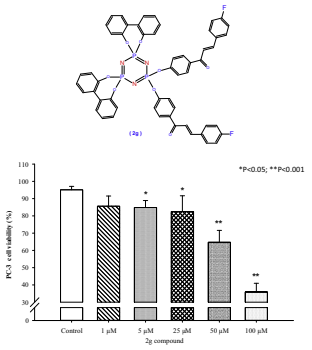
[a] Firat University, Faculty of Science, Department of Chemistry, 23169 Elazig, Turkey
[b] Inonu University, Faculty of Medicine, Department of Physiology, 44000 Malatya, Turkey

HIGHLIGHTS

- Compounds synthesized for the first time.
- And show antitumor activity.
- The effective dose is 100 μM.

GRAPHICAL ABSTRACT

The chalcone-cyclophosphazene compounds containing dioxybiphenyl groups (**2a–2h**) were synthesized. *In vitro* anti-carcinogenic activities of these compounds were performed by using MTT assay against PC-3 and LNCaP cancer cell lines. Results, these compounds (**2a–2h**) were found to have anti-tumor activity against PC-3 and LNCaP cancer cell lines.



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ABSTRACT

2,2-Dichloro-4,4,6,6-bis[spiro(2′,2″-dioxy-1′,1″-biphenylyl]cyclotriphosphazene **(2)** was synthesized from hexachlorocyclotriphosphazene **(HCCP)** and 2,2′-dihydroxybiphenyl. The mixed substituent chalcone/dioxybiphenyl cyclophosphazenes (**2a–h**) were obtained from the reactions of **(2)** with hydroxy chalcone compounds in K_2CO_3/acetone system. The chalcone-cyclophosphazene compounds were characterized by elemental analysis, FT-IR, ^1H, ^{13}C, ^{31}P NMR techniques. *In vitro* anti-carcinogenic activities of all compounds were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Anti-carcinogenic activity of the compounds **(2a–h)** against androgen-dependent (LNCaP) and independent (PC-3) human prostate cancer cell lines were investigated. Our results indicate that the chalcone-phosphazene compounds **(2a–h)** have anti-carcinogenic activity on PC-3 and LNCaP cell lines ($p < 0.05$). The effective dose of the compounds was determined as 100 μM.

Introduction

Phosphazenes are molecules which contain —P=N— bonds. There are three important types of phosphazenes, such as linear,

* Corresponding author at: Department of Chemistry, Firat University, 23119 Elazig, Turkey. Fax: +90 424 2330062.
E-mail address: kumfosfit@gmail.com (K. Koran).

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cyclic and poly. Trimer, tetramer and linear polyphosphazenes are the most known and studied types of phosphazenes [1].

The phosphazene derivatives have various physical and biological properties, for example liquid crystals [2,3], electrical conductivity [4], flame retardants [5–7], electrolytes for rechargeable batteries [8], fire resistant materials [9], dielectric properties [10], biomedical applications [11,12], antimicrobial, antibacterial [13–18], anti-leukemic [19] and strong anti-tumor activity [20–27].

Chalcones are compounds that can be prepared by the Claisen–Schmidt condensation reaction [28,29]. Because of the ketovinylenic group in chalcones and their analogs, they exhibit numerous physical and biological properties, for instance optical and fluorescence properties [30,31], dielectric properties [32,33], antioxidant and soybean lipoxygenase inhibitory activity [34], antimicrobial activity [35], Anti-HIV activity [36], antibacterial activity [37], anti-inflammatory [38] and anti-cancer activities [39–44].

The synthesis of different phosphazene compounds has been reported [13,45–53] but there are only four articles about synthesis of the phosphazene compounds bearing chalcone groups [10,54–56], there are, however, no studies about synthesis of dioxybiphenyl substituted chalcone-cyclophosphazene compounds. The cyclotriphosphazenes bearing 2,2′-dihydroxybiphenyl are much more stable to hydrolysis and thermal decomposition than hexachlorocyclotriphosphazene [1].

In this study, the chalcone compounds containing —OH groups were synthesized. And then these chalcone compounds **(1a–h)** were reacted with 2,2-dichloro-4,4,6,6,-bis[spiro(2′,2″-dioxy-1′,1″-biphenylyl)]cyclotriphosphazene in order to get substituted products. As a result, cyclophosphazenes bearing 2,2′-dioxybiphenyl groups and chalcone compounds were synthesized and characterized by elemental analysis, FT-IR, ^{1}H, ^{13}C, ^{31}P NMR techniques. Antitumor properties of these compounds were investigated by MTT ([3-(4,5-dimethylthiazol)-2-yl]-2,5-diphenyl-2H-tetrazolium bromide]) assay. The MTT assay is a simple procedure to determine living and growing cells without using radioactivity. Our results indicate that the chalcone-phosphazene compounds displayed potential antitumor activity towards on human prostate cancer cell lines (PC-3 and LNCaP).

Experimental

Materials and methods

Solvents and other liquids were purified by traditional methods. Hexachlorocyclotriphosphazene, $N_3P_3Cl_6$ (TCI), was crystallized from *n*-hexane. The chemicals were purchased from Merck and Sigma Aldrich. All reactions were monitored using thin-layer chromatography (TLC). The prostate carcinoma (PC-3 and LNCaP) and human breast (MCF-7) cancer cell lines were retrieved from the American Type Culture Collection (ATCC). Calf serum, trypsin, penicillin and streptomycin were purchased from Hyclone (Waltham, MA, USA).

FT-IR spectra were recorded on Perkin Elmer FT-IR spectrometer. Microanalysis was carried out by a LECO 932 CHNS-O apparatus. 1D (^{1}H, ^{13}C, ^{13}C APT and ^{31}P NMR) spectra were recorded using a Bruker DPX-400 spectrometer. The ^{1}H, ^{13}C and ^{31}P NMR chemical shifts were measured using TMS as an internal standard, whereas those for ^{31}P were measured using 85% H_3PO_4 as an external standard. For the NMR studies acetone-d6 was used as solvent for the compounds **2a** and **2d**. The chloroform-d was used as solvent for the compounds **2b**, **2c**, **2e**, **2f**, **2g** and **2h**.

Synthesis

4′-Hydroxy chalcone compounds were prepared by reaction of 4′-hydroxyacetophenone with various benzaldehydes [28,29]. 2,2-Dichloro-4,4,6,6-bis[spiro(2′,2″-dioxy-1″,1″-biphenyl]cyclotriphosphazene **(2)** was made as defined by Carriedo et al. [57]. The reaction of $[N_3P_3Cl_6]$ with the 2,2′-dihydroxybiphenyl took place under inert atmosphere.

Preparation of substituted chalcone-phosphazenes

Chalcone-phosphazene compounds **(2a–2h)** were synthesized by similar methods; therefore, the experimental method for the synthesis of these compounds is only explained in detail for the first case.

*Synthesis of 2,2-(4′-oxychalcone)-4,4,6,6-bis[spiro(2′,2″-dioxy-1′,1″-biphenylyl] cyclotriphosphazene **(2a)**.* A mixture of compound **2** (1.0 g, 1.75 mmol) and K_2CO_3 (0.97 g, 7.0 mmol) in 50 mL dry acetone was slowly added, over 0.5 h, to a stirred solution of 4′-hydroxychalcone **(1a)** (0.9 g, 4.03 mmol) in 20 mL of dry acetone at 0 °C and then refluxed for 7 h. The solvent was evaporated. The residue was extracted with CH_2Cl_2 (4 × 25 mL) and then washed with 5% KOH solution four times and then dried over anhydrous magnesium sulfate. The solvent was concentrated on a rotary evaporator. After the solvent was removed, a white solid (**2a**) formed 1.49 g (90%). *Anal.* Calc. for $C_{54}H_{38}N_3O_8P_3$ (MW = 949.82): C, 68.28; H, 4.03; N, 4.42. Found: C, 68.02; H, 4.12; N, 4.49%. IR (KBr, cm^{-1}): 3061 and 3027 $\nu_{C-H(Ar.)}$, 2933 $\nu_{C-H(Aliphatic)}$, 1664 $\nu_{C=O}$, 1605, 1576 and 1567 $\nu_{C=C}$, 1175 and 1206 $\nu_{P=N}$, 1273 ν_{P-N-P}, 936 ν_{P-O-C}. ^{31}P NMR (Aceton-d_6) δ/ppm: 25.02 (2P, d, $P_a(O_2C_{12}H_8)$), 9.62 (1P, t, $P_b(O_4C_{30}H_{22})$). ^{1}H NMR (Aceton-d_6) δ/ppm: 8.40 (4H, d, H^9), 8.12 (4H, d, H^{13}), 7.98–7.76 (10H, m, H^{15}, H^{16} and H^{17}), 7.68 (2H, d, H^{12}), 7.62 (4H, d, H^3), 7.53–7.42 (8H, m, H^4 and H^5), 7.24 (4H, d, H^6), 7.0 (4H, d, H^8). ^{13}C NMR (Aceton-d_6) δ/ppm: 187.66 C^{11}, 153.94 C^7, 147.72 C^1, 144.06 C^{13}, 135.47 C^{14}, 134.90 C^{10}, 130.52 C^9, 129.88 C^5, 129.60 C^3, 128.76 C^{16}, 128.53 C^{15}, 128.32 C^2, 128.29 C^{17}, 126.33 C^4, 121.61 C^6, 121.13 C^{12}, 115.13 C^8.

*Synthesis of 2,2-(2′-oxy-2-methylchalcone)-4,4,6,6-bis[spiro(2′,2″-dioxy-1′,1″-biphenylyl]cyclotriphosphazene **(2b)**.* 4′-Hydroxy-2-methylchalcone **(1b)** (0.95 g, 4.03 mmol), 9 h. Yield: 1.27 g, 75%. *Anal.* Calc. for $C_{56}H_{42}N_3O_8P_3$ (MW = 977.87): C, 68.78; H, 4.33; N, 4.30. Found: C, 68.82; H, 4.26; N, 4.35%. IR (KBr, cm^{-1}): 3063 and 3027 $\nu_{C-H(Ar.)}$, 2947 and 2924 $\nu_{C-H(Aliphatic)}$, 1662 $\nu_{C=O}$, 1597, 1500 and 1477 $\nu_{C=C}$, 1175 and 1203 $\nu_{P=N}$, 1274 ν_{P-N-P}, 936 ν_{P-O-C}. ^{31}P NMR (chloroform-d) δ/ppm: 25.41 (2P, d, $P_a(O_2C_{12}H_8)$), 8.93 (1P, t, $P_b(O_4C_{32}H_{26})$). ^{1}H NMR (chloroform-d) δ/ppm: 8.15–8.20 (6H, m, H^9, H^{13}), 7.74 (2H, d, H^{12}), 7.54–7.56 (8H, m, H^3 and H^5), 7.52 (2H, d, H^{19}), 7.40–7.44 (4H, m, H^{17} and H^{18}), 7.33–7.37 (6H, m, H^4 and H^{16}), 7.28 (4H, d, H^6), 7.14 (4H, d, H^8), 2.51 (6H, s, H^{20}). ^{13}C NMR (chloroform-d) δ/ppm: 189.12 C^{11}, 154.29 C^7, 147.96 C^1, 142.76 C^{13}, 138.49 C^{15}, 135.35 C^{14}, 133.82 C^{10}, 130.99 C^{16}, 130.48 C^9, 130.43 C^{17}, 129.84 C^5, 129.71 C^3, 128.68 C^2, 126.46 C^4, 126.26 C^{19}, 122.72 C^{18}, 121.77 C^6, 121.30 C^{12}, 115.45 C^8, 19.92 C^{20}.

*Synthesis of 2,2-(4′-oxy-3-methylchalcone)-4,4,6,6-bis[spiro(2′,2″-dioxy-1′,1″-biphenylyl]cyclotriphosphazene **(2c)**.* 4′-Hydroxy-3-methylchalcone **(1c)** (0.95 g, 4.03 mmol), 8 h. Yield: 1.19 g, 70%. *Anal.* Calc. for $C_{56}H_{42}N_3O_8P_3$ (MW = 977.87): C, 68.78; H, 4.33; N, 4.30. Found: C, 68.70; H, 4.35; N, 4.39%. IR (KBr, cm^{-1}): 3062 and 3031$\nu_{C-H(Ar.)}$, 2954 and 2920$\nu_{C-H(Aliphatic)}$, 1663 $\nu_{C=O}$, 1601, 1584, 1500 and 1477$\nu_{C=C}$, 1175 and 1201$\nu_{P=N}$, 1273ν_{P-N-P}, 936 ν_{P-O-C}. ^{31}P NMR (chloroform-d) δ/ppm: 24.83 (2P, d, $P_a(O_2C_{12}H_8)$), 8.99 (1P, t, $P_b(O_4C_{32}H_{26})$). ^{1}H NMR (chloroform-d) δ/ppm: 8.14–8.16 (6H, m, H^9, H^{13}), 7.83 (2H, d, H^{12}), 7.54–7.58 (8H, m, H^3 and H^5), 7.49 (2H, d, H^{19}), 7.40–7.44 (4H, m, H^{17} and H^{18}), 7.32–7.37 (6H, m, H^4 and H^{15}), 7.28 (4H, d, H^6), 7.14 (4H, d, H^8), 2.43 (6H, s, H^{20}). ^{13}C NMR (chloroform-d) δ/ppm: 189.29 C^{11}, 154.25 C^7, 147.97 C^1, 145.40 C^{13}, 138.70 C^{16}, 135.38 C^{14}, 134.73 C^{10}, 131.57 C^{15}, 130.47