GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gscbps.gsconlinepress.com/

(RESEARCH ARTICLE)

EVALUATION OF DNA CLEAVAGE AND BINDING ACTIVITIES OF A PYRIDINE-CONTAINING CYCLOPHOSPHAZENE LIGAND

Nurşen Çördük [1,*], Diğdem Erdener Çıralı [2] and Osman Dayan [2]

[1] Department of Biology, Faculty of Sciences, Çanakkale Onsekiz Mart University, Çanakkale 17020, Türkiye.
[2] Department of Chemistry, Faculty of Sciences, Çanakkale Onsekiz Mart University, Çanakkale 17020, Türkiye.
* Corresponding Author

ORCID Details
Nurşen Çördük: https://orcid.org/0000-0001-8499-4847
Diğdem Erdener Çıralı: https://orcid.org/0000-0002-4226-0455
Osman Dayan: https://orcid.org/0000-0002-0764-1965

GSC Biological and Pharmaceutical Sciences, 2026, 36(03), 139–144

Publication history: Received on 08 August 2026; revised on 13 September 2026; accepted on 16 September 2026

Article DOI: https://doi.org/10.30574/gscbps.2026.36.3.0328

ABSTRACT

Phosphazenes are a class of chemical compounds characterized by -N=PX_2- repeating units, in which the phosphorus atom is covalently bonded to a nitrogen atom by a double bond and to two additional substituents by single bonds. These units can assemble into cyclic or linear chain architectures, forming one of the most distinctive classes of inorganic molecules. Compounds formed by the repetition of this group in the molecule, which can be in ring or straight chain structure, constitute one of the most remarkable classes of inorganic molecules. In this study, it was aimed to determine the DNA cleavage and binding activity of a previously synthesized and structurally elucidated cyclophosphazene compound containing pyridine. It was determined that cyclophosphazene ligand was hydrolytic and oxidative cleavage effects on pBR322 plasmid DNA. Furthermore, the changes in absorbance of the ligand upon increasing DNA concentrations were analyzed by UV-Vis spectroscopy, and it was determined that the compound binds to Calf Thymus DNA (CT-DNA) via intercalation. These findings demonstrate that the pyridine-containing cyclophosphazene ligand exhibits measurable DNA binding and DNA cleavage activity, thus providing a basis for further studies on its biological properties.

Keywords: Phosphazene, Pyridine, DNA, Cleavage Activity, Biological Activity

1 INTRODUCTION

Phosphazenes are chemical compounds containing the [-N=PX_2-] structural unit in which a phosphorus atom is covalently bonded to a nitrogen atom through a double bond and to three other atoms through single bonds. The phosphorus atoms are typically bonded to two substituent groups (X), giving the general formula $(NPX_2)_n$, where X may be halogens, alkoxy groups, amino groups, aryloxy groups, or other organic substituents. Depending on the value of n, phosphazenes can exist as cyclic (cyclophosphazenes) or linear polymeric (polyphosphazenes) structures. Repetition of this structural motif produces either cyclic or linear frameworks, forming a versatile class of inorganic compounds distinguished by their structural diversity and tunable physicochemical properties [1-3]. Cyclophosphazenes are cyclic

phosphazene derivatives composed of alternating phosphorus and nitrogen atoms linked through P=N bonds to form ring structures. Owing to their highly reactive P–Cl bonds, cyclophosphazenes provide a versatile platform for the introduction of diverse organic and inorganic substituents, enabling precise control over their chemical and physicochemical properties. Phosphazenes can be used as ligands for transition metals because they have a skeletal structure that contains nitrogen atoms—which act as donor atoms—and to which side groups containing donor atoms can be attached. The nitrogen atoms in the ring are basic. The basicity of the nitrogen atom—and, consequently, the coordination ability of the phosphazene ring—increases with the addition of electron-donating groups on the phosphorus atom and with an increase in ring size, which enhances the flexibility of the cyclophosphazene ring [4-8].

Deoxyribonucleic acid (DNA) is the molecule that carries the genetic instructions used in the growth, development, functioning, and reproduction of all living organisms. DNA is made up of chemical building blocks called nucleotides. Each nucleotide consists of a five-carbon deoxyribose sugar, a phosphate group, and one of four nitrogenous bases: adenine (A), thymine (T), cytosine (C), or guanine (G). Nucleotides are covalently linked through their sugars and phosphate groups, forming an alternating sugar-phosphate backbone. DNA contains two polynucleotide strands that are held together by hydrogen bonds between complementary bases. The bases are located on the inside, while the sugar-phosphate backbones form the outside of the molecule. This arrangement gives DNA its characteristic three-dimensional double-helix structure [9]. DNA is one of the most important biological targets for a wide range of natural and synthetic compounds. Interactions between molecules and DNA play a crucial role in numerous biological processes. Compounds can interact with DNA by binding to different regions of the molecule through both covalent and non-covalent interactions. In covalent interaction, bonds are formed between molecules and the donor atoms of DNA molecules. Molecules can bind non-covalently to DNA through several distinct modes, including electrostatic interactions with the negatively charged sugar–phosphate backbone, binding within the major or minor groove, intercalation between adjacent base pairs via either groove, or threading intercalation. [10-11]. In addition to binding interactions, certain molecules can induce DNA cleavage. One or both strands of DNA can be cleavage through oxidative and hydrolytic. The generally accepted mechanism of the DNA hydrolytic reaction is a nucleophilic attack on the phosphate backbone. The hydrolytic process results in the breaking of phosphodiester bonds and the fragmentation of DNA. In the oxidative reaction, it involves the oxidation of deoxyribose through the removal of sugar hydrogen or the oxidation of nucleobases. Light or H_2O_2 are usually required to initiate oxidative damage. Oxidative damage to DNA can affect all four nucleobases or deoxyribose sugars, and no re-ligation occurs after breakage [12-13].

Among the available techniques for studying compound–DNA interactions, absorption spectroscopy is one of the most widely employed owing to its simplicity and rapidity. In this method, the binding mode can be determined by identifying shifts in wavelength and changes in absorption intensity after the compound interacts with DNA. Specifically, hyperchromic or hypochromic effects, as well as bathochromic or hypsochromic shifts, provide diagnostic evidence for the nature of the binding interaction [14]. Gel electrophoresis is widely employed to evaluate DNA–compound interactions, as it provides a straightforward means of visualizing and quantifying structural changes and cleavage events induced in DNA [15].

The interaction between synthesized compounds and biomolecules, especially DNA, has emerged as a significant focus of research. The interaction of complexes with DNA is of paramount importance, as DNA serves as a principal target for numerous therapeutic agents. A comprehensive understanding of the mechanisms underlying these interactions and the potential for DNA damage is essential for advancements in drug development and the evaluation of toxicological effects. Drugs or compounds can interact with DNA through electrostatic, intercalative, and non-covalent bonding, and these interactions vary depending on the structure of the compound [16].

The aim of this study is to determine the interaction of the cyclophosphazene compound, which was previously synthesized and whose structure was elucidated, with DNA.

2 MATERIALS AND METHODS

2.1 Synthesis of Cyclophosphazene Ligand

The cyclophosphazene ligand was synthesized using the method described below [17] (Figure 1).

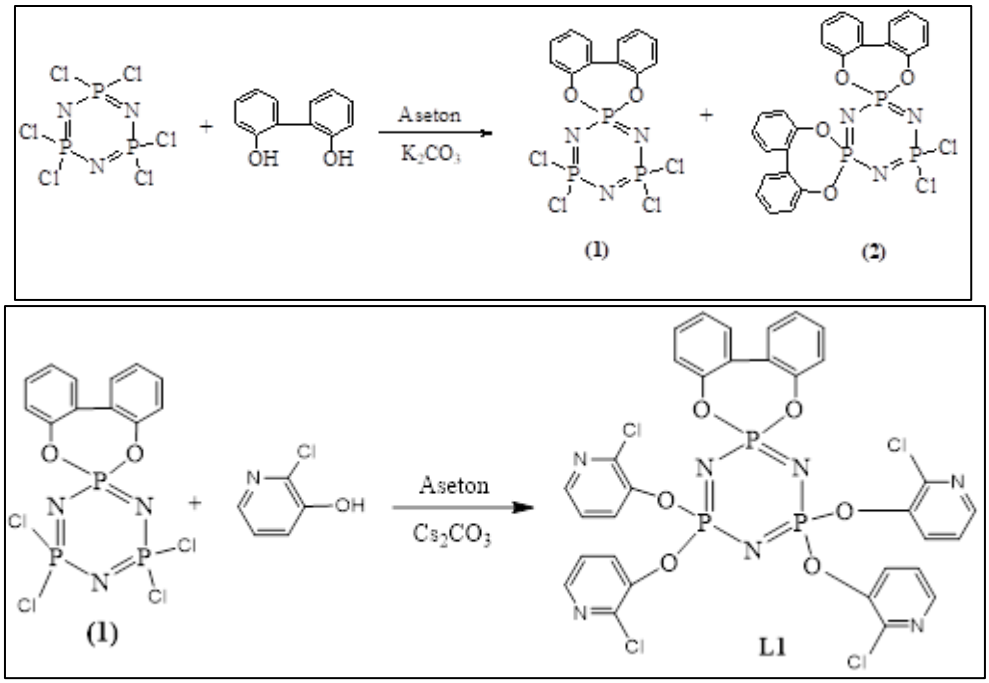

Figure 1*:* Synthesis of cyclophosphazene ligand containing pyridine derivative.

2.2 Cleavage Activity of Cyclophosphazene Ligand

The cleavage activity of the cyclophosphazene ligand was assessed at various concentrations, both hydrolytically and oxidatively by gel electrophoresis using supercoiled pBR322 DNA. For the hydrolytic cleavage assessment, 2 µL of pBR322 plasmid DNA was reacted with different concentrations of the ligand (156, 312, 625, 1250, 2500 and 5000 µM) in a final volume of 20 mL. For the oxidative activity, hydrogen peroxide (H_2O_2) was added to the DNA as an inducing agent. The mixtures were incubated with plasmid DNA in an incubator at 37°C for 24 h in the dark. A 1% agarose gel was prepared for electrophoresis. To analyze the reaction products, 10 µL of each sample was mixed with 2 µL of 6X loading buffer and loaded onto the gel. Gel electrophoresis was carried out at 70 V in 1 hour. After electrophoresis, the gel was stained with ethidium bromide and visualized with Quantum Gel Documentation and Analysis System.

2.3 Binding Activity of Cyclophosphazene Ligand

The DNA binding activity of the cyclophosphazene ligand was determined using the UV-Vis titration method with Calf Thymus DNA (CT-DNA). TNE buffer solution (10 mM Tris-HCl, 50 mM NaCl, and 1 mM EDTA, pH 7.4) was used throughout the experiment. A stock solution of the synthesized ligand was prepared in 25 mM DMSO. The ligand solution, diluted to 1.25×10-7 M, was titrated with increasing amounts of the DNA solution, added at 5-minute intervals, and absorbance measurements were recorded over the range of 200–600 nm using a UV-Vis spectrophotometer. CT-DNA additions continued until the absorption values stabilized.

3 RESULTS AND DISCUSSION

3.1 DNA Cleavage Activity of Cyclophosphazene Ligand

The DNA cleavage activity of the cyclophosphazene ligand has been determined using pBR322 plasmid DNA. All concentration of cyclophosphazene compound (156 µM, 312 µM, 625 µM, 1250 µM, 2500 µM, and 5000 µM) hydrolytically cleavage DNA, causing formation of linear form (Form III) as well as Form IV, which resulted from extensive DNA fragmentation (Figure 2).

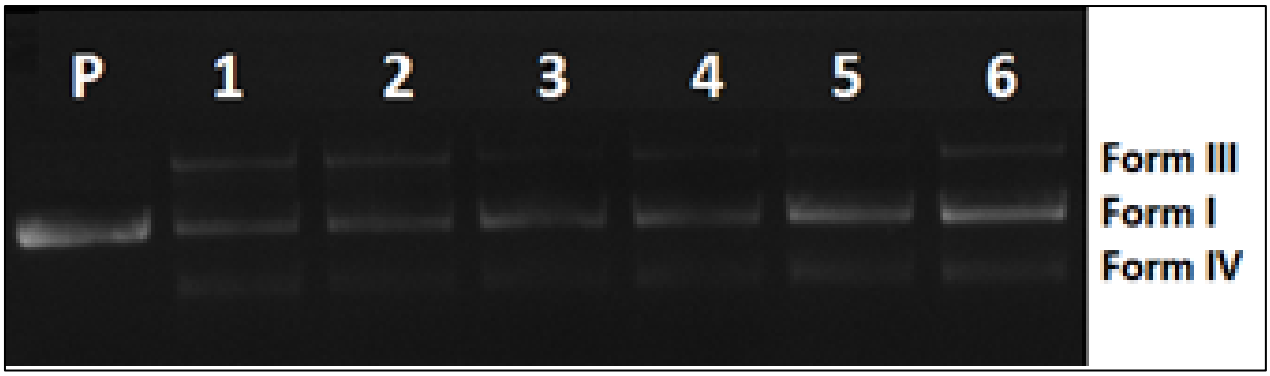

Figure 2*:* Hydrolytic DNA cleavage P: pBR322 plasmid DNA, 1: DNA+ 156 µM, 2: DNA+ 312 µM, 3: DNA+ 625 µM, 4: DNA+ 1250 µM, 5: DNA+ 2500 µM, and 6: DNA+ 5000 µM cyclophosphazene ligand.

As a result of the oxidative DNA cleavage at, Form III, along with Form I, as well as Form IV were observed at concentrations of 156 µM, 312 µM, 625 µM, 1250 µM, 2500 µM, and 5000 µM of the cyclophosphazene compound (Figure 3). It has been determined that the hydrolytic and oxidative cleavage activity of the cyclophosphazene ligand is higher at concentrations of 156 µM, 312 µM, 625 µM, 1250 µM, 2500 µM, and 5000 µM, causing DNA breaks.

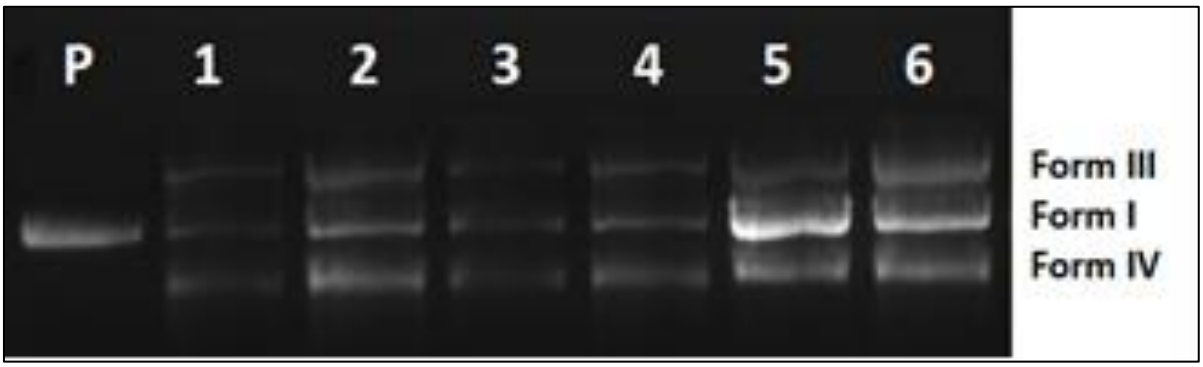

Figure 3: Oxidative DNA cleavage (B) P: pBR322 plasmid DNA, 1: DNA+ 156 µM, 2: DNA+ 312 µM, 3: DNA+ 625 µM, 4: DNA+ 1250 µM, 5: DNA+ 2500 µM, and 6: DNA+ 5000 µM cyclophosphazene ligand.

In recent years, significant emphasis has been placed on studies encompassing the interaction of these compounds with DNA to understand the chemotherapeutic effect of new phosphazenes on DNA and their potential for use as anti-cancer drugs. Tümer et al. [18] determined that the phosphazene derivatives they synthesized interacted with DNA and emphasized that this activity was due to oxidative DNA damage caused by redox-active ferrocene units. Okumuş et al. [19] determined that the molecules they synthesized bind to G/G and A/A nucleotides via hydrogen bonds and inhibit enzyme cleavage. Furthermore, it was emphasized that the biological activity of these molecules was expected due to possible oxidative DNA damage caused by the redox-active nitro unit. Akbaş et al. [20] determined that some of the synthesized compounds reduced the density and mobility of plasmid DNA, while others cleaved the DNA. Furthermore, based on restriction enzyme cleavage results, it was observed that the synthesized compounds did not bind to G/G and A/A nucleotides in DNA.

3.2 Binding Activity of Cyclophosphazene Ligand

When the absorption spectrum obtained by adding increasing amounts of DNA to the ligand was examined, a progressive increase in absorption was observed with increasing DNA concentration. The hyperchromic effect observed at 258 nm suggests that the ligand binds to CT-DNA through an intercalative binding mode (Figure 4).

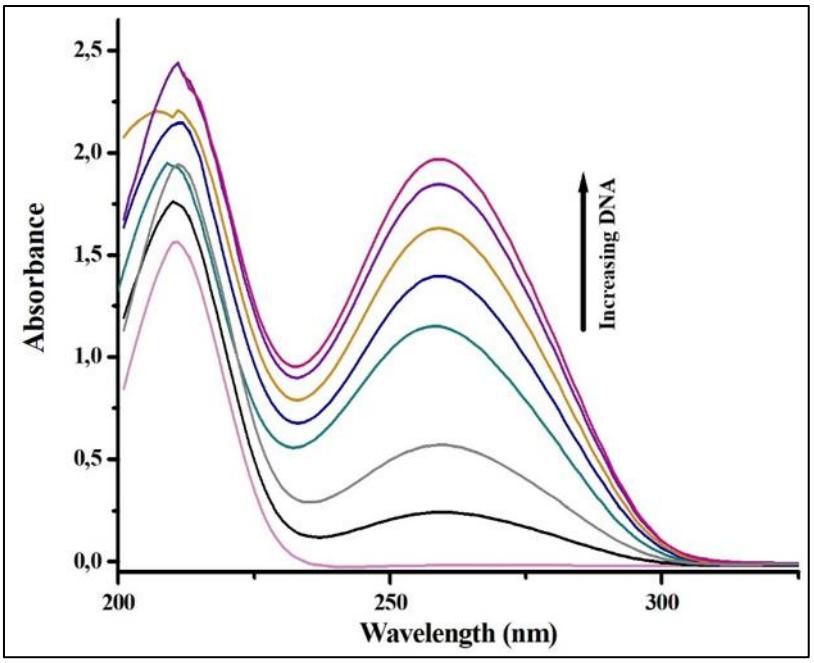

Figure 4: Absorption spectral changes on increasing CT-DNA amount addition to the cyclophosphazene compound.

UV-Vis absorption spectroscopy revealed that the ligand binds to CT-DNA through intercalation, as evidenced by the hyperchromic effect at 258 nm upon successive DNA additions. This binding mode is consistent with the insertion of planar aromatic moieties between adjacent DNA base pairs, which is a characteristic feature of intercalative agents. Given the planar aromatic character of pyridine, the presence of pyridine substituents in the phosphazene ring likely contributes to the observed intercalation.

4 CONCLUSION

The results of this study demonstrate that the investigated cyclophosphazene ligand interacts effectively with DNA through multiple modes. The cyclophosphazene compound containing pyridine induced both hydrolytic and oxidative cleavage of pBR322 plasmid DNA over the concentration range examined, while UV–Vis spectroscopic studies indicated an intercalative binding mode toward CT-DNA, as evidenced by the hyperchromic effect observed at 258 nm. The combination of DNA-cleaving and DNA-binding properties highlights the potential of this cyclophosphazene scaffold as a DNA-targeting agent. Further investigations, including cytotoxicity studies against cancer cell lines and assessments of cellular selectivity, are warranted to better understand its biological activity and evaluate its potential for therapeutic applications.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Funding Source

This study was financially supported by the Scientific Research Project Coordination Unit of Çanakkale Onsekiz Mart University, Çanakkale, Türkiye (FHD-2023-4410).

Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

REFERENCES

[1] Allcock HR. Phosphorus-Nitrogen Compounds: Cyclic, Linear, and High Polymeric Systems. Academic Press, New York, 1972.

[2] Stewart FF. Phosphazenes. In: ed. D.W. Allen, D. Loakes, and J.C. Tebby Organophosphorus Chemistry, The Royal Society of Chemistry, 2015. vol. 44, ch. 9, p. 397-430.

[3] Chakraborty A, Ahmed N, Chandrasekhar V. Phosphazenes. In: ed. L.J. Higham, D.W. Allen, and J.C. Tebby. Organophosphorus Chemistry. The Royal Society of Chemistry, 2022; 51: 355-397. Doi:10.1039/9781839166198-00355

[4] Allen CW. Linear, Cyclic and Polymeric Phosphazenes, Coordination Chemistry Reviews. 1994;130 (1-2): 137–173. Doi:10.1016/0010-8545(94)80004-9.

[5] Allcock HR. Chemistry and Applications of Polyphosphazenes, Wiley: New York, 2003.

[6] De Jaeger R, Gleria M, (Eds.). Phosphazenes. A Worldwide Insight, Nova Science Publishers, Inc. New York, 2004.

[7] Chandrasekhar V, Nagendran S, Baskar V. Cyclophosphazene-Based Multi-Site Coordination Ligands. Coordination Chemistry Reviews. 2007; 251 (9-10): 1045–1074. Doi:10.1016/j.ccr.2006.07.005.

[8] Patil BR, Machakanur SS, Badiger DS, Hunoor RS, Gudasi KB, Nethaji M, Bligh ASW. Hexa-arm star shaped hydrazone derivatives from hexakis(4-formylphenoxy)-cyclotriphosphazene core. Journal of Molecular Structure. 2011; 1003(1-3): 52-61. Doi: 10.1016/j.molstruc.2011.07.020.

[9] Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002.

[10] Strekowski L, Wilson B. Noncovalent interactions with DNA: An overview, Mutation Research. Fundamental and Molecular Mechanisms of Mutagenesis. 2007; 623 (1–2): 3-13. Doi:10.1016/j.mrfmmm.2007.03.008.

[11] Andrezálová L, Országhová Z. Covalent and noncovalent interactions of coordination compounds with DNA: An overview. Journal of Inorganic Biochemistry. 2021; 225:111624. Doi: 10.1016/j.jinorgbio.2021.111624.

[12] Gupta T, Patra AK, Dhar S, Nethaji M, Chakravarthy AR. Effect of copper–sulphur bond on the DNA photo-cleavage activity of 2-(methylthio)ethylpyridine-2-carbaldimine copper(II) complexes. Journal of Chemical Sciences. 2005;117(2):123–132. Doi:10.1007/BF03356106.

[13] Sangeetha Gowda KR, Mathew BB, Sudhamani C, Naik HB. Mechanism of DNA Binding and Cleavage. Biomedicine and Biotechnology. 2014; 2(1): 1-9. Doi:10.12691/bb-2-1-1.

[14] Rehman S U, Sarwar T, Husain M A, Ishqi H M, Tabish M. Studying non-covalent drug–DNA interactions. Archives of Biochemistry and Biophysics. 2015; 576: 49-60. Doi: 10.1016/j.abb.2015.03.024.

[15] Hangan AC, Oprean LS, Dican L, Procopciuc LM, Sevastre B, Lucaciu RL. Metal-Based drug–DNA interactions and analytical determination methods, Molecules. 2024; 29: 4361. Doi:10.3390/molecules29184361.

[16] Arıcı M, Nazır H. Synthesis and Investigation of DNA Effect of [N,N'-bis (salicylidene)-1,3-propanediaminato]nickel(II) Complex. Süleyman Demirel University Faculty of Arts and Science Journal of Science. 2010;5(1): 67-74. https://izlik.org/JA45XN72LX.

[17] Çıralı DE, Dayan O. Synthesis of Tetranuclear Ruthenium (Ii) Complex of Pyridyloxy-Substituted 2,2 '-Dioxybiphenyl-Cyclotriphosphazene Platform and Its Catalytic Application in The Transfer Hydrogenation of Ketones. Phosphorus Sulfur and Silicon And The Related Elements. 2015;190(7):1100-1107. Doi: 10.1080/10426507.2014.966190.

[18] 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 cytotoxic activities and DNA interactions of vanillinato-substituted NN or NO spirocyclic monoferrocenyl cyclotriphosphazenes. JBIC Journal of Biological Inorganic Chemistry. 2015; 20:165–178. Doi:10.1007/s00775-014-1223-5.

[19] Okumuş A, Akbaş H, Kılıç Z, Koç LY, Açık L, Aydın B, Türk M, Hökelek T, Dal H. Phosphorus–nitrogen compounds part 33: *in vitro* cytotoxic and antimicrobial activities, DNA interactions, syntheses, and structural investigations of new mono(4-nitrobenzyl)spirocyclotriphosphazenes. Research on Chemical Intermediates. 2016; 42:4221–4251. Doi: 10.1007/s11164-015-2271-3.

[20] Akbaş H, Okumuş A, Karadağ A, Kılıç Z, Hökelek T, Koç LY, Açık L, Aydın B, Türk M. Phosphorus-Nitrogen Compounds Part 32. Structural and thermal characterizations, antimicrobial and cytotoxic activities, and *in vitro* DNA binding of the phosphazenium salts. Journal of Thermal Analysis and Calorimetry, 2016; 123; 1627–1641. Doi: 10.1007/s10973-015-5001-6.