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An Investigating on DNA Binding Activity of Zn(II) Phthalocyanine Complex Having Tetra Substituted Phenoxy-3-methoxybenzoic Acid Group

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ABSTRACT

The tetra substituted Zn(II) phthalocyanine complex having the dicyanophenoxy)-3-methoxybenzoic acid group had been obtained from 4-(3, 4-dicyanophenoxy)-3-methoxybenzoic acid and analyzed with the application of FT-IR, NMR, UV/Vis techniques in compliance with reported literature. The interacting property of 2,10,16,24–tetrakis (phenoxy-3-methoxybenzoic acid) phthalocyaninato) Zn(II) by CT-DNA was examined with absorption bands, emission titrations, melting temperature, viscosity, and gel electrophoresis procedures. The obtained findings from these techniques demonstrated that the complex containing the dicyanophenoxy)-3-methoxybenzoic acid binds to the DNA by means of intercalation attachment mechanisms.

Keywords:
CT-DNA; Zinc phthalocyanine; Absorption spectra; Gel electrophoresis

INTRODUCTION

The phthalocyanine metal complexes have distinct pharmacologic and biologic activities such as anticancer, enzyme inhibition, and antimicrobic activities [1-7]. Currently, the investigations over the attachment activities of metallic phthalocyanine complexes by DNA have gotten great attention to develop new anticancer medicines [8,9]. Due to their unique pharmacologic and biologic activities, the metal complexes of phthalocyanines are used as anticancer medicines because of their very large π-delocalized surface area that are easily changed depending on biological system [10-13].

Carcinoma infection is an important healthiness issue around the globe and many people die because of this health problem. Nowadays, cancer treatment scientists concentrate their studies on aiming cell cycle and DNA interaction mechanisms. Therefore, DNA has been considered to be cellular target for therapeutic molecules. The interaction characteristics of transition metal complexes with DNA had been studied understanding how the acting of their tumor prevention activities of new anticancer medicine for cancer therapy [14,15]. A substantial part of cancer therapy comprises of metal complexes which bind to DNA or inhibit the DNA relaxation [16, 17]. The interaction of drugs with DNA molecule may modify the building of DNA [18]. Therapeutic drugs also binding to DNA can cause in difference in replication of DNA molecule and expression of gene [19, 20]. It is thought that there are fundamental interaction modes of tiny compounds with DNA molecule are intercalative and non-intercalative binding mechanisms [19, 21]. The physicochemical activities of metal phthalocyanine complexes may be arranged by altering the transition metal ions and the environmental substituent [22]. Studies in recent years, the binding characteristics of the phthalocyanine compounds to DNA because of preventing of direct or indirect growth of cancerous tumor had accelerated in the literature.

In this current study, the complex of 2,10,16,24–tetrakis (phenoxy-3-methoxybenzoic acid) phthalocyaninato) Zn(II) (Pc4) had been studied via NMR, FT-IR, UV/Vis techniques. The interaction properties of Pc4 with DNA (Calf Thymus DNA) had been analyzed by carrying out electronic spectra titration, fluorescence spectra, the melting temperature, viscosity and the electrophoresis methods. The obtained findings from this study could be a revelation for new investigation regarding cancer medication.

MATERIAL AND METHODS

Acetonitrile, DMF, DMSO, 4-hydroxy-3-methoxybenzoic acid, K_2CO_3, methanol, THF and $ZnCl_2$ reagents were commercially purchased from the commercial company and also the chemicals such as DNA, Tris-HCl and NaCl were supplied by Sigma Aldrich commercial company. The NMR experiments were carried out using Agilent Spectrometer and for IR measurements, Thermo Scientific FT-IR spectroscopy was conducted at room temperature. For the UV/Vis analyses, Cary UV/Vis spectroscopy was used and fluorescence spectroscopic measurements were recorded by a Perkin Elmer LS Fluorescence Spectroscope. In this study, for the electrophoresis measurements, Thermo Scientific owl electrophoresis instrument was used in a buffer solution at room temperature. Ubbelohde viscometer system was used for the viscosity measurements.

The Synthesis of 4-(3,4-dicyanophenoxy)-3-methoxybenzoic acid compound (3)

The compound of 4-(3, 4-dicyanophenoxy)-3-methoxybenzoic acid was synthesized in conformity with the published literature [23].

The synthesis of 2,10,16,24–tetrakis (phenoxy-3-methoxybenzoic acid) phthalocyaninato) Zn(II) complex (4)

The phthalocyanine complex of zinc (II) was synthesized by means of interaction of 4-(3,4-dicyanophenoxy)-3-methoxybenzoic acid compound in the presence of $ZnCl_2$ according to reported literature [23]. A mixture of 4-(3,4-dicyanophenoxy)-3-methoxybenzoic acid 3 (0.050 g, 0. 17 mmol) and $ZnCl_2$ (0.022 g) was powdered in a quartz crucible and heated in a sealed glass tube for 6 min under nitrogen at 270 °C. The reaction was terminated by pouring the solution into an aqueous solution of 2 M HCl followed by overnight storage. The precipitates were filtered and washed with water and acetic acid to a neutral pH. The product was washed with cold and hot methanol. The THF soluble were taken, and final product was obtained by solvent THF removal. The product is soluble in THF, DMF and DMSO. The yield was 0.019 g (38 %). MALDI-TOF MS: m/z[M]$^+$ Calcd. for $C_{64}H_{40}N_8O_{16}Zn$: 1242.43; found 1241.23[H]$^+$. IR spectrum (cm^{-1}):3525, 3078, 2935, 1691, 1591, 1467, 1400, 1267, 1213, 1176, 1112, 1093, 1029, 945, 881, 742. UV-Vis (THF) λmax (log ε): 674 (5.24), 608(4.65), 348(5.04). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.53, 7.78, 7.22, (Ar-H), 3.85(CH_3), 3.33(DMSO-d_6) 2.48(DMSO-d_6), 1.13 (CH_3) [23].

DNA binding experiments

The binding of Pc4 to CT-DNA was investigated by UV/vis, emission titration, melting point viscosity and gel electrophoresis experiments to analyze its binding activities with DNA. CT-DNA samples were prepared in the Tris-HCl buffer solution. UV absorbance of CT-DNA stock solution in buffer at 260 nm was measured and it was found that CT-DNA stock solution was free of protein. CT-DNA sample was kept at 4 °C overnight and used within 2 days. The solutions of the complex were prepared in DMF solvent and diluted in the Tris-HCl/NACl buffer solution at pH 7.03. The UV/vis absorption spectra titrations were carried out between 260 and 800 nm at 25 °C. Absorption spectra, emission titrations were performed fixed concentrations of the complex (20 μM) and spectra were recorded after each adding. For the all experiments, the solutions were incubated for 5 min for each run. Melting point study, the certain amounts of CT-DNA and Pc4 were heated up through 25 °C to 95 °C. The mixture of CT-DNA + Pc4 was incubated at the certain time for each 5 °C and the values of absorption titration were recorded.

For agarose gel electrophoresis studies was performed using Thermo Scientific Owl Electrophoresis System and for the viscosity measurements, Ubbelohde viscometer apparatus was used.

RESULTS AND DISCUSSION

The chemical synthesis route of Zn(II) phthalocyanine complex is indicated in Figure 1. The four peripheral substituted the Zn(II) complex was synthesized via cyclotetramerization of the compound by refluxing to $ZnCl_2$ at certain temperature and underneath nitrogen gas. The Pc4 complex was qualified with UV/Vis, FT-IR and the NMR measurements. The obtained findings were consistent with the prospective molecular structure. The characterization of the complex was reported in literature [23].

The characterization of the compound including a combining of techniques, involved elemental analysis, FT-IR, UV/Vis, and ^{1}H NMR spectroscopy. The spectroscopic data of the complex consistent with its proposed chemical structure. In the ^{1}H NMR spectrum, the expected aromatic protons for Pc4 are observed at 8.53, 7.78 and 7.22 ppm, while aliphatic CH_3 groups are observed at 3.85 and 1.13 ppm. IR spectral data of the compound as expected, OH groups at 3525 cm^{-1}, Ar-H vibrations at 3078 cm^{-1}, CH_3 vibrations at 2935 cm^{-1}, C=C vibrations at 1691 and 1591 cm^{-1}, Ar-O-Ar

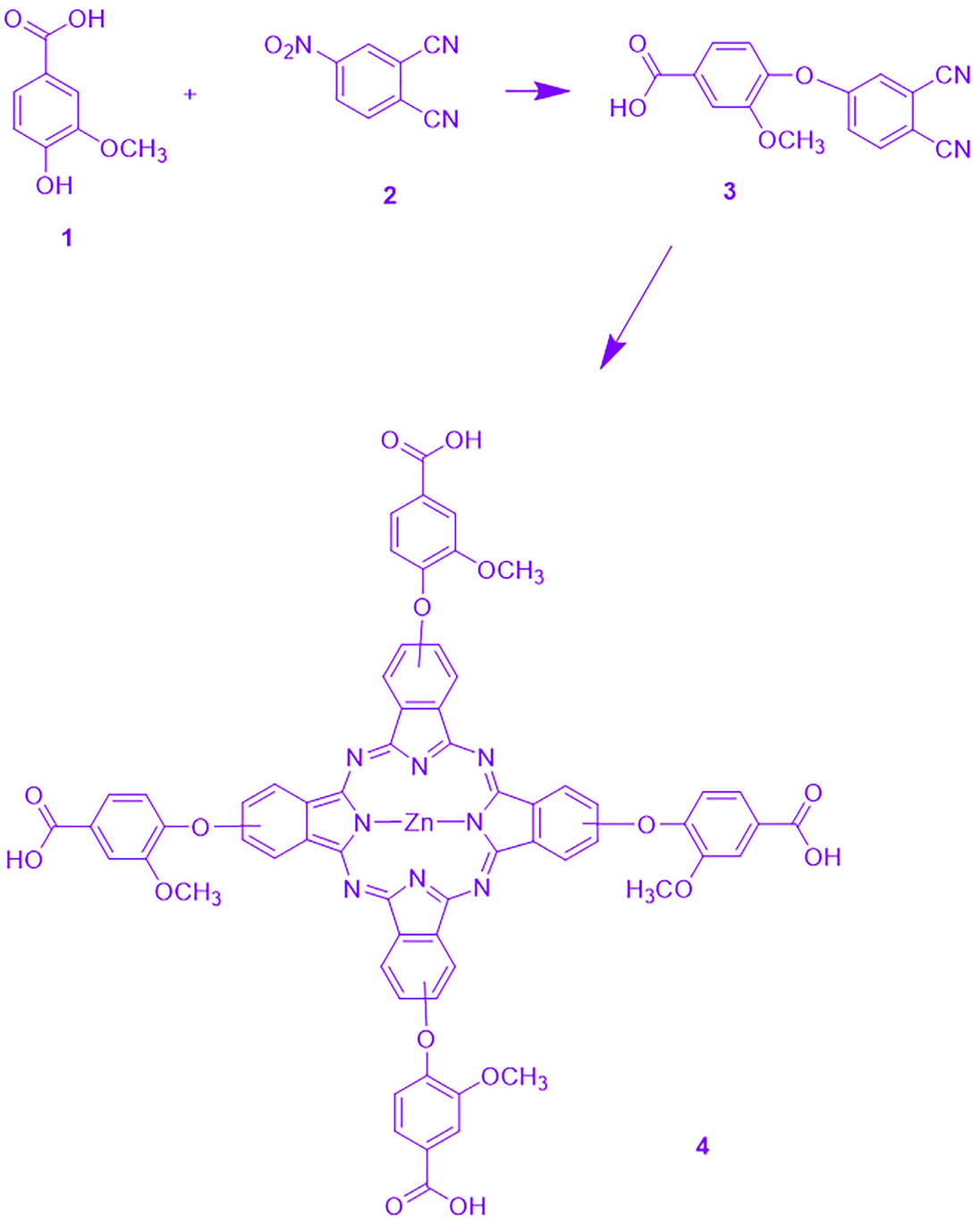

Figure 1. The chemical synthesis route of Zn(II) phthalocyanine complex.

vibrations at 1267 cm^{-1} is also observed. The UV-Vis absorption spectrum data of the zinc phthalocyanine compound give the characteristic absorption bands of the phthalocyanine compound, Q and B bands, at 674 and 348 nm, respectively. It yields the shoulder band at 608 nm as expected.

The binding study of Zn (II) phthalocyanine complex with the DNA

UV/Vis titrations were conducted by increasing concentration of the DNA with a constant concentration (20 μM) of Pc4 and electronic spectra had been recorded

afterward adding of the DNA sample. In this study, also the binding constant (K_b) was computed by using Wolfe-Schimer equation [24]. With increases in amount of the DNA, the absorbance values of Pc4 gradually dropped. The dropping of absorbance values demonstrated that the complex Pc4 interacts with CT-DNA and also three main absorbance bands were observed with hypochromism, and these bands were located at around 362, 625 and 682 nm related to the red shift as illustrated in Figure 2. The complex showed hypochromism, which reducing in absorption spectra is called as a hypochromism and incrementing in absorbance values is defined as a hyperchromism. Hypochromicity is related to a mild bathochromic shifting generally originated from the intercalation binding mechanism, comprising a packing interaction among a chromophore compound and DNA base pairs [13]. The Pc4 complex is quite planar central part and it can likely interact with the DNA by an intercalating binding mechanisms. The obtained results from absorption titration method showed that Pc4 complex binds to the DNA molecule through an intercalative mechanism, and the K_b value for Pc4 was obtained as 2. 17 x 10 6 M^{-1} as indicated in Figure 2. The calculated Kb value for the complex also demonstrated that Pc4 binds to the DNA with the intercalation mechanisms.

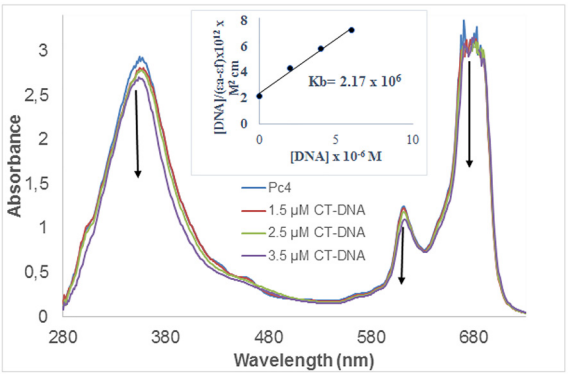

Figure 2. Electronic titrations of Pc4 in the buffer system on addition of calf thymus DNA. [Pc4] = 20µM and [CT-DNA] = 0–3.5µM. The arrows represent the dropping in absorption intensities on increasing the calf thymus DNA amount.

The emission titration studies

The emission study is frequently used to determine the investigating of DNA-drug binding activity because this technique is a very sensitive to explain DNA interaction probes and it can provide further information about the intercalation of molecular compounds [25]. In this present study, the DNA binding activity of Pc4 to the DNA was investigated using emission titration spectra. When the complex Pc4 was interacted with the DNA, it was seen that the intensities of emission spectra were dropped gradually as indicated in Figure 3. The dropping in emission intensity demonstrated that the complex binds to the DNA using hypochromic mechanism. It is seen in Figure 3 that the complex Pc4 gave a strong emission spectra in the absence of CT-DNA at pH 7.03 at around 457 nm. The strong fluorescence spectra could be originated from the ligand [25]. On the adding of the DNA, the decreases in intensity of emission spectra for Pc4 were illustrated in Figure 3. The findings from this technique demonstrated that Pc4 interacts with the DNA via the

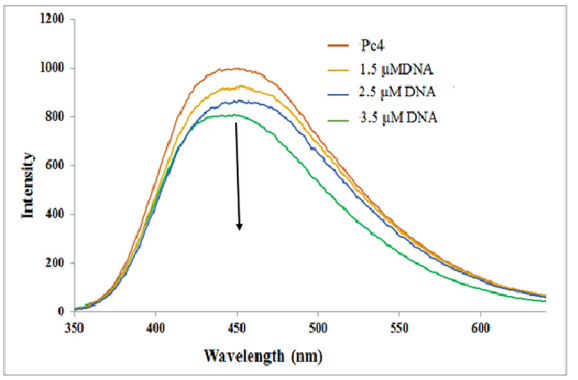

Figure 3. Fluorescence titrations of Pc4 in the buffer solution in the absence and presence of the DNA. [Pc4] = 20µM and [CT-DNA] = 0 – 3.5µM. The arrow displays the intensity change on mounting the DNA concentration.

mechanism of intercalative binding.

The viscosity studies for DNA Binding

In addition, the above methods, the viscosity technique was also applied to search DNA binding activity of Pc4. The viscosity method could supply more information about DNA interaction mechanisms that is very precise to change in length of DNA molecule. Generally, when a chemical complex inserts into the base pairs of DNA, DNA molecule elongates because the base pairs of DNA are decomposed to adapt the attached ligand, which causes to increases in the DNA viscosity [25]. On the other hand, chemical compounds react with DNA by non-intercalative binding mode may decrease the length of DNA by twisting the DNA [26] but, non-intercalative and electrostatic binding mechanisms cause to very a little impact upon DNA viscosity.

In the present study, the changing in the DNA viscosity in the presence of Pc4 was monitored. When the complex reacts with the DNA molecule in an intercalative binding mechanisms, it has an impact upon the DNA viscosity. It is indicated in Figure 4 that on the adding of Pc4 to the DNA, the surge in the DNA relative viscosity was observed. The increasing in CT-DNA viscosity that could relate to the reacting of Pc4 with the DNA molecule. The obtained findings proved that Pc4 attaches to the DNA via intercalative binding mechanisms with a strong affinity.

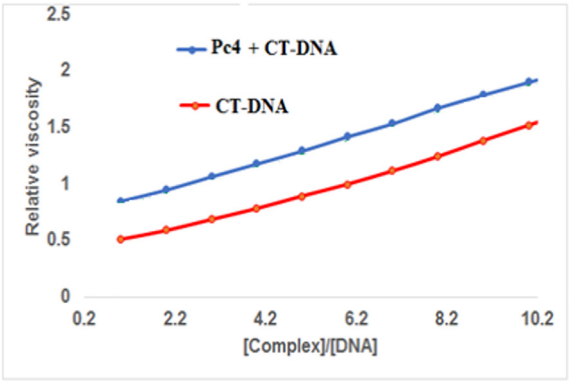

Figure 4. The viscosity study of Pc4 indicating the impact of surging amounts of the complex upon the DNA viscosity.

Thermal denaturation studies for DNA binding

The solution of CT-DNA + Pc4 was incubated at the certain time for each 5 °C and the values of absorption titration were recorded. The recorded absorbance values versus temperature chart were plotted as illustrated in Figure 5. It is observed in Figure 5 that thermal melting temperature of CT-DNA was recorded as approximately 70.40 °C, and the Tm value of CT-DNA + Pc4 was observed as 78.63 °C. Mostly, if the thermal melting variation of the DNA sample and CT-DNA + Pc4 is great, the DNA binding activity is believed to be an intercalation. If this value is not great, the DNA binding mechanism is considered as a non-intercalation. The obtained result from the viscosity measurement demonstrated that Pc4 reacts with the DNA by an intercalative binding mechanism.

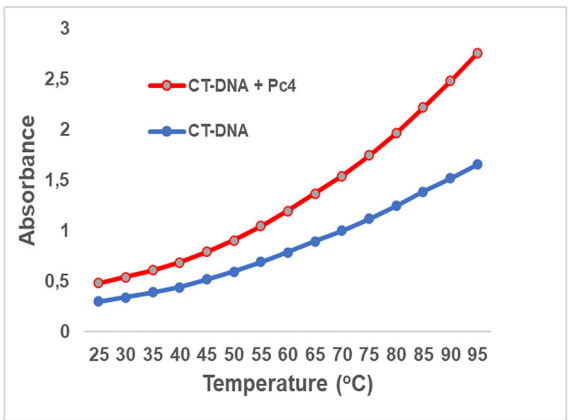

Figure 5. T_m study of the DNA showing impact of the compound on melting temperature of the DNA. The Tm measurements of the DNA (blue line) and DNA + Pc4 (red line).

The agarose gel electrophoresis study for DNA binding

In the literature, the binding activities of the compounds to CT-DNA were studied using agarose gel electrophoresis technique analyzing of the impact of different amounts of the Pd(II) compounds on CT- DNA. In this study, results showed that the intensities of CT-DNA bands obtained for the compounds after interacting with CT-DNA were dropped, as compared with control CT-DNA band. The drop in the intensities of the DNA bands observed after interacting of the compounds with the DNA is thought to be damage deformation of CT-DNA [27].

In addition to above studies, DNA binding activity for Pc4 complex was studied using agarose gel electrophoresis technique. First of all, the migrating of CT-DNA + Pc4 was recorded afterwards GelRed staining as shown in Figure 6. The lane M refers the ladder of DNA, and the lanes 1, 2 and 3 refer the compound Pc4, respectively with varied amounts of the DNA. The amount of DNA surged from the lanes 1 to 3. The concentration of Pc4 was hold fixed at 25 μM, whereas the amounts of DNA was changed from 15 to 25 μM. Then, the intensity of the DNA band was recorded in the absence of Pc4 and also the band intensity of the DNA was monitored in the presence of Pc4. It is clearly seen that in Figure 6, the DNA bands intensities were dropped and the migrating of CT-DNA bands were slightly vanished because of the DNA neutralization. The results demonstrated that Pc4 interacts by the DNA.

CONCLUSION

In the present study, the objective of the study was to explain the binding activity of the metal complex to CT-DNA for potential use of an anticancer medicine. First of all, the zinc (II) phthalocyanine compound was synthesized and analyzed with electronic spectra, FT-IR and NMR instruments according to the reported procedure in literature. The DNA binding activity of the complex was evaluated using with various methods such as absorption spectra, fluorescence titrations, melting point, viscosity

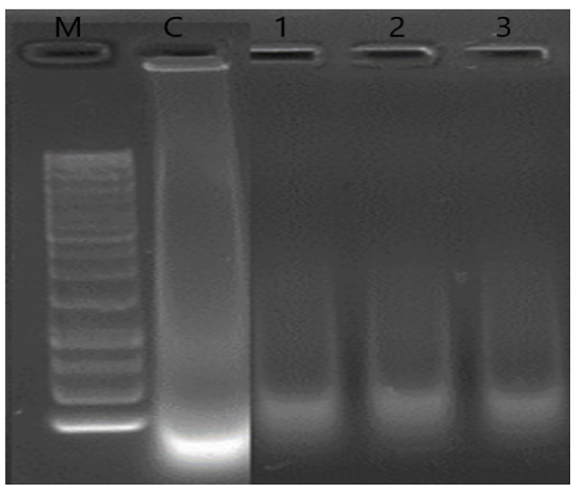

Figure 6. Agarose gel electrophoresis studies for Pc4 complex at pH 7.03 on surging the amount of CT-DNA. Lane M: DNA ladder, Lane C: control CT-DNA, Lanes 1 to 3: (Pc4 (20 μM) + CT-DNA (15, 20, 25 μM), respectively.

and the electrophoresis experiments. The obtained findings from these methods demonstrated that the metal complex binds to the DNA through the intercalative binding mechanisms. In addition to above techniques, the electrophoresis study was also studied to evaluate the DNA binding to mode for this compound. The results from gel electrophoresis method showed that the compound interacts with the DNA. The obtained results demonstrated that the complex can be evaluated as a potential candidate of anticancer medicine due to its binding activity to DNA molecule.

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CONFLICT OF INTEREST

Authors approve that to the best of their knowledge, there is not any conflict of interest or common interest with an institution/organization or a person that may affect the review process of the paper.

AUTHOR CONTRIBUTION

All sections including methodology, the experiments, analysis, writing, review and editing the manuscript was organised and performed by Ali Arslantaş. Mehmet Salih Ağırtaş and Zekeriya Ballı synthesized and characterized the compound.

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