



Template Synthesis, Characterization, Antioxidant Effects, DNA Cleavage and Antimicrobial Studies of Zn^{II}, Ni^{II}, Mn^{II}, Cu^{II}, and Co^{II} Complexes Containing Pyridazinone Moiety

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Abstract

In this study, novel five Schiff base metal complexes derived from Zn^{II}, Ni^{II}, Mn^{II}, Cu^{II}, and Co^{II} metal salts and 5-benzoyl-4-hydroxy-2-methyl-6-phenyl-2H-pyridazine-3-one with o-aminophenol have been synthesized using a template method. Metal complexes were characterized by elemental analysis, FT-IR, UV-Vis, Mass and magnetic susceptibility.

Antioxidant effects, DNA cleavage, and antimicrobial studies of synthesized compounds were performed. DPPH scavenging and metal chelating activity was found to increase with concentration. The highest DPPH activity and ferrous ion chelating activity were obtained from compound Zn. DNA cleavage studies indicated that all tested compounds were cleavage active at



the concentration of 100 mg/L. While all the synthesized compounds displayed antibacterial activity against *E. hirae* in order of Mn>Zn=Ni=Co>Cu. The compound Ni only showed antimicrobial activity against all microorganisms tested, especially against *L. pneumophila* subsp. *pneumophila* with inhibition zone value equal to 13 mm.

Keywords: Template synthesis; Schiff base complex; Antioxidant activity; DNA cleavage; Antimicrobial activity.

Piridazinon Yapısı İçeren Zn^{II}, Ni^{II}, Mn^{II}, Cu^{II} ve Co^{II} Komplekslerinin Şablon Sentezleri, Karakterizasyonları, Antioksidan Etkileri, DNA Klevajı ve Antimikrobiyal Etkilerinin Araştırılması

Öz

Bu çalışmada template metodu ile o-aminofenol, 5-benzoil-4-hidroksi-2-metil-6-fenil-2H-piridazin-3-on ve Zn^{II}, Ni^{II}, Mn^{II}, Cu^{II} ve Co^{II} metal tuzlarından türeyen beş yeni Schiff baz kompleksi sentezlenmiştir. Metal kompleksler elemental analiz, FT-IR, UV-Vis, kütle ve manyetik duyarlılık ile karakterize edilmiştir. Sentezlenen bileşiklerin antioksidan etkileri, DNA bölünmesi ve antimikrobiyal çalışmaları yapılmıştır. DPPH süpürme ve metal şelatlama aktivitesinin konsantrasyona bağlı olarak arttığı bulunmuştur. En yüksek DPPH aktivitesi ve demir iyon şelatlama aktivitesi Zn bileşiğinden elde edildi. Sentezlenen tüm bileşikler, Mn>Zn=Ni=Co>Cu sırasına göre *E. hirae*'ye karşı antibakteriyal aktivite sergilerken, sadece Ni bileşiği test edilen tüm organizmalara karşı, özellikle de *L. pneumophila* subsp. *pneumophila*'ya 13 mm'ye eşit inhibisyon zon değerinde antimikrobiyal aktivite göstermiştir.

Anahtar Kelimeler: Template sentez; Schiff baz kompleksi; Antioksidan aktivite; DNA bölünmesi; Antimikrobiyal aktivite.

1. Introduction

Oxidative stress is associated with production of reactive oxygen species (ROS) that are responsible for the damage of a range of cellular components [1]. Therefore, antioxidants play a very significant role in the living system in terms of protecting from oxidative stress and other chronic diseases. Nowadays, many scientists have been working on newly synthesized compounds or compounds derived from natural sources due to their antioxidant, anticancer, anticonvulsant, antitubercular, antimicrobial and anti-inflammatory activities [2, 3]. Therefore, the newly synthesized compounds are used in different fields such as molecular biotechnology, genetic engineering, drug designing. Schiff bases containing pyridazine ring are known to possess

a range of biological properties, including antibacterial, antifungal activities etc. [4]. Moreover, pyridazine derivatives and their metal complexes have various pharmacological activities [5-7]. For example, some drugs that contain the pyridazine ring in the structure are used as antihistaminic and sedative drug [6]. Pyridazinone metal complexes are rarely encountered in the literature. The complexes obtained by the template method are very limited. However, when the literature is examined, it has been observed that pyridazinone metal complexes also have antifungal, antibacterial and antitumor activity [4, 7].

In this study, we aimed to examine the antioxidant effects, DNA cleavage, and antimicrobial activities of five newly synthesized Schiff base Zn^{II} , Ni^{II} , Mn^{II} , Cu^{II} , and Co^{II} metal complexes. In order to initiate this investigation the template synthesis and characterization of Schiff base complexes of Zn^{II} , Ni^{II} , Mn^{II} , Cu^{II} , and Co^{II} was initiated and these results are first presented.

2. Materials and Methods

2.1. Chemicals and materials

All the reactants and solvents (95-99 % purity) were purchased from Aldrich or Merck and used without further purification. 5-Benzoyl-4-hydroxy-2-methyl-6-phenyl-2H-pyridazine-3-one was prepared according to the literature procedure [8]. Elemental analyses were measured on a Thermo Scientific Flash EA 2000 CHNS analyzer. Electronic absorption spectra were recorded on a PG Instruments T80+UV/Vis spectrometer. Infrared spectra were measured on a Perkin-Elmer Spectrum 100 FTIR spectrophotometer with an ATR sampling accessory. Mass spectra were obtained on a LC/MS/MS AB/SciEx 3200 Q Trap spectrometer.

2.2. General synthesis method of the complexes (1-5)

A methanolic solution (5 mL) of metal chloride ($ZnCl_2$ (0.068 g), $NiCl_2$ (0.064 g), $MnCl_2 \cdot 2H_2O$ (0.080 g), $CuCl_2 \cdot 2H_2O$ (0.085 g) and $CoCl_2$ (0.064 g), 0.5 mmol) was mixed with a suspension of 5-Benzoyl-4-hydroxy-2-methyl-6-phenyl-2H-pyridazine-3-one (0.153 g, 0.5 mmol) and o-aminophenol (0.055 g, 0.5 mmol) in 15 mL of methanol. The reaction mixture was stirred for 2 hour at 70-80 °C, the precipitate was filtered off, washed with cold MeOH- H_2O (1:1) and Et_2O , the complex was purified from slowly vapor diffusion of Et_2O with a THF-methanol solution of complex and then dried in vacuo (Scheme 1).

[ZnL(H₂O)] complex (1): White solid; Yield 0.043 g (18 %); 185 °C decompose; IR, (ATR)_v, cm^{-1} : 3285 (O-H); 3059 (C-H)_{arom.}; 3028, 2945 (C-H)_{aliph.}; 1662 (C=O); 1577 (C=N); 1359 (C-O); 1547 (C=C); 541 (M-O); 514 (M-N). μ_{eff} : Diamagnetic; ¹H NMR (400 MHz, d_6 -

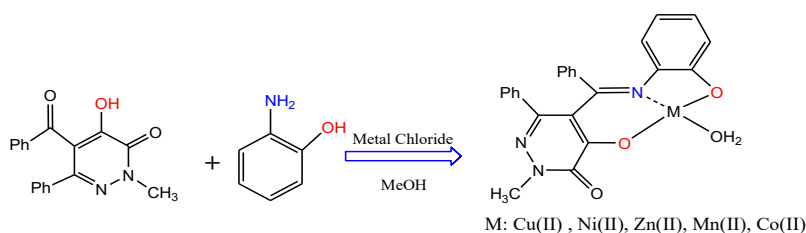
DMSO) δ (ppm);s, singlet; m, multiplet: 7.69-7.25 (m, 14H, Harm), 3.80 (s, 3H, CH₃ protons); ¹³C NMR (400 MHz, d₆-DMSO) δ (ppm); δ 196.19 (C-O)_{pyridazine}, 163.06 (C=N), 160.31 (C=O)_{pyridazine}, 149.19 (C-O)_{phenyl}, 138.00 (C=N)_{pyridazine}, 137.63 (C-N=), 133.48, 129.63, 128.97, 128.69, 128.47, 116.37 (Aromatic carbons), 40.51 (CH₃); UV (DMF, λ_{\max} nm, (Abs.)): 328 (0.223), 284 (0.693), 245 (1.724) nm; ESI-MS, m/z: 480.8 [Zn+L+H₂O+1]⁺; Anal. Calc. C₂₄H₁₉N₃O₄Zn (478.82): C, 60.20, H, 4.00, N, 8.78 Found: C, 61.07, H, 4.19, N, 8.50%.

[NiL(H₂O)] complex (2): Green solid; Yield 0.054 g (23 %); 250 °C decompose; IR, (ATR)_v, cm⁻¹: 3300 (O-H); 3062 (C-H)_{arom.}; 2940 (C-H)_{aliph.}; 1664 (C=O); 1578 (C=N); 1362 (C-O); 1548 (C=C); 544 (M-O); 514 (M-N); μ_{eff} : 2.55 B.M.; UV (DMF, λ_{\max} nm, (Abs.)): 336 (0.237), 285 (0.796), 245 (1.872) nm; ESI-MS, m/z: 473.0 [Ni+L+H₂O+1]⁺; Anal. Calc. C₂₄H₁₉N₃NiO₄ (472.12): C, 61.06, H, 4.06, N, 8.90 Found: C, 61.46, H, 4.63, N, 8.69%.

[MnL(H₂O)] complex (3): Yellow solid; Yield 0.069 g (30 %); 187 °C decompose; IR, (ATR)_v, cm⁻¹: 3350 (O-H); 3059 (C-H)_{arom.}; 3027, 2826 (C-H)_{aliph.}; 1651 (C=O); 1578 (C=N); 1378 (C-O); 1540 (C=C); 532 (M-O); 510 (M-N); μ_{eff} : 1.75 B.M.; UV (DMF, λ_{\max} nm, (Abs.)): 345 (0.068), 288 (0.339), 245 (1.020) nm; ESI-MS, m/z: 469.7 [Mn+L+H₂O+1]⁺; Anal. Calc. C₂₄H₁₉MnN₃O₄ (468.36): C, 61.55, H, 4.09, N, 8.97 Found: C,61.75, H, 4.50, N, 8.46%.

[CuL(H₂O)] complex (4): Green solid; Yield 0.039 g (17 %); 335 °C decompose; IR, (ATR)_v, cm⁻¹: 3150 (O-H); 3059 (C-H)_{arom.}; 3027, 2826 (C-H)_{aliph.}; 1651 (C=O); 1578 (C=N); 1378 (C-O); 1540 (C=C); 532 (M-O); 510 (M-N); μ_{eff} : 2.05 B.M.; UV (DMF, λ_{\max} nm, (Abs.)): 310 (0.367), 288 (0.339), 245 (1.020) nm; ESI-MS, m/z: 478.4 [Cu+L+H₂O+1]⁺; Anal. Calc. C₂₄H₁₉CuN₃O₄ (476.97): C, 60.43, H, 4.02, N, 8.81 Found: C,60.23, H, 3.89, N, 8.38%.

[CoL(H₂O)] complex (5): Brown solid; Yield 0.097 g (42 %); 220 °C decompose; IR, (ATR)_v, cm⁻¹: 3327 (O-H); 3059 (C-H)_{arom.}; 3027, 2935 (C-H)_{aliph.}; 1663 (C=O); 1578 (C=N); 1360 (C-O); 1546 (C=C); 541 (M-O); 512 (M-N); μ_{eff} : 5.54 B.M; UV (DMF, λ_{\max} nm, (Abs.)): 349 (0.222), 285 (0.767), 245 (1.938) nm; ESI-MS, m/z: 472.9 [Co+L+H₂O+1]⁺; Anal. Calc. C₂₄H₁₉CoN₃O₄ (472.36): C, 61.02, H, 4.05, N, 8.90 Found: C,61.53, H, 4.57, N, 8.72%.



Scheme 1: Template synthesis of Schiff base metal complexes

2.3. DPPH radical scavenging assay

Different solutions (0.5 ml) in dimethylformamide (DMF) of compounds and 2 mL of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) solution were incubated at 25 °C in the dark for half an hour [9]. DPPH scavenging activity was measured spectrophotometrically at 517 nm. A control reaction without DMF solution of compounds was performed under the same conditions. DMF solution was used as blank control. Trolox and Ascorbic Acid were used as standards in order to compare with the results. DPPH scavenging activity was determined using following equation:

$$\text{DPPH scavenging activity (\%)} = [1 - (A_{517 \text{ nm, sample}}/A_{517 \text{ nm, control}})] \times 100 \quad (1)$$

2.4. Ferrous ion chelating activity

Metal chelating effects on ferrous ions displayed by the compounds were applied as reported by Hsu et al. [10]. 1 mL of the synthesized compounds at different concentrations, FeCl₂·H₂O (0.1 mL 2 mM), ferrozine-1,2,4-triazine (0.2 mL 5 mM), and methanol (3.7 mL) were mixed in test tube and kept waiting for 10 min. The activity was measured spectrophotometrically at 562 nm. A control reaction without extract was performed under the same conditions. Metal chelating effect was calculated as follows:

$$\text{Metal chelating effect (\%)} = [(A_{562 \text{ nm, control}} - A_{562 \text{ nm, sample}}) / A_{562 \text{ nm, control}}] \times 100 \quad (2)$$

Ethylene diamine tetra acetic acid (EDTA) was used as the positive control.

2.5. DNA cleavage activity

The DNA cleavage studies were carried out by using agarose gel electrophoresis. A mixture of pBR 322 plasmid DNA (0.1 mg/mL), Tris buffer (50 mM Tris–HCl and NaCl buffer, pH 7.2) and the metal complexes (100 mg/L) were incubated at 37 °C for 1.5 hour. After the incubation, the loading buffer was added to the mixture prior to electrophoresis and the reaction mixture was electrophoresed for 1.5 hour at 80 V by using agarose gel. Tris-boric acid-EDTA was used as an electrophoresis buffer. The electrophoresis bands were visualized by UV-A light.

2.6. Antimicrobial activity

Antimicrobial activity of compounds was evaluated by using disk-diffusion method [11] against *Enterococcus hirae* (ATCC 10541), *Bacillus cereus*, *Staphylococcus aureus* (ATCC 6538), *Legionella pneumophila* subsp. *pneumophila* (ATCC 33152), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 10536), and *Candida albicans*. *B. cereus* and *Candida albicans* were obtained from Siirt Public Hospital.

3. Results and Discussion

Schiff base metal complexes occurred with template effect between coordination sites of *o*-aminophenol and 5-benzoyl-4-hydroxy-2-methyl-6-phenyl-2H-pyridazine-3-one on a metal center (Scheme 1). New synthesized metal complexes were obtained to the general formula of $ML(H_2O)$ where L is the anion of H_2L . These metal complexes were investigated using various spectroscopic techniques, such as FT-IR, UV-Vis, mass and magnetic susceptibility, elemental analysis, and molar conductivity. The investigation showed that the molar ratio obtained by the complexes was a 1:1 metal-ligand ratio. All of the complexes are air-stable at room temperature in the solid state, soluble in DMF, methanol and insoluble in water. Molar conductivity values measured for 10^{-3} M solution in DMF of these complexes fall in the range 1.41-2.83 $\mu S/cm$, indicate the non-electronic nature for the metal complexes [12].

3.1. FT-IR spectra

The infrared spectral bands in the region 3150-3350 cm^{-1} for O-H stretching indicate the presence of coordination water molecule in all the complexes. At the same time, in the spectra of all the complexes show in $\nu(C-O)$ at 1359-1378 cm^{-1} on complexation indicate that the phenolic OH group undergoes deprotonation and is coordinated to the metal ions [7]. A broad intensity sharp bands due to $\nu(C=O)$ group pyridazine ring appeared at 1651-1664 cm^{-1} in the complexes. The spectra of complexes displaying a band around 1577-1578 cm^{-1} was observed to C=N group stretching. Additionally, the bands in the complexes at 544-532 cm^{-1} and 510-514 cm^{-1} are attributed to the $\nu(M-O)$ and $\nu(M-N)$ vibrations, respectively [13].

3.2. Electronic spectra and magnetic measurements

In the electronic spectrum of the metal complexes showed bands between 245 and 288 nm, which can be attributed to $\pi-\pi^*$ transitions of the pyridazine and phenyl aromatic rings. The bands in the 310-349 nm range can be attributed to $n\rightarrow\pi^*$ transitions of imine and carbonyl groups [14]. As expected according to magnetic measurement value of Zn^{II} complex, this complex is diamagnetic, therefore, tetrahedral geometry is suggested for **1**. The observed magnetic moment values (μ_{eff}) of Ni^{II} complex was 2.95 B.M. which this magnetic moment value lie within the range normally found for other tetrahedral Ni^{II} complexes [15]. The magnetic measurement values of Co^{II} , Cu^{II} , and Mn^{II} complexes are 4.02, 2.30 and 5.75 B.M respectively which reveal with tetrahedral geometry [16-18].

3.3. Mass spectra

Given that the synthesized complexes, their molecular ion peaks in the mass spectrum have a distinctive; the appearance of this molecular ion peak is strong evidence for the identity of the complexes. Molecular ion peaks of the metal complexes appeared to be in agreement with formula weight after adding on one proton at: m/z : 480.8 $[\text{Zn}+\text{L}+\text{H}_2\text{O}+1]^+$, m/z : 473.0 $[\text{Ni}+\text{L}+\text{H}_2\text{O}+1]^+$, m/z : 469.7 $[\text{Mn}+\text{L}+\text{H}_2\text{O}+1]^+$, m/z : 478.4 $[\text{Cu}+\text{L}+\text{H}_2\text{O}+1]^+$, m/z : 472.9 $[\text{Co}+\text{L}+\text{H}_2\text{O}+1]^+$. The mass spectrums of the Ni, Co and Zn metal complexes were shown in Fig. 1-3.

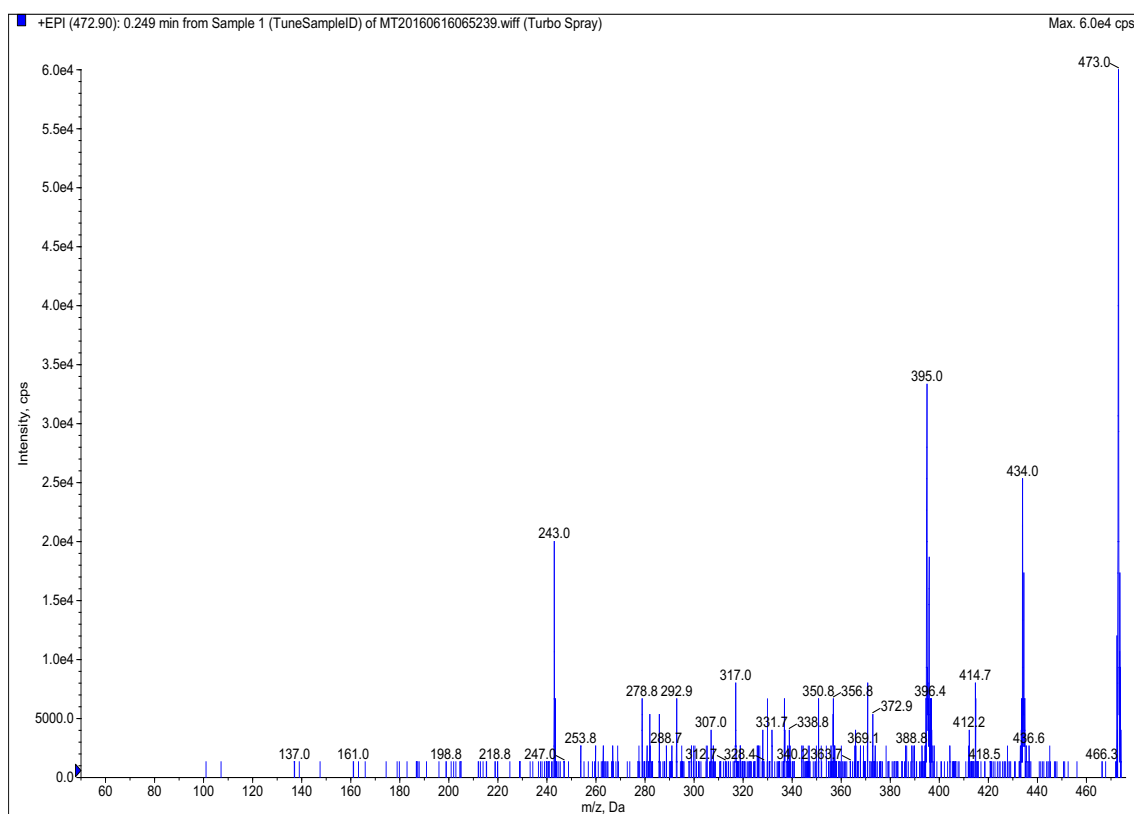


Figure 1: Mass spectrum of Ni(II) complex

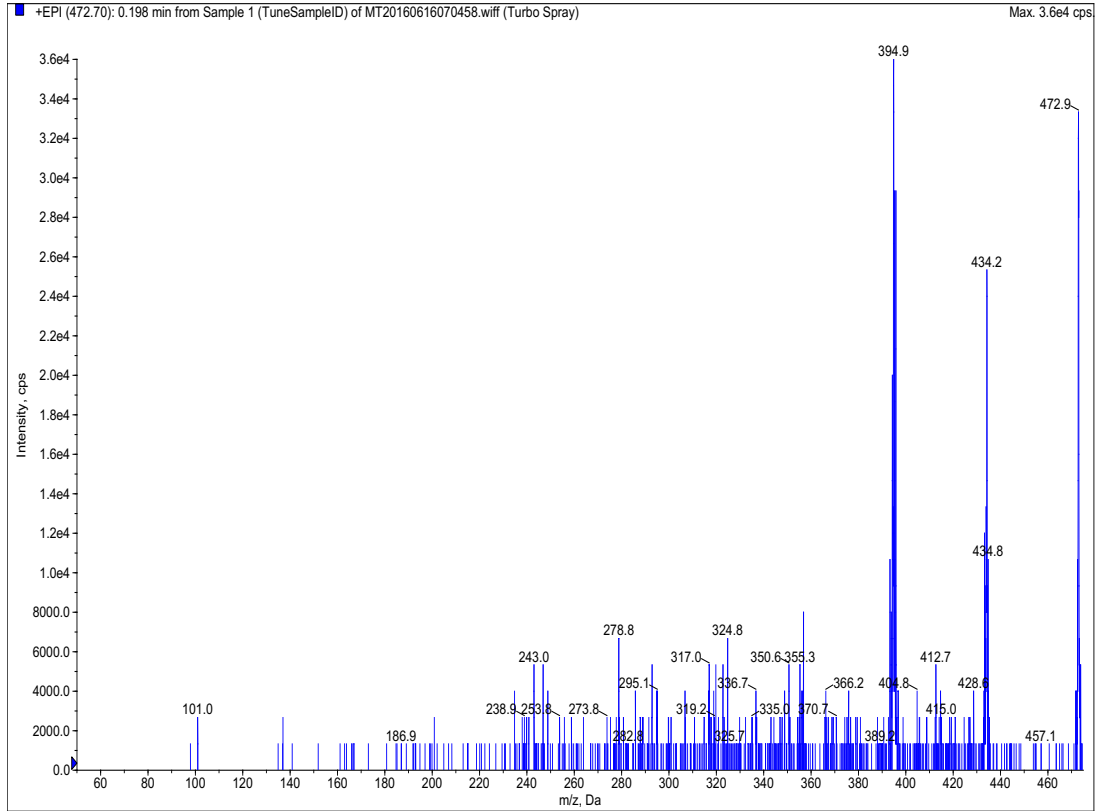


Figure 2: Mass spectrum of Co(II) complex

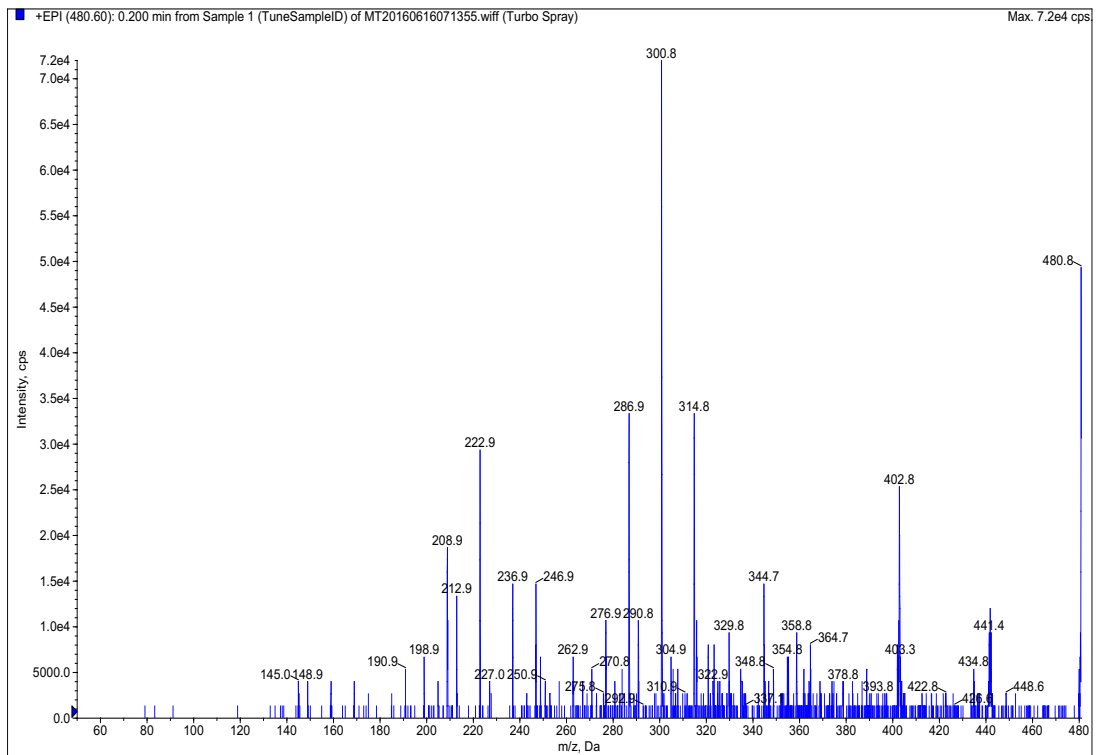


Figure 3: Mass spectrum of Zn(II) complex

3.4. Proton and carbon nuclear magnetic resonance spectra

^1H -NMR and ^{13}C -NMR spectrums of the Zn complex (**1**) were recorded in d_6 -DMSO as a deuterated solvent. The ^1H -NMR spectra of the Zn complex (**1**) exhibits signals due to protons of phenyl and pyridazine rings as multiplex at δ 7.69-7.25 ppm (14 H) and CH_3 protons as singlet at 3.80 ppm (3H) (Fig. 4). Water peak was found at 2.51 ppm. Since there are many aromatic rings in the structure of the Zn complex, the aromatic region is complicated in the ^1H -NMR spectrum. The ^{13}C -NMR spectra of the Zn complex (**1**) exhibits signals due to of pyridazine ring (C-O) carbon at 196.19 ppm, azomethine carbon at 163.06 ppm, pyridazine ring carbonyl carbon at 160.31 ppm, phenyl ring (C-O) carbon at 149.19 ppm, pyridazine ring (C=N) carbon at 138.00 ppm, (C-N=) carbon at 137.63 ppm, aromatic carbons at 133.48-116.37 ppm and CH_3 carbon at 40.51 ppm (Fig. 5).

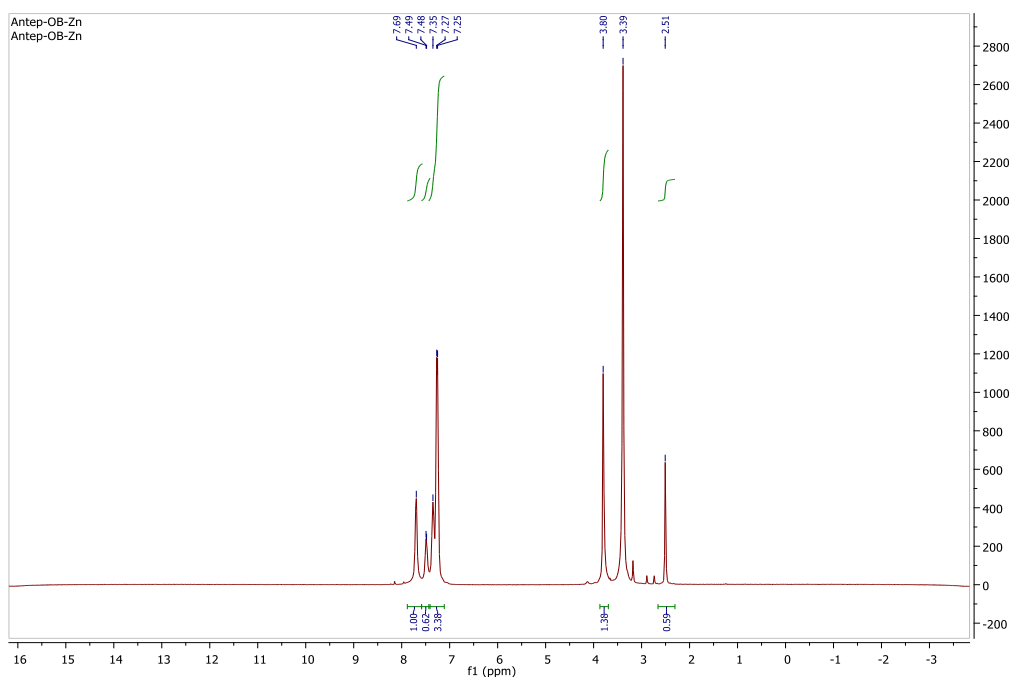


Figure 4: ^1H -NMR spectrum of Zn(II) complex

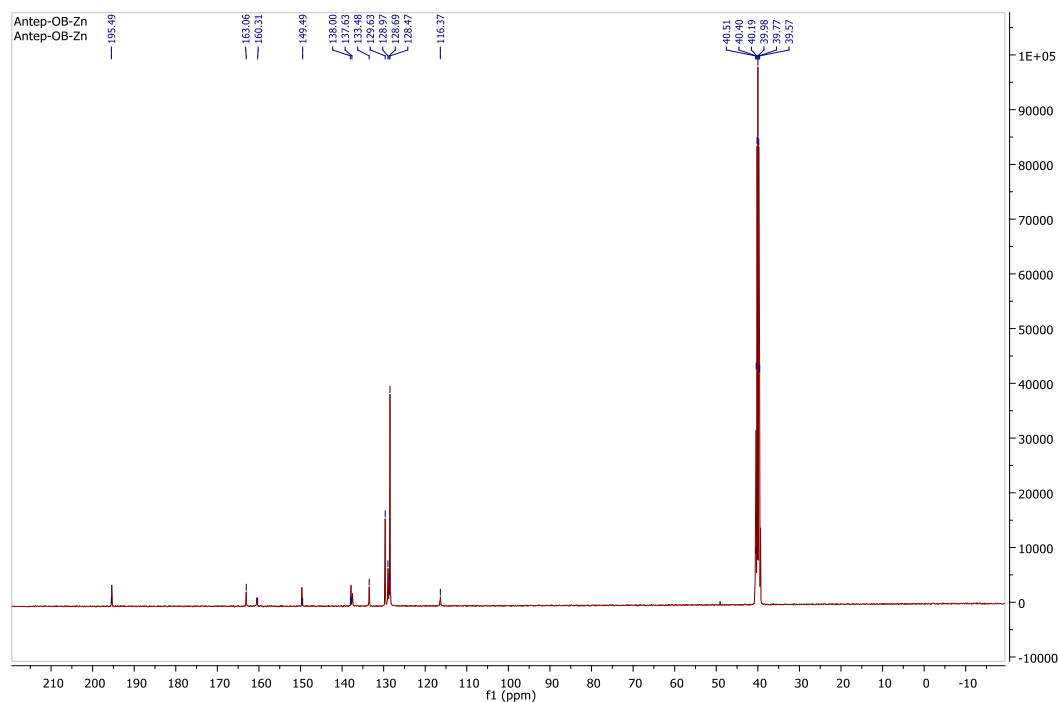


Figure 5: ^{13}C -NMR spectrum of Zn(II) complex

3.5. Antioxidant studies

3.5.1. DPPH scavenging activity

One of the most important factors that cause biological damage is free radicals. DPPH, a free radical compound that is stable at 25 °C and was widely used as a screening method for free radical scavenging activity of organic and inorganic compounds [19]. To determine the DPPH scavenging ability, various concentrations of compounds were tested by comparing with standard Ascorbic acid and Trolox. In Fig. 6, obtained results of the compounds on DPPH radical scavenging activity are presented. While the concentration was increased from 10 mg/L to 200 mg/L, DPPH ability increased from 6.1% to 38.6% mg/L and 17.8% to 84.3% mg/L for Cu and Zn, respectively. It was found that Cu had the lowest DDPH activity, while Zn had the highest. Trolox and Ascorbic acid displayed highest scavenging activity than tested other compounds. Similar results have been reported by Ağırtaş et al. [20]. The IC_{50} values of the novel pyridazine-based Zn^{II} , Ni^{II} , Mn^{II} , Cu^{II} and Co^{II} complexes were found as 46.54, 120.09, 168.99, 269.59, and 152.66 mg/L, respectively. The results indicated that the existence of Zn^{II} may contribute to the antioxidant activity.

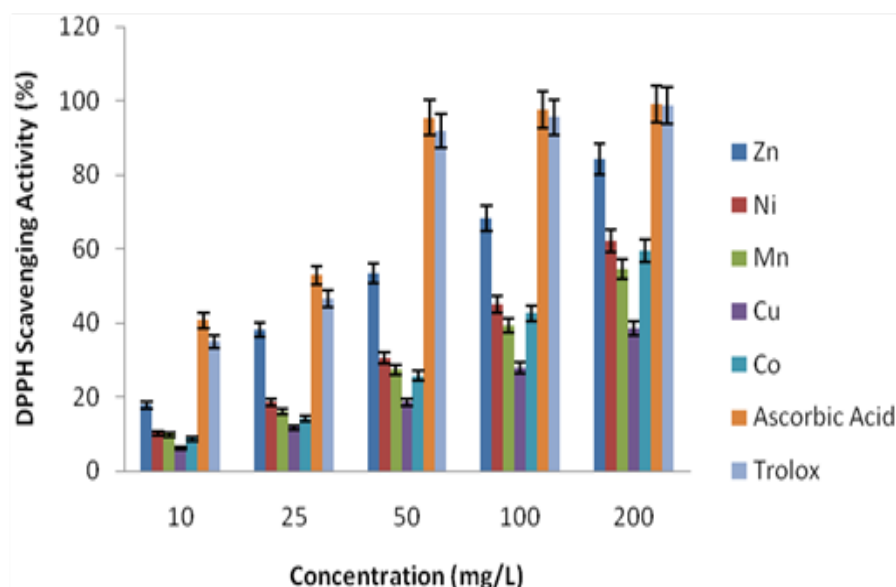


Figure 6: Free radical scavenging activity of the compounds on DPPH radicals

3.6. Metal chelating activity

In order to determine the capability of the tested compounds to bind the oxidation, catalytic ferrous ion was applied in ferrous ion chelating activity test [10]. In this study, chelating activities of DMF solutions of compounds were investigated. As can be seen in Fig. 7, when the concentration of the compounds increased, the metal chelating activities of all tested compounds increased at all tested concentration. The chelating activities of the DMF solutions of compounds at 200 mg/L concentration were 88.9% for Zn^{II} , 80.1% for Ni^{II} , 76.3% for Co^{II} , 53.4% for Mn^{II} and 31.6% for Cu^{II} . The IC_{50} values of pyridazine-based Zn^{II} , Ni^{II} , Mn^{II} , Cu^{II} , and Co^{II} were also determined as 48.82, 52.86, 184.46, 335.42, and 98.52 mg/L, respectively. According to ferrous ion chelating activity of Schiff base metal complexes, Zn^{II} was almost as good chelator as positive control (standard) EDTA.

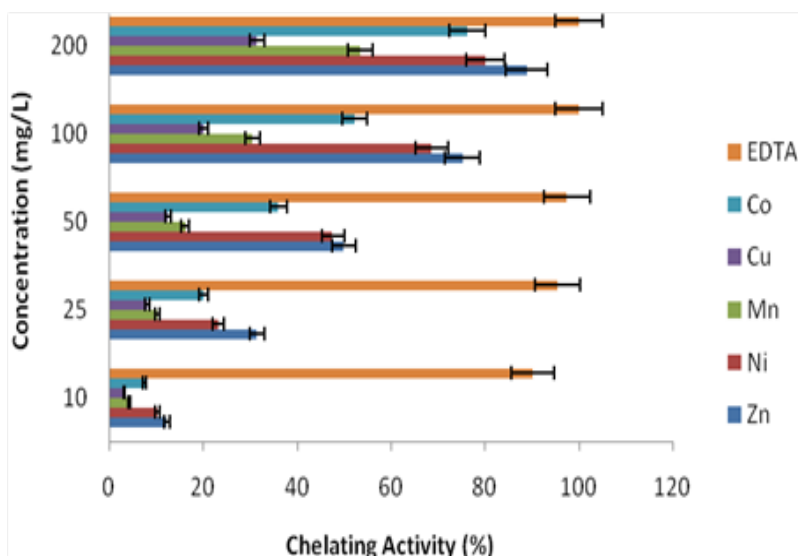


Figure 7: Metal chelating activity of compounds

3.7. Antimicrobial activity

Antimicrobial activity of the five newly synthesized compounds were examined by using disk-diffusion method and results are shown in Fig. 8. According to the results, only *E. hirae* was inhibited by all compounds and the antimicrobial activity was in order of Mn>Zn=Ni=Co>Cu. Compound 2 inhibited all microorganisms and displayed the highest antimicrobial activity against *L. pneumophila* subsp. *pneumophila* with inhibition zone value equal to 13 mm.

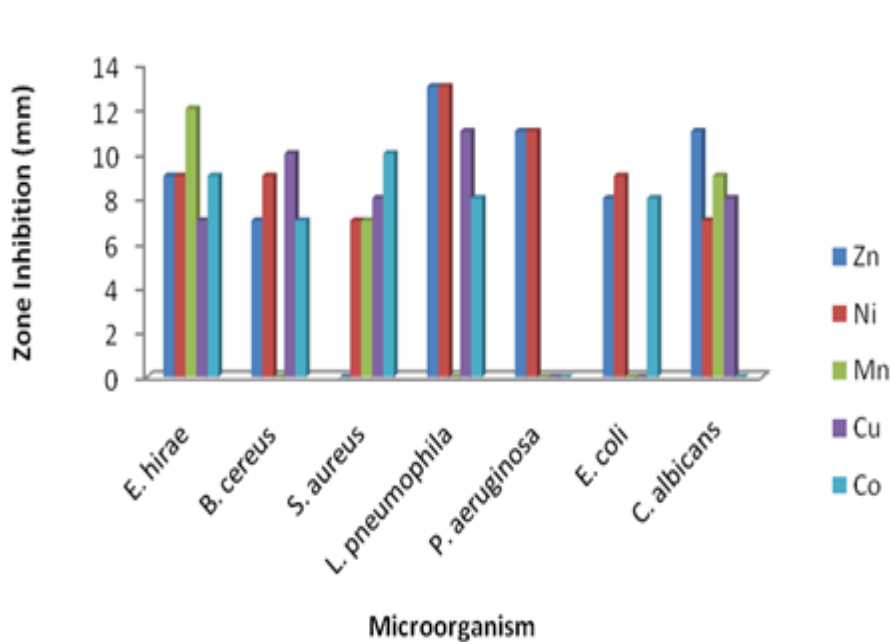


Figure 8: Antimicrobial activity of compounds

3.8. DNA cleavage studies

As seen in Fig. 9, all tested compounds cleaved the supercoiled DNA in varying extents (Lane 2, 3, 4, 5 and 6). The untreated plasmid DNA (Lane 1) and plasmid DNA with 3% DMF (Lane 7) were performed as control groups. Form I (supercoiled DNA) was converted to form II (nicked circular DNA) and form III (linear DNA) at 100 $\mu\text{g}/\text{mL}$ concentrations of all complexes. From experimental results we concluded that all of the tested compounds were active in cleavage. Therefore, these metal complexes can be used as alternative anti-cancer medicine after following toxicological test systems.

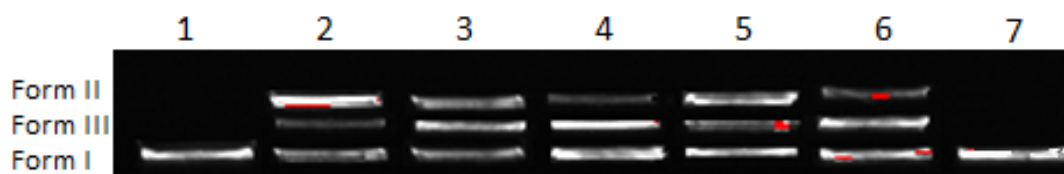


Figure 9: DNA cleavage of the metal complexes. Lane 1, pBR 322 DNA; lane 2, pBR 322 DNA + 100 $\mu\text{g}/\text{mL}$ of **Zn**; lane 3, pBR 322 DNA + 100 $\mu\text{g}/\text{mL}$ of **Ni**; lane 4, pBR 322 DNA + 100 $\mu\text{g}/\text{mL}$ of **Mn**; lane 5, pBR 322 DNA + 100 $\mu\text{g}/\text{mL}$ of **Cu**; lane 6, pBR 322 DNA + 100 $\mu\text{g}/\text{mL}$ of **Co**; lane 7 pBR 322 DNA + 3%DMF

4. Conclusion

In conclusion, the novel pyridazine-based Zn^{II} , Ni^{II} , Mn^{II} , Cu^{II} , and Co^{II} Schiff base metal complexes have been synthesized using the template method. The metal complexes were characterized by elemental analysis, UV-Vis, FT-IR, Mass, magnetic susceptibility. The newly synthesized compounds displayed antioxidant activity that directly proportional to the concentration of compounds. These compounds displayed moderate DPPH scavenging and metal chelating activity except compound Zn which displayed a potent antioxidant activity compared to the standards. On the basis of DNA cleavage results, all compounds completely broke the structure of form I and form II DNA. Inhibition efficiencies of the compounds were tested against seven microorganisms. According to the inhibition results, compound Ni inhibited all microorganisms and displayed the highest antimicrobial activity against *L. pneumophila* subsp. *pneumophila*. Besides, all compounds inhibited only one microorganism called *E. hirae*. As a result this novel compounds, especially Zn compound can be used as antioxidant agents and anti-cancer drug after mutagenic test assay.

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